Doctoral Thesis

In Silico Investigation of Bone Adaptation in Health and Disease

Author(s):
Levchuk, Alina

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In silico investigation of bone adaptation in health and disease

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presented by
Alina Levchuk
Master of Science in Biomedical Engineering, ETH Zürich
Bachelor of Science in Biomedical Engineering, The City College of New York
born on 28th May, 1986
citizen of the United States of America

accepted on the recommendation of
Prof. Dr. Ralph Müller, examiner
Dr. Bert van Rietbergen, co-examiner

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Alina Levchuk
Summary

The interplay between trabecular bone structure and function has been investigated for more than one hundred years. The ability of this tissue to adapt to the external mechanical environments has become the focus of many investigations ranging from molecular signaling to whole bone mechanics. While, many details of healthy and unbalanced bone remodeling processes are still to be elucidated, medical and societal benefits such knowledge would bring about are invaluable. Osteoporotic changes in bone threaten the lives and the well-being of millions of patients annually, and this number is bound to further increase as the world population ages. Clinical prediction and prevention of the consequences of the bone loss associated with osteoporosis could help doctors save millions of lives and decrease growing social and economic burdens. While accepted clinical tools make it difficult to innovate current state of the art practices in bone disease diagnosis, computational tools can provide the so far unavailable support in patient-specific risk assessment.

Therefore, the main objectives of this thesis were to contribute to the current understanding of bone remodeling processes and to develop in silico tools capable of simulating bone changes in health and disease.

Within the first aim of the thesis phenomenological simulations of bone remodeling in three dimensional human vertebrae and femora were performed. Age-related bone loss and bone gain associated with preventive and curative treatments with clinically available therapies were captured in the in silico simulations. Furthermore, a reference database supporting clinical assessment of bone quality was generated. While the objectives of this study were met, the inherent lack of mechanical feedback in the algorithm allowed us to establish a preference for a model that incorporated this mechanism, and thus led to the next aim of the thesis.

Therefore, the second aim comprised large-scale in vivo validation of the strain-adaptive algorithm for simulation of bone remodeling using an in vivo study
of 181 animals. Scenarios of early and late bone loss, as well as treatment with mechanical and pharmaceutical interventions were simulated. The outcomes of this study established capabilities of the model to reflect in vivo changes in bone quality and architecture. For example, average errors in bone volume fraction across all investigated groups after correlative simulation of a 4 week in vivo study remained under 5%. On the other hand, dynamic morphometric parameters were more difficult to capture in the simulations. Further analysis of these results provided a clear direction for the third objective.

Within the final aim of the thesis, iterative strain-adaptive algorithm was expanded to incorporate current knowledge of dissimilarity between bone formation and resorption processes. The following in vivo derivation of the model settings from the experimental data allowed simplified control of the algorithm. The development also comprised the first attempt to control a computational algorithm with model parameters directly derived from in vivo measurements. An update from step-wise linear to logarithmic functions to describe the relationships between bone remodeling and mechanical signal was performed as the next step in the study. This process facilitated not only improvement in the simulation outcomes, but also revealed valuable insight into computational bone mechanics. Finally, a novel statistical model for the prediction of individual remodeling processes was developed. The full scope of the predictive capacity of the in silico framework was then validated using an independent in vivo dataset. The outcomes of the study were encouraging in their accuracy in prediction of bone remodeling. For example, after 8 weeks of predictive simulations errors in bone volume fraction for healthy, osteopenic, and mechanically treated groups remained under 3%, a result which has not been achieved previously even with the correlative approach.

In summary, the results of the work associated with this thesis lead to better understanding of bone remodeling in healthy and diseased bone, and provided novel tools for the in silico simulations of changes in bone in human and animal models.
Résumé

La relation entre la structure de l’os trabéculaire et sa fonction est étudiée depuis plusieurs siècles. La capacité de ce tissu à s’adapter aux contraintes mécaniques extérieures a fait l’objet de nombreuses recherches allant de l’étude de la signalisation moléculaire à l’étude mécanique de l’os dans son ensemble. Cependant, une connaissance plus précise des processus de remodelage osseux dans les cas sains et pathologiques est susceptible d’apporter des bénéfices médicaux et sociaux considérables. Les conséquences de l’ostéoporose sur la structure osseuse menacent en effet chaque année la vie et le bien-être de millions de patients, et ces chiffres sont appelés à croître avec l’allongement de la durée de vie de la population mondiale. Prédire et prévenir la perte de masse osseuse associée à l’ostéoporose pourrait permettre de sauver des millions de vies et de diminuer ce fardeau social et économique toujours plus grand. Bien que les outils cliniques existants et largement acceptés rendent difficile l’innovation en matière de diagnostic osseux, de nouveaux outils numériques sont en mesure de fournir pour la première fois une prédiction de risque personnalisée.

Par conséquent, les objectifs de cette thèse sont de contribuer à la compréhension des processus de remodelage osseux et de développer des outils numériques (in silico) capables de prédire l’évolution de la masse et de l’architecture osseuses dans les cas sains et pathologiques.

Dans le cadre du premier objectif de cette thèse, des simulations tridimensionnelles phénoménologiques du remodelage osseux de vertèbres et de fémurs humains sont développées. Les résultats de ces simulations in silico mettent en évidence la perte de masse osseuse liée à l’âge ainsi que son augmentation suite à des traitements préventifs ou curatifs. De plus, ils ont permis la génération d’une base de données de référence destinée à l’évaluation clinique de la qualité osseuse. Bien que les objectifs de cette étude soient atteints, la nécessité de l’ajout d’un rétrocontrôle mécanique est constatée. Ceci amène le deuxième objectif de cette thèse.
Ce second objectif consiste en une validation in vivo et à grande échelle de l’algorithme simulant le remodelage osseux à partir d’une étude in vivo sur 181 animaux. Des scénarios avec perte de masse osseuse précoce ou tardive, avec traitements médicamenteux ou stimulations mécaniques, sont simulés. Les résultats de cette étude confirment la capacité du modèle à refléter l’évolution in vivo de la qualité et de l’architecture osseuse. Par exemple, l’erreur moyenne sur la fraction volumique osseuse sur l’ensemble des groupes soumis à une simulation corrélative in vivo de 4 semaines demeure inférieure à 5%. Cependant, les paramètres morphométriques dynamiques s’avèrent plus difficiles à prédire. Le troisième objectif de ce travail a été établi à partir d’une analyse approfondie de ces résultats.

Dans le cadre du troisième objectif de cette thèse, l’algorithme itératif avec déformations rétrocontrôlées est modifié afin d’incorporer les connaissances actuelles sur les dissimilarités entre les processus de formation et de résorption de la masse osseuse. L’obtention des paramètres du modèle à partir des données expérimentales permet alors une simplification du contrôle de l’algorithme. Dans un premier temps, un algorithme numérique contrôlé directement par les paramètres mesurés expérimentalement est développé. Dans un second temps, une relation logarithmique reliant remodelage osseux et stimuli mécaniques est implémentée en remplacement de la relation linéaire par morceaux initialement retenue. Cette modification améliore les résultats de la simulation et permet une compréhension plus poussée de la mécanique osseuse numérique. Enfin, un modèle statistique préliminaire est développé pour permettre une prédiction personnalisée du remodelage osseux. La capacité prédictive de ce procédé in silico est alors évaluée dans sa totalité à l’aide de résultats in vivo indépendants. Les résultats obtenus par cette étude présentent une précision encourageante dans la prédiction du remodelage osseux. Par exemple, les prédictions sur 8 semaines présentent une erreur inférieure à 3% pour les groupes sains, pour ceux présentant une ostéopénie, ainsi que pour ceux soumis à une stimulation mécanique. Un tel résultat qui n’avait jusqu’à présent jamais pu être obtenu ni avec une approche corrélatrice, ni aucune autre méthode.
En résumé, ce travail apporte d’une part une meilleure compréhension du remodelage osseux, dans les cas sains comme dans les cas pathologiques, et d’autre part de nouveaux outils pour la prédiction in silico du remodelage osseux humain et animal.
Chapter 1
Introduction

1.1 Motivation

Healthy bone is a dynamic tissue prone to constant adaptive processes in response to mechanical and systemic changes an organism undergoes [1]. Age-related metabolic alterations and reduced physical activity, such as prolonged bed rest, often lead to degenerative conditions of bone tissue, most prevalent of which is osteopenia, followed by osteoporosis [2]. While loss of bone in itself is not a life-threatening process, it often results in elevated risks of developing bone fractures [3-5]. While current medical technology provides effective treatment options [6, 7]; sustaining a fracture inevitably leads to decreased mobility, compromised quality of life, and financial expenditures [8-10]. Furthermore, such fracture-related complications and their consequences can lead to fatal outcomes. For example, the number of deaths reportedly related to fractures in the European Union in 2010 alone was estimated to be 43,000 [10]. Once an individual suffers the first bone fracture, the risk of the follow up event increases by 86% [11]. Global statistics are also striking, with 8.9 million fractures reported annually [12].

The most rational approach to alleviate health and economic consequences of bone fractures appears to be prevention of the initial fracture, which is only possible if the event can be foreseen. Fracture prediction is not currently available in clinics, in fact many patients are not appropriately diagnosed or treated for osteoporosis until it is too late to prevent its adverse effects [13, 14]. Incorporation of in silico models with the current diagnostic methods of quantifying bone quality could make patient-specific predictions of bone degeneration possible. Such computational approaches can be further utilized for the selection of appropriate treatment options based on their mechanisms of action.

Prior to integrating in silico algorithms in clinical diagnostics and treatment planning, the principles of bone remodeling have to be deciphered, translated into
mathematical format, and carefully validated against controlled experimental data [15, 16]. For this purpose, in vivo experiments to investigate the exact mechanisms of disease progression and drug action and interaction are often carried out in phenotypically controlled animals [17-19]. The outcomes of such studies not only enable better understanding of biological processes, but can be used to improve and validate computational algorithms [15].

A comprehensive review of existing in silico models for the simulation of bone remodeling has been compiled by Gerhard et al [20]. The main limitation for many algorithms remains the scale of the whole bone simulations, which often exceeds available computational resources. This in turn calls for geometrical simplifications and/or volume sub-selection, reducing both biological relevance of the developed tools and their applicability in clinics. In addition, lack of appropriate validation techniques leaves the legitimacy and accuracy of many of the algorithms unverified, further limiting their value [16].

Two of the in-house developed algorithms have been designed to process large data sets of three dimensional (3D) whole bone volumes [21, 22]. At the same time extensive experimental studies of bone adaptation in a mouse model, carried out as part of two pervious theses [23, 24], provide the necessary in vivo foundation for further development of these in silico algorithms.

The simulated bone atrophy (SIBA) algorithm was initially developed for the prediction of trabecular bone remodeling due to age-related bone loss, and was based on the phenomenological open-loop approach [22]. It was also successfully validated using a model for anabolic changes in mouse bones [25]. The next application that could show clinical relevance of this computational model would be evaluation of its capacities to simulate the effects of osteoporosis in combination with anabolic and antiresorptive treatments for whole human bone.

The second algorithm was implemented in a closed-loop mechanical feedback manner [21] and was based on the well-established Mechanostat Theory of bone regulation [26]. Briefly, the model allows iterative simulation of bone adaptation as a function of the local mechanical signals, and was designed using
experimental mouse data. However, *in vivo* validation of the algorithm function was missing. In addition, the control parameters required iterative selection due to the lack of biological linkage. These two shortcomings, therefore, comprised the next objective in the algorithm development process.

With the available computational tools, and supporting data from experimental animal studies, the overall goal of this thesis was to combine the available resources in the *in silico* investigation of bone adaptation in health and disease.

### 1.2 Specific Objectives

The main purpose of this thesis was to develop a framework facilitating the prediction of bone adaptation for clinically relevant scenarios, while also elucidating new mechanisms for simulating bone remodeling processes *in silico*.

In short, the phenomenological algorithm was used for predictive simulations of bone remodeling in whole human vertebrae and femora in an effort to test its clinical applicability. In addition, to address the lack of biological feedback, a model allowing for incorporation of mechanical signaling in an iterative loop was further developed. This computational algorithm was first subjected to an exhaustive *in vivo* validation using a mouse model for bone adaptation, followed by analysis of the underlying biological processes, with the consequent integration of the insights from the *in vivo* measurements into models control parameters. Finally, the new implementation of the algorithm was validated in its predictive capacity with an independent animal study.

Following, are the aims of the dissertation:

**Aim 1:** Simulations of bone remodeling in human femur and vertebra in health and disease using simulated bone atrophy (SIBA) algorithm

**Aim 2:** Large-scale validation of a closed-loop mechanical feedback algorithm against experimental animal data

**Aim 3:** Hypothesis-driven investigation of bone remodeling in a mouse model: algorithm development and validation
1.3 Thesis Outline

Chapter 2 provides the background information for the thesis. First, features of bone structure and function relevant to in silico model development and implementation are discussed. Second, the limited extent of human studies, and corresponding ubiquity of animal models in the investigation of bone diseases is covered. Finally, a review of the current in silico models in bone biomechanics and the gold-standard for their validation are presented.

Chapter 3 describes implementation of the previously developed SIBA algorithm for the simulation of bone adaptation in human femora and vertebra. This chapter provides evidence of the clinical relevance of the approach, and gives an overview of the methodology. In short, high resolution (82 µm for the femora and 37 µm for the vertebrae) 3D scans of whole organ sample were used for the prediction of the effects of menopause and consequent development of osteoporosis in bone architecture. In addition, preventive and curative treatments with anabolic and anti-catabolic treatments have been included. Finally, study outcomes, as well as data analysis and significance to the scientific community are presented.

Chapter 4 introduces in-house developed mechanical feedback model and presents validation of the algorithm using an in vivo mouse study. The results cover large-scale simulations of experimental studies of bone loss accompanied by mechanical loading, anabolic (PTH: parathyroid hormone) and anti-catabolic (BIS: bisphosphonate) treatments. Quantitative and qualitative analyses are discussed with respect to the accuracy of the computational model.

Chapter 5 comprises a hypothesis-driven examination of biological relevance of the algorithm, as well as its long-term validation. First, the algorithm is expanded to decouple the control mechanisms of bone formation and resorption processes. Second, incorporation of the in vivo derived parameters into the control settings of the model is presented, followed by the update of the algorithm format from linear into natural logarithm function to further strengthen its links to the experimental
observations. Fourth, a statistical model for the prediction of algorithm settings on the animal-specific basis is outlined. Finally, model validation though group and animal-specific predictions of bone remodeling in an independent animal study are presented.

Chapter 6 synthesizes the results of the thesis. Major outcomes, their value to the scientific community and society, as well as limitations, and trajectories for future research are outlined.

References


Chapter 2
Background

2.1 Bone biology in reference to computational modeling

*In silico* modeling is often referred to as a “third pillar of science”, and is positioned right after deductive investigation and empirical approaches. More and more it is becoming the method of choice, particularly in studies where theoretical and inductive investigations are too costly, too complex and time consuming, or where the understanding of the underlying processes is too poor. Multidisciplinary computational approaches bridge natural sciences and applied mathematics with the technological tools that not only allow researchers to overcome these drawbacks, but also facilitate discovery of the new insights in the biological realm.

The human body is a complex physical system, in which the skeleton is responsible for locomotive and postural functions, as well as for mineral homeostasis, and hematopoiesis. On the organ level bones are composed of dense cortical and porous trabecular compartments. These two types of tissue are specific to their physiological location and function (*Figure 2.1*). Thus, it is believed that cortical bone, which comprises the outer surfaces of short bones, and the diaphysis of long bones, is mainly employed in protective and supportive role [1, 2]. Trabecular, or cancellous, bone, on the other hand, is located internally in the vertebrae, ribs, iliac crest, and metaphysis of the long bones. The most marked attribute of this tissue type is its elaborate architecture based on the rod- and plate-like trabecular arrangement (*Figure 2.1*), which is responsible for the light weight of the material, and its surprisingly high stress-bearing capabilities. Thus, material and structural properties of trabecular bone have received continuous attention in research, and can be characterized using a standardized metric system, which includes trabecular number (Tb.N), trabecular thickness (Tb.Th), trabecular separation (Tb.Sp), specific bone surface (BS/BV), and bone volume fraction (BV/TV) [3]. In the clinical setting, bone quality is often evaluated in terms of bone mineral density.
(BMD), which allows physicians to recognize health conditions in which the ratio of plate-like to rod-like structures is unbalanced, thus disrupting the ability of bone to withstand stress.

One of the most prevalent bone diseases, characterized by decreased bone mass and corresponding increased risk of developing bone fractures, is osteoporosis. The World Health Organization reported that in 2004 more 75 million people in the United States, Europe, and Japan were affected by osteoporosis [4]. While not life-threatening in itself, the disease resulted in almost 9 million annual bone fractures, with many leading to recurring accidents and complications that have often left patients bed-ridden. Aside from personal impairments, economic burdens must be taken into account. It has been reported that in the EU alone in 2010 the cost of osteoporosis treatment including pharmaceutical interventions was €37 billion [5]. While the human and financial tolls of the disease are growing along with the global population aging, the most effective measure of dealing with osteoporosis is prevention of the disease.

*Figure 2.1: (Left) Cortical and trabecular bone in a human vertebra (T12). (Right) Sub-volume of human trabecular bone showing heterogeneous plate-like and rod-like structure.*
General clinical practice in bone loss prevention involves administration of bisphosphonates [6-8]. These are therapeutic compounds with anti-catabolic qualities, which cause a reduction in bone resorption while keeping bone formation constant [9, 10]. However, such a combination of decreased resorption and stable formation has been shown to have detrimental effects on bone quality due to reduced bone turnover and corresponding increases in mineral content tissue brittleness [11, 12]. Therefore, a tailored approach to identify patients, for whom the benefits of such pharmaceutical interventions would outweigh the side effects, could potentially prevent fragility-induced bone fractures and lead to better selection of bone-loss therapies. While such capabilities are unavailable in current clinical practice, advances in *in silico* modeling can provide a solution for patient-specific prognosis and treatment selection.

Aside from endocrine pathways, dynamic bone remodeling is often discussed in the context of mechanical stresses, which are believed to be the driving mechanism in homeostatic and imbalanced formation and resorption processes. Osteocytes, simplistically referred to as “bone cells”, are widely believed to be a key player in mechanosensation in bone tissue. Despite dedicated efforts to unravel their true function and exact mechanisms of actions in experimental and computational studies [13-16], the complete comprehension of the cellular involvement in bone remodeling processes has not been achieved yet. Nevertheless, the established bone remodeling phenomena at the tissue level state that bone will be removed if mechanical stimulus is absent or insufficient, for example in the event of extended bed rest [17] or in the microgravity environment [14]; and correspondingly, that bone will be added if the mechanical stimuli are increased on a permanent or long-term basis, as has been observed in competitive athletes [18] or in animal models of mechanical loading [19, 20]. While these observations have formed the basic premise of the mathematical and computational models of bone remodeling, the precise relationship between mechanical loading and the corresponding amount of bone remodeling remains unknown. Mechanostat Theory [21], which suggests ranges of mechanical strain capable of inducing
local formation, resorption, or quiescence in bone, is often integrated into *in silico* models of bone remodeling [22, 23]. However, the biological relevance of these models, as well as confidence in the chosen implementations, remains an active area of research.

In addition, before such models can be used in clinics, they should be validated with the corresponding *in vivo* data in line with the current gold standards [24]. While long-term monitoring and non-destructive tracking of bone changes in human patients is difficult and costly, animal models can provide valuable supporting *in vivo* data for model development and validation.

### 2.2 Animal models in bone research

A major requirement for animal models in pre-clinical research is the ability to mirror corresponding biological processes in humans. For the purposes of bone remodeling investigations at the tissue and molecular level, it is also preferred to have genetically controlled animals, as this allows researchers to control for all environmental factors, isolating only the effects that are being investigated. In terms of bone adaptation studies, mouse models have been established for both genetic and physiological investigations, with dense genetic maps of the whole genome [25, 26] and models for osteopenia [27, 28], mechanical loading [29-31], and drug development [32] readily available.

In addition to the convenience of genomic modification, mouse models offer very short generation time, with animals taking only 10 weeks to go from birth to reproductive maturity [33], in turn reducing maintenance costs. Laboratory mice can also give birth to up to 12 pups in a single litter with an average of 3-4 litters per mouse [34], further reducing breeding and purchasing expenses. Finally, mice are social animals that can be housed together and are easy to handle, reducing both space requirements and breeding costs. One of the limitations of the mouse model for osteoporosis is that with removal of the ovaries (OVX), the resulting estrogen depletion results in weight gain in some animals, which in turn leads to increased mechanical loading, and a corresponding increase in bone mass. As this does not entirely correspond to
the progression of human osteoporosis, the bone changes in mouse models are usually classified as osteopenia rather than osteoporosis as is the case in humans [35]. In addition, younger animals have different bone growth and remodeling patterns compared to humans; this discrepancy is diminished with age, and the inability of mice to maintain bone mass in the OVX model is similar to the menopause effects observed in humans [36]. Finally, as tissue changes and loading scenarios are markedly different in mice and humans, direct comparisons of mechanical properties between the two models are limited. Nevertheless, mouse models remain the most frequently used animal system in osteoporosis investigations [37], and can provide fast and reliable comparative outcomes provided the limitations are taken into account.

In computational applications, mouse models provide invaluable benefits by reducing size, complexity, and computational times of the calculations. In fact, similar to clinical trials, the performance of the in silico model is easier to access and validate using more readily available animal in vivo data. For example, an average mouse caudal vertebra (CV6) scanned at 10.5 µm isotropic resolution, would require approximately 1.8 million elements for conversion into 8 node hexahedral elements. A corresponding meshing procedure of a human lumbar vertebra (L2) scanned at 41 µm resolution would require 360 million elements. In terms of computational time, a mouse model can be solved with ParFE [38] with 128 CPU’s in less than 60 seconds, while a human model requires 960 CPU’s and about 2400 seconds of solving time with the same solver.

The benefits of using mouse models in computational studies of bone remodeling are numerous. A recent validation of the in vivo mouse model for osteopenia [28] and an earlier established model for mechanical loading [39] provide further justification for using mouse models in computational investigations. Animal models can also provide support not only in the algorithm development process, but, perhaps even more importantly, in supplying data for the direct in vivo validation of the model assumptions and in
silico outcomes. The next section of this chapter discusses gold standard and common practices in model validation in the computational bone

References


2.3 In vivo validation of predictive models for bone remodeling and mechanobiology

Alina Levchuk and Ralph Müller


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Abstract

In silico modeling is a powerful tool for the prediction of bone remodeling and mechanobiology. As the method is gaining popularity a standardized measure for the in vivo validation of the quality of the produced simulations is required. In this review, we discuss current validity assessment approaches, as well as the validation “gold standard”, in which the experimental and computational parts are carried out concomitantly, and by the same research team. A novel validation framework for the tissue level model, based on the true geometry is introduced.

Introduction

Our understanding of bone remodeling and its governing mechanisms has come a long way since the first attempts to explain these complex processes [1, 2]. In fact, it is now often left to the biologists to characterize the elaborate signaling processes in bone, while a new branch of computational biomechanics has emerged, with the focus on creating realistic models of these biological events. In silico modeling, supported by experimental investigations, is a powerful tool that allows translation of biological phenomena into mathematical laws, thus
facilitating detailed analyses of distinct biological processes. The true value of in silico modeling is, however, in its predictive power, which, if close enough to the in vivo events can not only save large efforts in the experimental domain both resource and time wise, but also introduce treatment prediction options in clinics, thus improving therapeutic outcomes.

The transition from theoretical modeling to in silico simulations required a major improvement in the available computational capabilities. The advent of the finite element (FE) analysis in the second part of the 20th century has become such a breakthrough for the field of biomechanics. The first published investigation, which incorporated the technique, was performed by Brekelmans et al. [3]. The popularity of the application grew exponentially ever since, many of the prominent publications of the first decade of its existence being reviewed in Huiskes and Chao (1983).

Micro-computed tomography (micro-CT), introduced several years later [4], allowed not only three-dimensional (3D) visualization of bone architecture, but also a more reliable image-based validation method for the computational models. However, the first validation of an in silico model against the corresponding biological data was reported only in 1997, in a study that compared FE models of trabecular bone with contact radiographs both quantitatively and qualitatively [5].

In the meantime, both computational and visualization advances have provided a framework for accurate simulations of bone remodeling and mechanobiology throughout the hierarchical levels of its complexity, while algorithm validation with experimental data collected within the same study was deemed “gold standard” for model confirmation [6]. This review focuses on different approaches of in vivo validation across multiscale modeling of bone remodeling and mechanobiology from cell to tissue and on to the organ level.

**Cell Level**

Bone is a tissue subject to frequent remodeling due to various mechanically triggered remodeling processes as well as micro- and macro-fractures. The study
of bone mechanobiology, thus, remains relevant throughout the lifetime of the organism. However, understanding and prediction of the cell mechanics is particularly significant for the multiscale applications, such as implant selection and fixation, and fracture healing [7]. Nevertheless, even with the focus of research narrowed down to the single cell level, *in silico* studies range from the simulations of cellular interactions, to signaling pathway modeling, and all the way to the intracellular predictions of cytoskeletal reorganization.

The first validated model in mechanobiology described osteocyte excitation by mechanical stresses in mathematical terms [8]. The model was based on the experimental observations, and attempted to quantify mechanical stimuli sensed by osteocytes within the bone tissue. For validation purposes, the calculations were compared to experimentally measured results, reported by a collaborating group [9].

Incorporation of the FE analysis into the mechanobiological models was initially an attempt to provide analytical perspectives on observed *in vivo* events; this trend later developed quantification and even prediction of the mechanical changes on the local level. Nevertheless, comparison with literature remained a preferred method of validation in the field [10-12].

In an effort to create more realistic and sophisticated *in silico* models researchers started incorporating true geometries obtained by various imaging methods [13, 14]. The study by Anderson and collaborators, for example, was based on high-resolution transmitted electron micrographs to analyze stresses imposed on osteocytes by fluid drag, while McGarry and colleagues incorporated previously reported images of cell spreading [15] to assess the effect of fluid shear stress and strain on the mechanical response of bone cells using FE analysis. Nevertheless, while computational and experimental components of the investigations are rarely carried out within a single integrative study addressing the same research question using both *in silico* and *in vivo* modalities concomitantly, the possibility of direct validation remains slim.
A computational model for signaling pathways and interactions between osteoblast and osteoclasts has attempted to predict the effects of catabolic treatment with parathyroid hormone (PTH), as well as to simulate the interaction between receptor activator of nuclear factor-κβ, its ligand, and osteoprotegerin (RANK – RANKL – OPG pathway), which is essential for osteoclast formation [16]. This complex in silico framework has reportedly been able to correctly predict cellular interaction, and the effects of the common metabolic diseases, such as estrogen deficiency, calcitriol deficiency, senescence and glucocorticoid excess. The results of the simulation find convincing evidence in the extensive comparison with literature; however no other direct validation has been undertaken. Other theoretical models with the focus on the prediction of molecular signaling pathways and mechanobiology have also been presented [17-19]; unfortunately, despite the fact that all of them strive to predict bone adaptation on the micro scale, none of them have been verified against corresponding in vivo data, and thus are lacking confirmation of the level of fidelity.

The need for validation has also been emphasized for in silico models of cellular chemotaxis and cytoskeletal reorganization [20, 21]. Both investigations compare results of the computational simulations with the in vitro experiments. In both reports sample geometries and boundary conditions for the models were derived directly from the experimental data. For example, the study by Landsberg and colleagues used a tetrahedral mesh for micro-CT reconstruction, as a starting point for the chemotaxis simulation, while Loosli and colleagues reconstructed the shapes of the adhesive islands from the in vitro study to computationally predict the adhesion sites of the cells (Figure 2.2). Such complementary experimental and in silico studies tend to enable better understanding of the model limitations. For example, Landsberg and colleagues refer to a similar ongoing experimental study, utilizing signaling molecules, for further model validation. Loosli and colleagues, on the other hand, mention the algorithm’s failure to predict adhesion formation at curved geometries, due to a missing model parameter, as one of the limitations, requiring further improvements.
The overall lack of adequate validation for the predictive value of microscale models in bone mechanobiology has also been noted by other authors [22-24]. A particular concern of validating models with \textit{in vivo} data provided by collaborating investigators is that important details of experimental setup, and measurements relevant for the confirmation of the computational results, might be omitted. This is often the case for boundary conditions and mechanical properties of the material, which generally hold true only for the exact conditions of the testing setup. Consequently, while numerous experimental reports can be found in literature, extrapolations or estimations of such data for validation purposes could be misleading and erroneous.

\textbf{Tissue Level}

It has long been shown that trabecular bone is more susceptible to the effects of osteoporosis than cortical bone [25]. Non-surprisingly, most predictive models for
bone adaptation on the tissue level focus on this particular component of bone. While a large number of existing models have already been extensively reviewed [24, 26], validation of those studies has never been comprehensively discussed. This section covers bone remodeling algorithms which have been validated in one way or another. Additionally, a new in vivo validation technique for a recently developed model of mechanically triggered trabecular remodeling is discussed.

The first algorithm based on true bone geometry comprised a phenomenological model for bone resorption and was performed on the high resolution quantitative computed tomography (QCT) images [27]. In the study, 3D FE models were paired with controlled Gaussian filtration to derive two models of moderate and pronounced bone atrophy. While the resulting apparent Young’s moduli were the only mode of validation for this early attempt of in silico simulation of bone adaptation, the reported methods became the foundation many subsequent models. Thus, a follow-up study introduced further improvement to the application by using 3D micro CT scans as the input for the algorithm [28]. The isotropic resolution of the input images increased from 170 to 14 µm. In addition, the original simulated bone atrophy (SIBA) model was expanded to follow the mechanostat hypothesis [29], allowing controlled formation and resorption. This, in turn, facilitated simulation of various stages of bone loss, as well as a more realistic “age-match”. For validation the results were compared to the experimental measurement of the post-menopausal group both qualitatively and quantitatively, and proved to be in good agreement. Finally, the model was applied to the 3D micro CT scans of human iliac crest and lumbar spine biopsies selected from the pre- and post-menopausal groups, in an attempt to simulate pre-, peri-, and post-menopausal bone states [30]. In this study strong emphasis was placed on the validation of the results against biological data. Thus, visual comparison after the simulation of 43 years confirmed that the model produced realistic trabecular architecture when compared to the in vivo group, while quantitative bone morphometry, carried out for both groups produced a 100% match for the bone volume density (BV/TV) parameter, and excellent agreement for the other parameters.
Another *in silico* simulation, based on true bone geometry and verified against *in vivo* biological data, employed a voxel-based surface adaptation under uniaxial compression [31]. In this algorithm, micro-CT measurements of canine cancellous bone were obtained from the previously published investigation [32], and the results of the simulation were compared to the corresponding animals at the end of the *in vivo* study [33]. The validation was performed based on a comparison of the calculated morphometric indices for the *in silico* and *in vivo* experiments respectively. The results from the two approaches were in good agreement with values for bone volume fraction (BV/TV) being 0.230 and 0.222 for the experimental and simulated samples, respectively.

Several other notable studies, presenting elaborate models with realistic results, should be mentioned. For example, long-term investigation performed on the 3D micro-CT scans of human vertebra modeled the period of 50 years [34]. Morphometric indices, calculated for the resulting structures, correlated closely with the values reported in literature. Unfortunately, no validation against experimental data has been performed for this study. Another remarkable algorithm has been presented by Ruimerman et al. [35]. The simulation was carried out on computer generated cubes of trabecular bone, and investigated the ability of bone to adapt in response to elevated strains. In addition to implementing the theory for metabolic expression under load [36], an extensive examination of osteocytic stimuli, such as maximal principal strain, volumetric strain, and strain energy density (SED) have been carried out as part of the study [37]. While the model parameters themselves are largely based on the values found in literature, the results of the simulations have not received any validity confirmation past the “circumstantial evidence” validation, a term the authors used to summarize the similarity of the assumption-based prediction and biological reality.

A different algorithm for stochastic simulation of bone adaptation via the exchange of discrete bone packets and an accompanying novel approach for validation has recently been introduced [38]. The study was subdivided into the investigation of the most effective signal integration for this *in silico* model, as
well as validation of the results with quantitative backscattered electron imaging (qBEI) data. The model assumed that resorption takes place randomly on the bone surface, while deposition is mechanically controlled. The investigation of collective (summed), individual (maximal) and total (the sum of the previous two) signaling modalities indicated that using collective signal from the osteocyte network will introduce effective surface tension, which the authors argue plays a key role in bone morphogenesis and cell sorting. Validation of the simulation results against experimental qBEI data centered on correlating the values of quantified material heterogeneity. For this purpose, the age of bone packets (voxels) has been converted to represent corresponding mineral content [39]. When comparing simulated structures to the experimental images, bone mineralization density distribution (BMDD) exhibited similar trends, where older bone was enclosed under layers of younger bone. Notably, this validation method helped identify one of the limitations of the algorithm, as it did not comply with the proposed theory that older bone is more likely to be remodeled than younger bone [40], and was capable of remodeling only bone surface voxels.

More recently, Schulte et al. [41] introduced an algorithm to simulate bone thickening in response to cyclic mechanical loading using an open control loop. This in silico model is based on the assumption that a single remodeling signal submitted as an input for the simulation is sufficient to predict the long-term outcome of the remodeling process. Micro-CT scans of whole murine caudal vertebrae measured at the beginning of the in vivo study were used as the input for the simulation and the results computed from the time-lapsed in vivo images were compared to the simulated time points. This approach allowed not only comparison of the morphometric indexes and relative geometries in vivo and in silico, but also quantification and spatial distribution of the errors produced by the algorithm for each individual animal. The authors report a maximum error of 2.4% for bone volume fraction and 5.4% for other morphometric parameters. In addition, similarly to the previous study, the appropriate validation method helped detect one of the less obvious model limitations, namely that in the simulation
remodeling occurred rather homogenously in the surface layers, while a similar assessment of the in vivo data revealed localized areas of stronger deposition.

Finally, a similar approach has been extended for the validation of a newly developed algorithm for bone remodeling employing Frost’s mechanostat theory and using SED values calculated after each remodeling iteration, in a closed feedback loop [42]. The growth velocity was calculated with a set of iteratively solved non-linear equations, and the mechanical thresholds for resorption, formation or homeostasis were selected interactively. The algorithm was applied for a short-term prediction of the effects of hormone depletion due to ovariectomy, cyclic loading, and pharmaceutical treatments with anabolic (parathyroid hormone (PTH)), and anti-resorptive (bisphosphonate (BIS)) agents, as well as for the control studies for all groups. The model is also capable of long-term prediction (Figure 2.3). The input micro-CT images of the murine caudal vertebra were obtained from a concomitant in vivo study. The results of both in silico and in vivo study have been assessed qualitatively as described elsewhere [42]. For the quantitative evaluation, both static and dynamic morphometric parameters were calculated, and comparative physiome maps were constructed for each parameter showing again strong agreement between experiment and simulation.

Organ Level

Due to their place in the hierarchy of bone modeling, organ-level simulations often treat bone as a continuum, disregarding local architecture, and biological events. Instead, such models focus on global stresses and strains, as well as on the interaction of bone tissue with other materials. Thus, computational models on the organ level can be an invaluable tool for the pre-clinical studies of orthopedic implant performance and whole bone fracture healing. Since such investigations have the potential to go to clinics, thorough and well planned validation is mandatory in order to assess practicality and applicability of such in silico efforts.

The questions of importance of validation and its existing modalities have been comprehensively discussed by Huiskes [43]. In the same study, the author
presented a three-tier approach to validation of the augmented femur model. According to the model, periprosthetic remodeling runs under the effects of stress shielding, and can be estimated according to the adaptive bone-remodeling theory. The levels in the proposed validation included quantitative validity of the results at large, the validity of the outcome in a specific population, which was verified again with results from a canine study [44], and the validity of the prediction relative to a single specimen in the population, assessed with the human post-mortem retrieval study [45]. Remarkably, the suggested model performed well on all levels, and was deemed clinically relevant. Another two studies that attempted to predict pre-operative implant fit and fill [46], as well as to analyze different mechanical signals [47] for the total hip arthroplasty application have been validated with a similar approach. The presented algorithms were first validated against the in vitro CT data for the surface assessment and distance map accuracy in the first study, and performance of the FE code in the second study. Following this validation step, both algorithms were tested in vivo on patient specific clinical data. Eventually, both models were declared clinically applicable, with only the cortical penetration parameter performing slightly lower than expected in the sensitivity test of the first investigation. The second study also demonstrated that SED and deviatoric strains were the best candidates for the in silico mechanical signal used in the remodeling algorithm.

In addition, as both the need for computational modeling, and validation of such models is getting increasing recognition among researchers, several groups have focused on generating and collecting potential validation data [48-50], where micro CT imaging was performed either pre-operatively, and/or in the follow up studies, with the hope of making this data useful for future in silico studies, needing validation.

Another common validation method for the organ level computer models is comparison with the in vivo performed dual-energy X-ray absorptiometry (DXA). Several studies reported successful use of bone mineral density (BMD) measurements obtained from the 2D DXA images to validate and improve their algorithms [51-53]. Thus, Kerner and colleagues were able to demonstrate that the
results of both *in silico* patient specific model and clinical results agree in that bone loss corresponds to the inverse of the pre-operative bone mineral content (BMC). This result suggests that the model can be used to improve current implant design, by taking into account predicted bone loss. An investigation by Coelho et al. proposes a hierarchical model on the organ and tissue levels, where each scale of complexity was represented by density based variables. The model correlated apparent density distribution with that measured with clinical DXA, and found good agreements in both quantitative and qualitative results. The latest of the validated studies [53] was based on the patient-specific model for the prediction of BMD (Figure 2.4).

*Figure 2.3* Long-term simulation of the catabolic effect of PTH treatment combined with cyclic mechanical loading in a murine caudal vertebra.
When quantitatively compared to the DXA derived results for the normal, osteopenic, and osteoporotic bone, the maximum discrepancy between the *in silico* and *in vivo* measurements was only 3.92%. In addition, the authors report, that comparison with the clinical data has helped them improve the model by selecting the parameters that lead to the biologically relevant results.

![Methodology framework for comparative analysis of the in silico and in vivo studies on the organ level. Reprinted with permission from [53].](image)

Fracture healing is another area of interest that borders on the cellular and organ levels, in that it focuses on an event associated with the cellular level, such as sheer forces or angiogenic processes, but the algorithm is still based on a continuum assumption. One of the first computational models attempting to simulate tissue differentiation during fracture healing was based on the biphasic poroelastic FE algorithm that started at granulation and traced the process all the way to bone resorption [54]. The model was validated against histomorphometric data from literature, with different fracture gap sizes. The validation confirmed that the proposed mechanobiological model produced realistic results for different gap sizes and loading magnitudes on the rate of reduction in interfragmentary strains. Isaksson and colleagues have performed a comparative review of the existing approaches, and determined that deviatoric strain is the most significant
parameter for the modeling of tissue differentiation [55]. Unfortunately, since fracture healing is an inflammatory time-dependent process that is difficult to monitor, this investigation relied only on previous reports for validation.

**Conclusions**

While *in silico* simulations are gaining popularity, and have even been referred to as the “third method of science”, following logic and experiment [56], adequate validation is the only way to ascertain the level of fidelity, and thus, the advantage of such studies. It is generally accepted that the “gold standard” method of validation is a complementary *in vivo* investigation, ideally be carried out within the same research group. Both *in silico* and *in vivo* approaches should focus on the same research questions, and match boundary conditions, time scales, and other relevant parameters on the sample basis. Additionally, current imaging capabilities allow the use of the experiment data as direct input for the computational models, an improvement that should be taken advantage of for all suitable studies. Finally, it is important that both qualitative and quantitative modules of validation are comprehensively evaluated for the convincing evidence of the algorithm’s capability to produce realistic results.

**Index**

*In vivo, in silico*, bone, remodeling, mechanobiology, simulation, validation

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**References**


Chapter 3
Simulations of bone remodeling in human femur and vertebra in health and disease: open-loop phenomenological approach

Levchuk A, Badilatti SD, Webster DJ, van Rietbergen, B, Hazrati Mangalou, J, Ito, K, Müller R

1Institute for Biomechanics, ETH Zurich, Zurich, Switzerland
2Department of Biomedical Engineering, Eindhoven University of Technology, Eindhoven, The Netherlands


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Abstract

It is generally accepted that trabecular architecture plays a pivotal role in the mechanical behavior of bone. With age, bone undergoes structural changes resulting in osteoporosis, which can lead to life-threatening fractures, and inevitable decrease in the quality of life. As the mathematical representations of bone remodeling remain poorly understood, the aim of this study was to apply a previously reported phenomenological *in silico* model to simulate changes in the bone architecture due to age in a clinical setting. In addition, the effects of the
current recommended treatments were incorporated into the model. Using high-resolution three-dimensional μCT scans of whole human vertebrae and femora, age-related bone loss and recovery simulations have produced realistic models of structural changes over the period of 30 years. Finally, a database comprising 27 femoral and 20 vertebral samples with the corresponding computational simulation of osteoporosis, as well preventive and curative anabolic and anti-catabolic treatments, was compiled for clinical reference.

**Keywords**

Bone microstructure, simulation, osteoporosis, pharmaceutical treatment, *in silico* medicine

**Introduction**

Mechanical behavior of bone depends on the internal trabecular structure and its ability to withstand applied loads [1-3]. Resistance to stress often becomes impaired due to age-related osteopenia. If left untreated, the condition can develop into osteoporosis and lead to potentially life-threatening bone fractures and reduced quality of life [4, 5]. Despite the ability of current clinical practice to lead to accurate diagnosis of reduced bone quality, it remains unable to identify individuals that are prone to developing fractures. Patient-specific analysis of bone architecture and long-term prediction of bone adaptation could lead to improved treatment planning and fracture prevention.

Extensive efforts have been made to provide deeper insight into the biological phenomena involved in bone remodeling, as well as to translate those processes into mathematical terms [5, 6]. *In silico* medicine attempts to apply this knowledge to artificially reproduce and predict *in vivo* events in patients using modelling simulation and visualization techniques of biological and medical processes. Development and long term use of such an approach could not only reduce the need for animal studies, but also aid in clinical diagnostics and treatment planning.
This study was based on the previously established simulated bone atrophy (SIBA) algorithm [5], which was initially developed for the prediction of trabecular bone remodeling due to age-related bone loss. The model allows use of three dimensional (3D) whole bone samples, and is capable of handling large volume datasets of high-resolution micro-computed tomography (μCT) scans of human specimens. The phenomenological basis for the parametric selection within the algorithm carries both limitations and benefits. On one hand, this allows use of clinical measurements extracted from literature or experimental studies to be integrated directly into the model. On the other hand, it makes the model contingent on the availability of the clinical measurements. Nevertheless, the algorithm was successfully validated against in vivo data through the simulation of anabolic changes due to mechanical loading in a controlled mouse population [6]. This in turn led to the first aim of the thesis, namely, a test of model capacity to produce realistic outcomes of the simulation. Specifically, this was assessed in bone degeneration, as well as in bone formation in response to anabolic or anti-resorptive treatments.

In the clinical setting, vertebral fractures are reported to be the most prevalent, yet the least understood type of fractures [7]. This type of fracture often goes undiagnosed and is very difficult to predict [8]. Femoral fractures, on the other hand, are the most relevant with respect to decreased quality of life, functionality, and mortality [7, 9]. Hip fractures pose the highest risk in the elderly population due to high rate of falls in this age category. Diagnosis and prognosis of the fracture risk usually is based on the bone mineral density (BMD) measurements, which explain a large part of material properties of the tissue [10]. A measure of degree of anisotropy (DA) however, can help link bone mechanics to trabecular architecture, thus improving fracture prediction efficacy [10]. However, the reliance of a clinical prognosis on population-based databases [8] creates the need for a resource that would establish a connection between clinically linked BMD and prediction-relevant DA.

With respect to available treatment options, estrogen replacement therapies remain the most effective preventive therapy for of the decrease in bone mass.
However, this method is only effective for women in the stage of menopause. The two other widely used clinical therapies that have been shown to counteract the effects of osteoporosis are bisphosphonate agents (BIS), which reduce the amount of bone resorption, and parathyroid hormone (PTH), which was shown to improve bone quality through a number of different mechanisms of action [11, 12].

With this in mind, the aim of this project was to extend the application of the SIBA algorithm to simulate the progression of osteoporosis, as well as the effects of the BIS and PTH therapies in preventive and curative scenarios. The modelling was performed on high-resolution images of whole human vertebrae and femora from both male and female donors with the algorithm settings based on the reports of the clinical data. The final output of the study was then arranged in a database of 1598 bone samples, allowing for the clinical referencing of bone architectures to the corresponding bone quality.

Methods

Human Samples

The sample pool for femora consisted of 27 human cadaveric specimens obtained from 15 male and 12 female donors. The mean donor age was 77.4, with the range of 61–93 years (Figure 3.1). The twelfth thoracic vertebra (T12) sample pool comprised 20 specimens obtained from a previous investigation (9 male and 11 female donors). The mean age was 76.5, within the range of 64–92 years (Figure 3.2). All donors were of Caucasian origin, with no known bone or metastatic disorders. All experimental procedures were carried out at the Eindhoven University of Technology, Eindhoven, The Netherlands. The complete methodologies were previously published within associated studies [13, 14].

High Resolution Imaging

All samples were stored in buffered formalin solution for preservation purposes prior to imaging. The femoral samples have been scanned with an HR-pQCT system (XtremeCT, Scanco Medical AG, Brüttisellen, Switzerland) according to the previously published protocol [14]. In short, a region of approximately 90mm
was visualized from the proximal end of the femora, making sure that the femoral head was fully included in the field of view. The scans were recorded with the isotropic voxel size of 82 μm. Images were processed using the standard procedure, as previously described [14], which consisted of a Laplace–Hamming filter (epsilon = 0.5, frequency cutoff = 0.4) followed by segmentation with the default threshold setting of 400 ‰.

The vertebral samples were scanned using a different μCT setup (microCT 80, Scanco Medical AG, Brüttisellen, Switzerland), according to the previously described protocol [15]. This system allowed visualization of the vertebral microstructure with an isotropic resolution of 37 μm. Contrary to the femoral investigation, higher resolution was shown to be necessary for the analysis of vertebral trabecular microstructure [16, 17]. As part of image processing protocol, all scans were treated with Gaussian filtration (sigma=0.6, support=1) followed by thresholding to reduce the noise.

**Computational Algorithm**

Simulated bone atrophy (SIBA) algorithm comprised a combination of Gaussian filtration and thresholding [5], where the settings of the model were linked to the biological parameters. For example, sigma of the Gaussian filter was linked to the osteocyte penetration depth, while support, which corresponds to the radius of voxels affected by the Gaussian kernel, controlled maximum penetration depth. Threshold values were related to the efficiency of the osteocyte in refilling the resorption cavities, and iteration length reflected to the activation frequency of the basic multicellular unit (BMU) formation rate [5, 18]. For the current implementation of the algorithm we relied entirely on the clinical reports of those biological values in humans, and attempted to differentiate between femoral and vertebral sites if corresponding data was available.

**Parameter Selection**

Within the study, simulations of osteoporosis (30 years), antiresorptive (BIS) treatment (11 years), and anabolic (PTH) treatment (11 years) have been performed.
Two treatment scenarios were considered to mimic realistic clinical settings: in the preventive treatment case, pharmaceutical intervention was started instantaneously with the onset of menopause; in the late treatment case, simulation of treatment began after 9 years of bone loss.

Figure 3.1 Donor database for the femoral samples based on the ratio between the DA and BV/TV. Age and gender of each individual are displayed next to the corresponding sample.

The first year of pharmaceutical treatment was simulated separately for both treatment options, due to the initial increase of up to 6%/year in BV/TV shown clinically. In the following two years the effect was allowed to plateau in accordance with the clinical reports [19-21]. Despite the fact that treatment with PTH is currently approved for a maximum of 18 months, simulations were projected to reflect 10 years of treatment for both BIS and PTH cases to allow comparison of microstructural changes due to different simulation scenarios. As one of the objectives of the study was to populate the morphometric database correlating DA and trabecular BV/TV, biological age and gender of the donors were not considered in the simulations, allowing the use of the same method for all samples. Table 3.1 lists simulation
assumptions, corresponding sources from literature, and selected parameter settings for each scenario.

Figure 3.2 Map of the donor distribution for the T12 vertebral samples based on the ratio between the DA and BV/TV. Age and gender of each individual are displayed next to the corresponding sample.

**Data Analysis**

Simulation outcomes were assessed both morphometrically and visually to compared to the clinically reported outcomes. BV/TV and DA were computed for all samples to generate a reference database comprising 1598 simulation outcomes.

**Results**

Following the selection of corresponding algorithm settings for each scenario, the long-term effect of bone loss and pharmaceutical treatments have been simulated in 20 vertebral and 27 femoral human samples (Figures 3.1 and 3.2). In addition, a
database of resulting outcome was constructed for each anatomical side, comprising a total of 1598 samples (Figures 3.3 and 3.4).

In our simulations, we observed an average decrease of 37% in the vertebral, and 59% in the femoral BV/TV due to 30 years of osteoporosis-related bone loss. The first years of treatment, either BIS- or PTH-related, was simulated with identical settings due to the similarity of the values reported in literature, and resulted in average increases of 6% in the femora and 5.5% in the vertebra. The variance between preventive and late treatment scenarios was 2%.

Total increase of 5-9% and 10-12% in bone volume fraction was simulated in response to BIS treatment in femora and vertebrae, respectively, for the period of additional 10 years regardless of the start of treatment. In the case of PTH therapy, an average increase in BV/TV simulated for the femora for 11 years from the start of the treatment was 0-2%. For the vertebra, an increase of 8-11% was observed over the total treatment course of the same length. Figure 3.5 shows an example of changes in femoral BV/TV for a 63 year old female as a result of untreated bone loss and related preventive and curative treatments. Figure 3.6 depicts a similar example of changed in vertebral BV/TV for a different 63 year old individual.

Table 3.1 SIBA algorithm parameters used for the simulation of osteoporosis and associated treatments with BIS or PTH.

<table>
<thead>
<tr>
<th>Scenario</th>
<th>Sigma</th>
<th>Support</th>
<th>Threshold</th>
<th>Iteration Length</th>
<th>Literature source</th>
</tr>
</thead>
<tbody>
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<td></td>
<td>FEM</td>
<td>T12</td>
<td>FEM</td>
<td>T12</td>
<td></td>
</tr>
<tr>
<td>Osteopenia</td>
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<td>1.2</td>
<td>3</td>
<td>2</td>
<td>0.5</td>
</tr>
<tr>
<td>1st year of treatment</td>
<td>1.5</td>
<td>1.5</td>
<td>3</td>
<td>3</td>
<td>0.4</td>
</tr>
<tr>
<td>BIS</td>
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<td>1.5</td>
<td>2</td>
<td>3</td>
<td>0.4</td>
</tr>
<tr>
<td>PTH</td>
<td>0.7</td>
<td>0.7</td>
<td>2</td>
<td>2</td>
<td>0.49</td>
</tr>
</tbody>
</table>
Figure 3.3 Database of all in silico outcomes of the femoral simulations. The purpose of such a visualization is to facilitate sample referencing comparing BV/TV and DA measures.

Discussion

The goal of this study was to simulate microstructural changes in bone architecture due to osteoporosis and related pharmaceutical treatments in whole human bone samples. SIBA has been successfully employed to model long-term effects of both osteopenia and bone formation processes in 3D high-resolution images. The outcomes of the simulations performed in 20 vertebrae and 27 femora have both realistic trabecular architecture, and morphometric indices comparable to clinical findings.

For example, Müller [5] reported an average decrease of 38% in vertebral BV/TV following 30 years of menopause, a results that could be recreated exactly in the current implementation of the algorithm. Clinical reports of bone changes after the first year of treatment with either PTH [19, 21] or BIS [20], were in the range of 6% increase across all studies, and could also be accurately reflected in the simulations of femora and vertebrae alike.
Projected *in silico* result for the BIS treatment matched expected changes in clinics, as reported by Seeman and Delmas [20], despite the challenge of a single 10 year-long iteration required to match the physiological parameters. Simulated PTH treatment aligned well with the data reported for the 18 month long administration of the drug, in which average increases of 2.3% and 6.5% were reported for the femoral neck and lumbar spine measurements, respectively [21].

![T12: Populated Database](image)

*Figure 3.4 Database of all in silico outcomes of the T12 vertebral simulations. The referencing system allows sample tracing based on BV/TV and DA measurements.*

The large scale simulations have also resulted in the creation of a sample database, capable of tracing of the individual bone architectures based on the measurement of BV/TV. The purpose of this approach is to include a measure of trabecular DA into diagnosis and treatment of bone loss in clinics, a measure that would allow physicians to include structural arrangement in addition to bone density into the diagnostic process.
Figure 3.5 In silico modelling of microstructural changes in human femur due to osteoporosis and pharmaceutical treatments. Top left: Initial bone structure prior to treatment or bone loss (62 y.o.). Top right: Bone structure affected by 30 year of untreated osteoporosis (92 y.o.). Bottom left: Effect of the preventive treatment scenario (73 y.o.). Bottom right: Corresponding results for the late treatment scenario (82 y.o.). Lower section: Simulated changes in bone volume fraction (BV/TV) due to osteoporosis and pharmaceutical treatments in a 62 year old female.
Figure 3.6 In silico modelling of microstructural changes in human T12 vertebra due to osteoporosis and pharmaceutical treatments. Top left: Initial bone structure prior to treatment or bone loss (62 y.o.). Top right: Bone structure affected by 30 year of untreated osteoporosis (92 y.o.). Bottom left: Effect of the preventive treatment scenario (73 y.o.). Bottom right: Corresponding results for the late treatment scenario (82 y.o.). Lower section: Simulated changes in bone volume fraction (BV/TV) due to osteoporosis and pharmaceutical treatments in a 62 year old female.
Currently, this parameter is not measured in patients, despite its potential to draw more accurate concussions about the state of bone health and lead to better diagnostic and therapeutic outcomes. Providing a guiding database to the physicians would allow them to trace clinically measured parameters to corresponding DA measures.

In conclusion, in silico medicine is a powerful tool that allowed us to accurately predict changes in bone microstructure using a previously presented phenomenological model for bone remodeling. With this implementation both bone formation and bone resorption could be realistically simulated for age-related bone loss, as well as for preventive and curative treatment scenarios. A database linking parameter describing bone structure and density was created in an attempt of establish a connection to the clinical practice. In the future, this and similar in silico application could not only improve the accuracy of the diagnosis, but also contribute to patient-specific treatment planning.

References


Chapter 4
Large-scale simulations of bone remodeling in a mouse model: closed-loop, mechanical feedback algorithm validation

4.1 Correlative closed-loop model for bone remodeling simulation

The relationship between external load and architectural alignment within the trabecular bone is one of the earliest phenomena that was recognized in the field of bone biomechanics [1, 2]. Computational tools in the form of finite element (FE) analysis are capable of using realistic geometries and experimentally-derived tissue properties to calculate local effect of external mechanical environments. At the same time, despite extensive investigation into the endocrine and molecular interactions that govern bone remodeling, our understanding of this domain remains rather limited.

It is therefore, a logical step to consider local mechanical signals, as the major driving force behind bone modeling and remodeling in computational applications. Indeed, a number of successful algorithms capable of producing realistic outcomes of changes in bone have been based on these principles [3, 4]. An in-house developed framework was based on Mechanostat Theory [5] and integrated local mechanical feedback loop directly into the remodeling decision making process [6]. Micro-computed tomography (µCT) scans of live animals were used as input into the model. The single voxels of each segmented scan were then converted into 8 node brick elements for the FE calculation. Tissue properties derived from literature and realistic boundary conditions were then used to solve micro FE models and derive local strain energy density (SED) values. The signal was then incorporated into the so called “advection equation”, which also factored in the normal direction from the surface and the pre-set remodeling control parameters to produce final output of surface adaptation.
The framework was previously validated through correlative comparison of in silico outcomes against the results of the concurrent in vivo study using both quantitative and qualitative techniques. Mechanical loading and estrogen-depletion related bone loss studies were used in the model development. The authors reported maximum errors of 2.4% in bone volume fraction after four weeks of the simulation for the mechanical loading group, and 12.1% for the ovariectomy group. In the group that underwent mechanical loading, the most challenging index within the static morphometric evaluation was trabecular spacing. The maximum error between in vivo and in silico results for this parameter reached 8.4%. In the hormone depletion group, bone volume fraction resulted in the highest errors, while other static parameters were simulated with errors less than 4.6%.

The main challenge the model faced was capturing the dynamic morphometric indices, and correspondingly the local remodeling sites. Statistical evaluation showed no significant differences between in vivo and in silico values for bone formation and bone resorption rates, however, the large errors of over 50% in the mineral apposition and resorption rates proved to be significantly different between the simulation and the experiment. Therefore, the objective of the study presented in the following section of the chapter was to optimize parametric settings for the improved simulation outcomes for catabolic, anabolic, and anti-resorptive processes in bone, and thus to validate the model in its capabilities to reproduce those effects.

References:


4.2 Algorithm validation using mouse model for bone loss and associated treatments

Alina Levchuk\textsuperscript{a}, Alexander Zwahlen\textsuperscript{a}, Claudia Weigt\textsuperscript{a}, Floor M. Lambers\textsuperscript{a}, Sandro D. Badilatti\textsuperscript{a}, Friederike A. Schulte\textsuperscript{a}, Gisela Kuhn\textsuperscript{a}, Ralph Müller*\textsuperscript{a}

Affiliation: \textsuperscript{a}Institute for Biomechanics, ETH Zurich, Wolfgang-Pauli-Strasse 10, 8093 Zurich, Switzerland


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Abstract

Microstructural simulations of bone remodeling are particularly relevant in the clinical management of osteoporosis. Before a model can be applied in the clinics, a validation against controlled \textit{in vivo} data is crucial. In this study, we present a strain-adaptive feedback algorithm for the simulation of trabecular bone remodeling in response to loading and pharmaceutical treatment, and report on the results of the large-scale validation against \textit{in vivo} reference data.

The presented algorithm follows the mechanostat principle and incorporates mechanical feedback, based on the local strain-energy density. For the validation of the algorithm, simulations of bone remodeling and adaptation in 180 osteopenic mice were performed, and included a permutation of the conditions for early (20th week) and late (26th week) loading of 8N or 0N, and treatments with
bisphosphonates, or parathyroid hormone. Static and dynamic morphometry and local remodeling sites from in vivo and in silico studies were compared.

For each study, an individual set of model parameters was selected. Trabecular bone volume fraction was chosen as an indicator of the accuracy of the simulations. Overall errors for this parameter were 0.1-4.5%. Other morphometric indices were simulated with errors of less than 19%. Dynamic morphometry was more difficult to predict, which resulted in significant differences from the experimental data.

We validated a new algorithm for simulation of bone remodeling in trabecular bone. The results indicate that the simulations accurately reflect effects of treatment and loading seen in respective in vivo data, and thus, following adaptation to human data, could be transferred into clinical setting.

**Keywords**

Bone remodeling, simulation, in vivo validation, mechanical loading, pharmaceutical treatment

**Introduction**

Osteoporosis is a systemic disease, characterized by reduced bone quality and increased susceptibility to fracture [1]. The symptoms are common in menopausal women and elderly of both sexes [2-5]. At the present, osteoporosis is considered a pandemic in the aging world population [6, 7]. The biggest challenge in the clinical handling of the disease is not identification of those individuals that are suffering from decreased bone quality due to the homeostatic shifts, but detection of those that are prone to developing fractures [1]. Once the first osteoporosis-related fracture is sustained, an individual becomes more likely to suffer repeated incidences of broken bones [8, 9], thus leading to ever decreasing mobility and quality of life, as well as growing financial costs [7, 10, 11]. If identified prior to fracture, osteoporosis can be clinically managed with appropriate physical therapy, pharmacological treatments, or a combination of both [12-14]. In this context, microstructural simulation models of bone remodeling, capable of
reflecting changes in the tissue over time, can become a valuable auxiliary tool in clinical prediction and prevention of osteoporosis.

Before a model can be practically applied in the clinics, however, a thorough validation is of major importance [15]. An extensive validation routine overview has been presented by Huiskes [16]. Several types and levels of validation for bone adaptation models are proposed prior to clinical transfer. Briefly, numerical validation should establish that the algorithm is functional and does not produce obscure artifacts. Mechanistic validation attests that the model accurately reflects realistic mechanisms and processes. Finally, predictive validation verifies the results of the simulation against controlled reference data. In the first step, model performance according to bone-remodeling phenomena at large should be tested. The next step of predictive validity assessment juxtaposes simulated predictions with the experimental results for a population. Lastly, a specimen-specific quantitative validation has to be performed. This last step has always been a bottleneck for *in silico* models in the past, mainly due to the lack of appropriate *in vivo* data [15, 16].

One of the earlier *in silico* models for the prediction of trabecular bone-remodeling focused on voxel-based surface adaptation by means of combined Gaussian filtration and thresholding as an open-loop control model, and did not include mechanical feedback [17]. In another study, mechanical feedback was incorporated in the form of strain gradients, and the algorithm was applied on the data from an ongoing *in vivo* study, thus allowing for a subsequent indirect validation of the results [18]. A different model for examination of bone metabolic expression under load was presented by Ruimerman et al. [19]. The same group of researchers has also introduced an algorithm where formation was coupled with strain energy density (SED), while resorption was randomized according to a certain probability [20]. Finally, a more recent *in silico* model has been introduced by Schulte and colleagues [21]. This approach is based on the previously established open-loop control model, which did not include mechanical feedback between iterations of the simulation [22]. In the new algorithm, on the other hand, SED stimuli for local control of formation and resorption sites are
calculated for each iteration using the large-scale micro finite element (µFE) analysis. The inherent approach to strain adaptation allows the algorithm to run in a closed-loop feedback mode [21].

In this study, we extended Schulte in silico framework to simulate trabecular bone remodeling in response to loading and pharmaceutical treatment. Scans obtained directly from in vivo micro-computed tomography (µCT) measurements were used input for the model, thus allowing systematic time-lapsed comparison of the simulation results with the biological changes in experimental subjects. The study incorporated a total of 180 genetically inbred mice, subjected to controlled experimental conditions. This parallel setup of in vivo and in silico studies has allowed us to carry out an extensive validation of the algorithm, covering full range of pre-clinical validity testing. Here, we report on the results of this large-scale validation of the closed-loop strain adaptive algorithm against three-dimensional (3D) in vivo reference data of bone adaptation due to pharmaceutical treatments and mechanical loading in mouse vertebrae.

Methods

In vivo reference data

All animals used in the validation study were ovariectomized at the age of 15 weeks to create a state of estrogen depletion, and mimic the effect of osteoporosis (CTR, control group). All animal procedures were performed under isoflurane anaesthesia (2-2.5%, 0.4 L/min) delivered through a nose mask, and were approved by the local authorities (Kantonales Veterinäramt Zürich, Zurich, Switzerland). The studies were subdivided into two time-dependent groups in order to emulate the consequences of and treatment effects during initial rapid bone loss, as well as at the later stage of osteopenia [23, 24]. In the early treatment (ET) group treatment intervention started 5 weeks post-operatively or at the 20th week of life for the animals. In the late treatment (LT) study, a lag period of 11 weeks was allowed prior to the start of treatment in the 26-week-old mice [25]. The animals in the ET group were losing bone during the first half of study period, and consequently recovering some of the loss in the second half. In the LT
group, on the other hand, the bone condition could be described as osteopenic at the onset of the treatment, and there was not further bone loss observed during the entire study period. Therefore, the in vivo experiments have been designed in this manner to investigate the effects of preventive and curative treatments. The experimental study was based on an established mouse model for bone adaptation [26], in which sixth caudal vertebra (CV6) of the female C57BL/6 mice underwent mechanical loading (ML) through the metal pins inserted into CV5 and CV7. In the experiment, a sinusoidal force of 8N was applied at the frequency of 10Hz for 5 min/day, three times a week, for 4 weeks. Pharmacological treatments included parathyroid hormone (PTH; hPTH 1-34, Bachem AG, Switzerland), administered at a dose of 80 µg/kg for 4 weeks, or bisphosphonate (BIS; Zometa 4mg/5ml; Novartis Pharma Schweiz AG, Bern) administered once at the start of the treatment at a dose of 100 µg/kg.

A total of 12 studies investigating single and combined effects of loading and pharmacological treatments at different stages of bone loss have been used in the validation, where all experimental procedures have been carried out as part of a doctoral thesis [27]. Changes in bone microstructure were monitored bi-weekly, starting at the beginning of treatments with in vivo µCT (vivaCT 40, Scanco Medical, Brüttisellen, Switzerland) at an isotropic voxel resolution of 10.5 µm.

**Computational algorithm and μFE analysis**

A strain-adaptive feedback algorithm for the simulation of trabecular bone remodeling has recently been developed in house [21]. While the experimental data in this project would allow investigation of biological changes in both cortical and cancellous bone, the algorithm was developed exclusively for the simulations of changes in the trabecular bone tissue. The model itself is based on the principles of mechanical control, which has not been shown to have the same impact on the cortical bone as it does on the trabecular bone [28, 29]. In silico bone adaptation was derived from the mechanostat theory proposed by Frost [30] and is computed by moving bone surface through adding or removing bone volume depending on local mechanical stimuli. In short, as represented in Figure
1, a segmented image of an *in vivo* μCT scan is used as input. μFE analysis is then applied on the input image for the calculation of local mechanical signals represented by strain energy density (SED). μFE analysis was performed following a previously established protocol [26], where simplified disks were applied on each side of the full vertebra to ensure even force distribution. Material properties of the segmented input images were based on the assumption of tissue homogeneity. Young’s Modulus of 14.8 GPa and a Poisson ratio of 0.3 were assigned to all elements [26], and the model was solved on a super computing system (CSCS, Lugano, Switzerland) [31]. The amount of bone loss or gain is calculated with the advection equation based on the model input parameters (*Table 4.1*) and SED values. A total of four parameters that control the algorithm can be adjusted individually for each simulation. These include bone formation and resorption rate per SED value (τ), upper and lower SED thresholds for the formation and resorption (SED\text{upp} and SED\text{low}), and maximum remodeling velocity (u\text{max}). The output image is created by moving the bone surface in the direction normal to the surface. This image is then used as input for the subsequent iteration loop (*Figure 4.1*).

*Selection of parameters*

For all simulations bone formation and resorption rate was kept constant, while the remodeling saturation level and SED thresholds were optimized to produce the best match of bone volume fraction (BV/TV) with *in vivo* data across all measurement time point. Matches in all other morphometric and dynamic parameters were the outcome of this optimization, and reflect the accuracy of the algorithm.

An initial parameter set was carried over from the previous study performed on C57B/6 data [21]. Similarly to the reported method, mechanostat theory was assumed to act on the osteocytic network, where signals for the remodeling are released in response to change in mechanical environment, and has a certain radius of action and a specific time interval. In this study, the iteration length was set to two weeks, which corresponds to the measurement interval of the *in vivo*
study. Gaussian filtration, performed at each iteration prior to solving the
advection equation was set to reflect the maximum osteocyte signal radius of 52
µm [32].

Due to lack of reference data, formation and resorption rate per SED value (τ)
was kept constant at 1 (mm/wk)/(J/mm$^3$) for all simulations. The strategy for
finding a matching set of parameters included adapting the maximum remodeling
velocity ($u_{\text{max}}$) first, while keeping SED thresholds constant. Once the best BV/TV
match with this set of parameters was found, SED threshold settings were adapted
one by one to refine the simulation outcome. An individual set of parameters was
selected for each treatment scenario; and subsequently applied for all subjects
within the group.

Comparison of static and dynamic morphometry

Static and dynamic morphometric parameters were computed for the trabecular
compartment of in vivo and in silico data sets, where trabecular masks were
created using distance transformation map from the cortex, based on the baseline
scans and according to the established protocol [33]. Static parameters such as
BV/TV, specific bone surface (BS/BV), trabecular thickness (Tb.Th), trabecular
number (Tb.N) and trabecular separation (Tb.Sp) were assessed at each
measurement point according to the guideline paper on the assessment of bone
microstructure in rodents using micro-computed tomography [34]. Dynamic bone
morphometry was calculated by overlaying the first and the last measurements,
identifying areas that were formed and removed in the course of the experiment.
3D bone formation rate (BFR), mineral apposition rate (MAR), mineralizing
surface (MS), as well as bone resorption rate (BRR), mineral resorption rate
(MRR) and eroded surface (ES) were computed for each treatment group.
Algorithms and parameters needed for the calculation of the dynamic indices have
been set according to Schulte and colleagues [35]. The thresholding value (22% of
maximum grayscale value) for the segmentation of formed and resorbed bone has
been chosen to yield accurate results as compared to an earlier study using
standard histomorphometry [36], where also precision and sensitivity of this method were determined to be very high.

Table 4.1. Model parameters: Selected parametric settings for early (5 weeks after ovariectomy) and late (11 weeks after ovariectomy) treatments, where formation and resorption rate per SED (τ) was kept constant at 1, and the remaining three parameters were optimized to produce the best match of BV/TV between in vivo and in silico groups. CTR stands for control group; ML, for mechanical loading; BIS, for bisphosphonate treatment; and PTH, for parathyroid hormone treatment.

<table>
<thead>
<tr>
<th>Study/Parameter</th>
<th>SED_{low}</th>
<th>SED_{upp}</th>
<th>u_{\max}</th>
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<td></td>
<td>Formation threshold [10^{-3} \cdot J/mm^3]</td>
<td>Resorption threshold [10^{-3} \cdot J/mm^3]</td>
<td>Saturation level [10^{-3} \cdot mm/week]</td>
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<td>7.5</td>
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</tr>
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<td>8</td>
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</table>
Figure 4.1. Top: Schematic workflow of the in silico algorithm for bone remodeling (adapted from [21]). The binary input image is used to calculate SED signal using μFE analysis. The advection equation solved with input parameters is then used to determine surface growth velocity and direction. An output image derived from the process is then used as input for the second iteration. Bottom: The Mechanostat plot showing model input parameters. The plot is used to determine remodeling events based on the calculated SED values. In the current implementation the value for the formation and resorption rate per SED (τ) was kept constant at 1.

For the statistical analysis, errors of means per group were calculated for all static parameters. Simulated and experimental static morphometry was compared using ANOVA adjusted for multiple comparisons, while dynamic morphometry was evaluated with paired Student’s t-test. Normal distribution of residuals was verified using Q-Q plots and histograms, while Levene’s test was applied to test for the quality of variances. In case of dynamic morphometric evaluation, where homoscedacity could not be confirmed Welch’s modification of Student’s t-test was used. p-Values less than 0.05 were considered significant.
Finally, visual investigation of the 3D surfaces of bone formation and resorption allowed direct qualitative comparison of the local remodeling sites between in vivo and in silico samples.

Results

Model parameters

All values assigned to the model can be found in Table 4.1. Validation of the in silico algorithm for bone adaptation was performed against experimentally collected data. SED thresholds for bone formation and resorption (SED_{upp} and SED_{low}), as well as remodeling saturation setting (u_{max}), were lower or equal to for the ET, compared to the LT group. ML studies in the ET group required higher “remodeling saturation level” than the control and treatment only cases. In the CTR and PTH studies of the ET group bone formation and resorption thresholds, SED_{low} and SED_{upp}, respectively, resulted in the same value following the optimization, thus eliminating the so called “lazy zone” [37].

Static morphometric evaluation

The simulations were optimized for the best match of the trabecular BV/TV, while other parameters were used to assess the accuracy of the algorithm (Table 4.2). In the ET group BV/TV errors ranged from 0.2-4.5% for all time points. The simulations of ML, BIS and BIS+ML resulted in the lowest errors in bone volume fraction, with error values under 1% for both measurement points. Meanwhile, CTR and PTH treated groups resulted in the highest BV/TV errors. Nevertheless, in silico BV/TV was not significantly different from the in vivo results for the ET studies, except for the first comparison point in the CTR group. In the LT group the errors in trabecular BV/TV ranged from 0.1-1.9%, and were not significant. CTR, PTH, BIS, and BIS+ML simulations resulted in less than 1% errors, while the highest error of 1.9% corresponded to the PTH+ML study. With respect to other static morphometric parameters, Tb.N, Tb.Sp and BS/BV produced errors in the range of 0-10% in all investigated scenarios, while accurate match in Tb.Th proved more difficult to achieve with the current parametric settings. The errors
for Tb.Th reached 18.1% in the LT, and 14.3% in the ET groups. These results in BS/BV and Tb.Th were also largely statistically significant (p<0.05). The full overview of the errors in the static morphometry between in silico and in vivo samples is provided in Table 4.2.

Results for in silico simulations have been organized to match physiome maps constructed for the static morphometric parameters of the experimental data [25]. This type of representation allows direct comparison of matches in the parameter of interest, and displays how the trends change over time (Figure 4.2). For the simplification of analysis all values have been normalized by the initial measurement. Since the first measurement has been used as input for the algorithm, the nominal value is 100% for all groups, with the consecutive values reflecting changes measured at the following time points. Errors or means greater than 1% have also been indicated where applicable, while errors of lesser values are not shown.

**Figure 4.2.** Normalized trabecular bone volume fraction (BV/TV) plots comparing experimental and simulated results. Listed numbers correspond to the average errors for the group of corresponding color code. Errors under 1% are not shown.
**Dynamic morphometric evaluation**

With respect to the dynamic morphometry, we were able to show that the algorithm is capable of reproducing experimental formation and resorption for both low and high levels of remodeling (*Figure 4.3*). The accuracy of the matches in this domain has been compromised to assure better fit in the static morphometry, as well as in the local remodeling sites. On average, dynamic parameters have been underestimated in the simulations to prevent excessive deposition and resorption on the trabecular surface, and thus preserve realistic structures. BFR and MS in the *in silico* samples resulted in the lowest average errors of 17% and 3%, respectively. MS produced the lowest average error of 3%, and was closest to the *in vivo* results in the studies involving anabolic treatments and mechanical loading. BFR was simulated with the least number of significant errors from the experimental results. On the other hand, BRR, MRR, and ES produced less accurate matches, where underestimations range was 11-100%. Dynamic morphometry was most accurately simulated for the LT ML study, for which BFR, BRR, and MS were not significantly different from the *in vivo* measurements, and errors across all parameters were 3-34%. On average, groups undergoing ML showed lower errors in all dynamic parameters in both ET and LT groups. All results for dynamic morphometry can be found in *Table 4.3*.

**Visual examination**

Visual analysis of the local remodeling sites was in agreement with morphometric results, and confirmed that *in silico* results often showed excellent matches for the areas of formation when compared to the *in vivo* samples, while resorbed areas seemed to be underestimated and less on target (*Figure 4.4*). In addition, bone remodeling matches were hindered by the removal of whole trabeculae, which appeared to be attached only on one side in the *in silico* cases, thus preventing load transmission in the FE calculation. In the experimental case, on the other hand, initiation of the growth of the previously undetected and visually disconnected trabecular structures was frequently observed from measurement to measurement.
Table 4.2. Static morphometric parameters: Absolute error of means [%] between simulated and experimental bone structural parameters for early (5 weeks after ovariectomy) and late (11 weeks after ovariectomy) treatments. The simulation errors at weeks 20 and 26, respectively, were 0, since the experimental measurement was used directly as input for the model. Simulations were optimized to produce the best match of the BV/TV between in vivo and in silico groups. *p<0.05, **p<0.01 from repeated measures ANOVA are considered significant.

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**Discussion**

The aim of this work was the validation of a novel strain-adaptive *in silico* algorithm for the simulation of trabecular bone adaptation. The unique comprehensive validation was achieved by means of direct comparison of the simulation results with the mouse *in vivo* µCT data from the osteopenia-inducing ovariectomy experiment, as well as a combination of ML, PTH and BIS therapies. Unlike many of the previously introduced algorithms that underwent population-based validation [16-18, 20], this study provides more advanced group-based validation, which included static and dynamic morphometric assessments, as well as comparison of the resultant bone architecture.

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In the current validation, *in silico* algorithm was able to reproduce changes in BV/TV with a maximum error of 4.5% for all investigated scenarios. This result is an improvement of the outcome of 12.1% reported when the algorithm was first introduced [21], and could be achieved with careful parametric selection. The agreement of other morphometric parameters was also better, with the remaining discrepancy in Tb.Th matches. The juxtaposition of *in vivo* and *in silico* results in a physiom map (Figure 4.2) provides an intuitive way to compare the success of algorithm performance for each of the investigated scenarios. For example, it becomes evident that simulations resulted in the most precise matches for the studies with the linear change in BV/TV, as most cases in the LT group are. On the other hand, if the trend changed along the course of the experiment, as in the CTR and PTH scenarios of the ET group, *in silico* results did not match both time points perfectly, but followed the average of two measurements. The amounts of *in vivo* bone formation and resorption were reproduced well in the simulations; however more accurate matches were compromised in favor of better static morphometry and local remodeling site agreements. In particular, to achieve the same formation rates as observed *in vivo* with the formation of new structures, resorption was often underestimated in the simulation. With the focus on optimization of overall bone volume fraction, this resulted in successful prediction of trabecular spacing and number, along with bone formation rates, and less accurate bone surface, trabecular thickness, and resorption rate estimations.

Comparison with literature is difficult, as this is the first *in silico* algorithm which has undergone a comprehensive validation against corresponding time-lapsed *in vivo* data. Other promising models for bone adaptation have been published [18, 20, 22, 38]; however, the presented approach reflects not only the effects of bone-loss, but also a variety of corresponding treatments based on different mechanisms of action. These features make the algorithm suitable for clinically relevant applications, such as prediction of bone changes in real patients, including the effects of pharmaceutical treatments or physical therapy, as well as assistance in identification of the individuals prone to fracture.
One of the limitations to the proposed approach with respect to the method is the assumption of the tissue homogeneity. In order to address this issue, more experimental data would be needed to evaluate mechanical properties of newly formed bone, as well as a way to incorporate this information in the FE modeling, something that to our knowledge is currently not available.

Another major limitation of the *in silico* algorithm is its inability to simulate complex biological events, such as formation of new trabecular structures, a phenomenon frequently induced by anabolic treatments *in vivo*. This, in turn, results in less accurate distribution of the remodeling sites, particularly for the cases of high bone formation, such as PTH and ML studies, where BV/TV of the missing trabecular structure has to be compensated for elsewhere on the bone surface. The difficulty of targeting formation and resorption rates is also exacerbated by the current simplistic coupling of the controlling parameters for formation and resorption rates ($\tau$), as well as for the remodeling saturation ($u_{\text{max}}$). Separate definition of those settings for formation and resorption would allow for better optimization of bone adaptation processes, at the same time preserving the mechanical-feedback principle of the model. While current selection of parameters followed the observations of the *in vivo* studies [25, 39], more realistic, decoupled setup of the control parameters could also make direct linking of the setting to the *in vivo* observed phenomena possible.

In conclusion, we presented a comprehensive *in vivo* validation of the predictive strain-adaptive computational model with the experimental animal data. The validation has been carried out for a hormone-related catabolic disease, as well as anabolic and anti-resorptive treatments. The simulation errors for the static and dynamic morphometric parameters have been determined, along with visual local remodeling site comparisons. With the current validation, and the on-going transfer of the control settings from the animal to the human scale, the model is on the way to clinical implementation. Therefore, the presented *in silico* algorithm could contribute to more accurate identification of fracture risk, as well as aid in long-term patient-specific treatment planning.
Figure 4.3. Visual comparison of spatial patterns of bone formation and resorption within the trabecular network (cortical bone not shown) after four weeks between simulation and experiment for the group with the lowest (CTR) and highest (PTH+ML) remodeling rates. (Top row) spatial patterns and changes in the trabecular bone volume for experimental (solid line), and simulated (dashed line) CTR sample. (Bottom row) spatial patterns and changes in the trabecular bone volume for experimental (solid line), and simulated (dashed line) PTH+ML sample.
Figure 4.4. Visual comparison of bone formation and resorption patterns within the trabecular network (cortical bone not shown) between in vivo (last column) and in silico (first and second columns) results in the LT study. The accumulation and adaptation of the remodeling sites can be traced from the first two weeks (first column) to the full four weeks (second column) of the simulation. Rows correspond to the denoted control and treatment scenarios.
Table 4.3. Dynamic morphometric parameters: Mean of the three-dimensional dynamic bone morphometry in the experiment compared to the simulation for early (5 weeks after ovariectomy) and late (11 weeks after ovariectomy) treatments. *p<0.05, **p<0.01 from paired Student’s t-test or Welch’s t-test are considered significant.

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Supplementary Material:

Table S4.1. Static morphometric parameters: Absolute error of means [%] between simulated and experimental bone structural parameters for early (5 weeks after ovariectomy) and late (11 weeks after ovariectomy) treatments. Simulations were optimized to produce the best match of the BV/TV between in vivo and in silico groups. The simulation errors at weeks 20 and 26, respectively, were 0, since the experimental measurement was used directly as input for the model. *p<0.05, **p<0.01 from repeated measures ANOVA are considered significant.

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Chapter 5
Predictive simulations of bone remodeling in a mouse model: closed-loop, mechanical feedback model update and validation

Alina Levchuk, Remo Sommer, Claudia Weigt, Floor M. Lambers, Gisela Kuhn, Ralph Müller

Institute for Biomechanics, ETH Zurich, Vladimir-Prelog-Weg 3, 8093 Zurich, Switzerland

Prepared for publication as: Hypothesis-based investigation of load-driven remodeling in trabecular bone

Reprinted with approval from all co-authors.

Abstract

Bone adaptation to external loads is often explained by the widely accepted Mechanostat Theory, which allowed simplified translation of biological process into mathematical and computational terms. While in silico simulations of bone remodeling can provide a reliable method for modeling of in vivo events, and thus contribute to alleviating experimental and clinical challenges, clear understanding of the underlying remodeling rules must be established and validated first. A previously developed algorithm using a mouse model for bone remodeling demonstrated consistent agreement between in vivo and in silico results. Nevertheless, biological relevance of the parametric settings, and verification of the predictive power of the model was missing. In this study we relied on the hypothesis-driven approach to guide the development of bone remodeling rules using serial in vivo mouse study. First, we hypothesized that bone formation and resorption require separate control parameters in the algorithm to reflect the distinct in vivo pathways. The second hypothesis was that linking of the control parameters to the experimental data could further improve simulation outcomes. Third, we hypothesized that accuracy and biological relevance of the model can
be enhanced through replacing linear relationships with the non-linear functions. Consequently, logarithmic remodeling rules were derived and implemented for controlling bone remodeling in the simulations. The results confirmed the hypothesis, when validated against corresponding *in vivo* data. Finally, to assess predictive power of the algorithm, a long-term study with an independent experimental dataset was carried out. The control parameters were calculated both individually and on the group level. While the outcomes of the individual predictions require further improvement, group-wise logarithmic remodeling rules, as well as biologically derived parameters present a necessary update to the existing *in silico* approaches, and help establishing firmer basis for the *in silico* technology transfer into clinics.

**Introduction**

Trabecular bone, synonymous with cancellous bone, is characterized as an ultimate load-bearing design, where weight is minimized through structural porosity, while stiffness and strength are maintained through the architectural arrangement and material properties of the tissue [1, 2]. It has been long hypothesized that this structural form is following the function of the organ, namely to support the body in the principal loading directions, while also maintaining the minimal acceptable weight. Extensive attempts both *in vitro* and *in situ* [9-11] have been made to characterize the mechanisms of local mechanosensation and mechanotransduction in bone, both of which are hypothesized to be a precursor of structural adaptation [3]. On the other hand, tissue level load-adaptive properties of bone have long been accepted, initially derived from the simple anatomical observations [4, 5], and later extended through further clinical and mechanical analysis [6-8].

Currently favored explanation of bone adaptation rests with the Mechanostat Theory, proposed in 1987 [9], and last updated in 2003 [10], according to which changes in bone mass follow mechanical stimuli on the tissue. In other words, there exist theoretical mechanical thresholds for bone resorption and formation,
separated by the so called “lazy zone”, a loading range at which bone remains quiescent and no net change of mass is incurred [11].

The Mechanostat Theory, although not without its sceptics [12, 13], has been implemented extensively in the computational models of bone adaptation [14, 15]. The value of experiment-based in silico models is often attributed to their ability to aid our understanding of biological phenomena and explaining complex ambiguous interactions of different variables involved in the bone remodeling process [16, 17]. The true potential of the computational approach, however, lies in its capacity to predict biological events, thus reducing the need for extensive experimental efforts, and allowing for control of the precise environmental factors. Adequately implemented and validated computational frameworks could facilitate better understanding of the governing mechanisms of bone adaptation, and if transferred into clinical setting, enable patient-specific predictions of disease and/or treatment outcomes. Yet, deeper comprehension of bone remodeling rules needs to be established before such level of reliability can be attained.

The first in silico model for bone adaptation incorporating mechanical feedback in a closed-loop feedback scenario was based on a symmetric implementation of the Mechanostat Theory, with respect to modeling of the formation and resorption phenomena [18]. The major drawback the authors cited was lack of biological relevance, as the corresponding experimental studies for derivation of the model parameters were unavailable at that time. In another model, mechanical signal threshold was implemented for the formation processes only, while resorption was modeled as a spatially random stochastic event [19]. While the implementation proved to be successful in replicating in vivo-like load-induced adaptation, remodeled surfaces of the trabecular crossings were rougher and sharper than realistically possible, which made comparison of the results to the in vivo measurements difficult. In the most recent model for bone adaptation, formation and resorption events have been represented by the identical linear functions with strain energy density (SED) chosen as the driving mechanical signal [14]. The model was built in a closed-loop mechanical feedback scenario,
developed on the three dimensional (3D) whole mouse vertebrae. While fully validated against the concurrent in vivo study [20], the model was shown to have limited capabilities in targeting the resorption side of remodeling events, leading to the conclusion that symmetric implementation of the remodeling rules might not be the best strategy. Additionally, similarly to the previously mentioned model by Adachi et al. [18], control settings in this implementation were subject to literature derivation and iterative fitting, as opposed to direct linking to the experimental data.

With this in mind, a series of hypotheses based on the experimental observation were used to drive this study. The project as a whole relied on the serial in vivo micro-computed tomography (µCT) scans, collected as part of the controlled study of trabecular bone adaptation in the caudal vertebra of inbred mice. Experimental groups allowed investigation of the healthy bone remodeling (SHM 0N), estrogen-depletion related bone loss (OVX 0N), and mechanically induced bone formation state (SHM 8N). Scans from the short-term (4 weeks) and long-term (8 weeks) studies were used for model training, and predictive validation, respectively (Figure 5.1). Simulations were carried out according to the previously published protocol [20]. Parametric settings of the computational model were decoupled to verify the first hypothesis, questioning the effects of using separate control parameters for formation and resorption processes (Figures 5.2A-B). Direct and implicit links between in vivo measurements and the control parameters were then established to address the second hypothesis of the study (Figure 5.2C). Third hypothesis stated that linear relationships, used to represent Mechanostat Theory in terms of rate of bone remodeling and measured mechanical signal, could be replaced with continuous mathematical functions without compromising the quality of the simulations. To test this hypothesis analytical and iterative investigation was used to formulate logarithmic functional relationships of bone remodeling parameters for each of the experimental groups (Figure 5.2D). The main motivation for this undertaking was on one hand, simplification of the control parameters, and on the other hand, an attempt to make the model more biologically relevant. With the fourth hypothesis the goal
was to investigate, whether prediction of individual control settings could be possible. Using statistical techniques of logistical regression, classification, and linear modeling with predictor variable interactions the hypothesis could be confirmed, and prediction of the individual remodeling parameters for each animal was achieved. Finally, group and individual predictive simulations were carried out and compared to the long-term in vivo data to validate the prognostic power of the algorithm, as well as to test the developed methods for parameter selection.

Results

Expanded linear model with in vivo derived control parameters (Hypotheses 1 and 2)

Once control parameters for formation and resorption processes of the in silico algorithm had been decoupled, model parameters were linked to or derived from experimental data, leading to the formulation of the new remodeling rules. (Table 5.1).

In the SHM 0N group, both SED thresholds were determined to be at the same point of 0.0024 MPa (Figure 5.3, Table 5.1). This outcome, derived from the probability plots, and verified based on the simulation outcomes, leads to the conclusion, that “lazy zone” was not necessary for simulations of bone remodeling for the control group with the current implementation. Bone formation and resorption rates were iteratively derived through incorporation of other parameters, and resulted in values of 1.2 (mm/week)/MPa and 5.0 (mm/week)/MPa, respectively. The maximal formation and maximal resorption velocities were extracted directly from the in vivo data at 0.0138 mm/week and 0.0167 mm/week, respectively. No significant differences between simulated and experimental data were observed. Errors of means for between in silico and in vivo results remained at 5% or lower across all static morphometric indexes (Figure 5.4, Table 5.2).
Table 5.1. In vivo derived parameters for the expanded linear model

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Unit</th>
<th>Symbol</th>
<th>Settings</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>SHM 0N</td>
</tr>
<tr>
<td>Resorption threshold</td>
<td>MPa</td>
<td>$SED_{res}$</td>
<td>0.0024</td>
</tr>
<tr>
<td>Formation threshold</td>
<td>MPa</td>
<td>$SED_{for}$</td>
<td>0.0024</td>
</tr>
<tr>
<td>Resorption rate</td>
<td>(mm/week)/M Pa</td>
<td>$\tau_{res}$</td>
<td>5.0</td>
</tr>
<tr>
<td>Formation rate</td>
<td>(mm/week)/M Pa</td>
<td>$\tau_{for}$</td>
<td>1.2</td>
</tr>
<tr>
<td>Resorption saturation</td>
<td>mm/week</td>
<td>$u_{res}$</td>
<td>0.0167</td>
</tr>
<tr>
<td>Formation saturation</td>
<td>mm/week</td>
<td>$u_{for}$</td>
<td>0.0138</td>
</tr>
</tbody>
</table>
Figure 5.1. Experimental design and algorithm development. (Top panel) The original 4 parameter algorithm for the simulation of bone remodeling was expanded through decoupling of the formation and resorption controls. Training of the algorithm using a short-term in vivo study commenced with the finite element analysis (FEA) of the input scan, followed by the simulation of bone remodeling using experimentally derived settings. This approach lead to the adjustment of the symmetric remodeling rules and development of the statistical model for the prediction of the algorithm settings. (Bottom panel) The expanded 6 parameter linear algorithm was matched with the representative logarithmic functions. The validity of the model developments as well as capability of individualized predictions were then verified using a long-term experimental study.
Figure 5.2. Progressive development of remodeling rules for the simulations in trabecular bone (A-D).

(A) A model with the coupled symmetric formation and resorption controls, 4 parameter linear implementation. (B) Decoupled formation and resorption controls, as seen in the 6 parameter linear model (C) 6 parameter linear model, shaped through the in vivo derivation of the parameters. (D) Logarithmic representation of remodeling rules and the equation describing the relationship between the mechanical signal (SED) and bone remodeling rates, where \( \alpha \) and \( \beta \) are constants.

\[ \text{SED} = \alpha \cdot \ln(\text{SED}) + n \]

\( \gamma \): remodeling rate, \( \text{SED} \): mechanical load threshold, \( u \): remodeling saturation, for: formation, res: resorption.
Bone resorption rate (BRR) was overestimated by over a factor of two, which resulted in the only dynamic morphometric parameter that differed significantly (p<0.05) between simulation and experiment when compared using Student’s t-test with the Bonferroni correction for multiple comparisons. The rest of the dynamic morphometric parameters were simulated with errors of less than 25% that were also not statistically significant compared to the in vivo measurements (Figure 5.4, Table 5.3).

In the OVX 0N case, SED thresholds for both formation and resorption were at 0.0041 MPa (Figure 5.3, Table 5.1). Similarly to the SHM 0N group, “lazy zone” was not critical for the simulations of bone remodeling for this group. Maximum formation velocity, extracted directly from the in vivo data was 0.0105 mm/week and the corresponding value on the resorption side was 0.0158 mm/week. The values for bone formation and resorption rates were determined to be 0.6 (mm/week)/MPa and 3.8 (mm/week)/MPa, respectively. Evaluation of the static morphology, resulted in the errors of means from the in vivo data ranging from 0.8% for trabecular thickness (Tb.Th) and specific bone surface (BS/BV) to 2.0% for bone volume density (BV/TV) (Figure 5.4, Table 5.2). None of the reported static morphometric indices differed significantly from experimental data. On the side of the dynamic morphometry, none of the parameters showed significant differences between simulation and experimental data either. Most errors remained under 5% with only bone formation rate (BFR) and BRR at 24.8% and 56.6%, respectively (Figure 5.4, Table 5.3).

Remodeling thresholds for SHM 8N group were 0.0095 MPa and 0.0110 MPa for resorption and formation, respectively (Figure 5.3, Table 5.1). Bone formation rate was determined to be 0.3 (mm/week)/MPa, while bone resorption rate for this group was 0.9 (mm/week)/MPa. Maximum formation and resorption velocities, extracted from the in vivo data, were both 0.0128 mm/week. Maximum errors of means for the static morphometric parameters in this group were under 4.5%, with no significant differences from the experimental data (Figure 5.4, Table 5.2). BRR was the only index in the dynamic morphometry to differ significantly between simulation and in vivo study (p<0.05) (Figure 5.4, Table 5.3).
Figures 5.3. Linear and logarithmic functions selected as remodeling rules for three investigated experimental scenarios. Each graph juxtaposes linear relationships based on the in vivo derived setting and a corresponding logarithmic function. Of note are the intersection points on the SED curve, which denote the absence of the remodeling “lazy zone”, as well as the areas under the curves, which correspond to the total amount of formation or resorption at any given value of the SED.
Figure 5.4. Static and dynamic morphometric parameters comparing linear and logarithmic models to the in vivo measurements. Top row summarizes errors of means from in vivo for linear and logarithmic remodeling model implementation in the static morphometric parameters. Lower row shows the results of the dynamic morphometric evaluation. The figure compares linear and logarithmic implementations of the computational algorithm with the experimental measurements. Significance was assessed using ANOVA with post hoc Bonferroni correction for the static morphometry, and Student’s t-test using Bonferroni correction for multiple comparisons for the dynamic parameters.
Table 5.2. Static morphometry for the training set: linear and logarithmic models

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>SHM 0N</td>
<td>Experimental</td>
<td>15.1 ± 2.4</td>
<td>30.1 ± 2.4</td>
<td>84.0 ± 6.0</td>
<td>2.18 ± 0.18</td>
</tr>
<tr>
<td></td>
<td>Linear</td>
<td>14.6 ± 1.1</td>
<td>29.5 ± 1.3</td>
<td>88.2 ± 4.1</td>
<td>2.09 ± 0.19</td>
</tr>
<tr>
<td></td>
<td>Logarithmic</td>
<td>15.0 ± 1.5</td>
<td>28.7 ± 1.1</td>
<td>87.1 ± 3.3</td>
<td>2.09 ± 0.19</td>
</tr>
<tr>
<td>OVX 0N</td>
<td>Experimental</td>
<td>8.5 ± 1.1</td>
<td>40.2 ± 5.4</td>
<td>72.9 ± 8.6</td>
<td>1.87 ± 0.25</td>
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<tr>
<td></td>
<td>Linear</td>
<td>8.7 ± 0.8</td>
<td>38.8 ± 3.6</td>
<td>73.5 ± 6.0</td>
<td>1.85 ± 0.24</td>
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<tr>
<td></td>
<td>Logarithmic</td>
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<td>40.3 ± 3.7</td>
<td>69.6 ± 5.5</td>
<td>1.86 ± 0.23</td>
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<tr>
<td>SHM 8N</td>
<td>Experimental</td>
<td>15.6 ± 2.3</td>
<td>31.4 ± 3.4</td>
<td>80.9 ± 7.9</td>
<td>2.30 ± 0.19</td>
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<tr>
<td></td>
<td>Linear</td>
<td>15.4 ± 1.0</td>
<td>30.5 ± 1.1</td>
<td>84.4 ± 2.2</td>
<td>2.28 ± 0.17</td>
</tr>
<tr>
<td></td>
<td>Logarithmic</td>
<td>15.8 ± 1.2</td>
<td>29.7 ± 1.5</td>
<td>84.7 ± 3.3</td>
<td>2.26 ± 0.15</td>
</tr>
</tbody>
</table>

No significant differences from ANOVA corrected for multiple comparison at p<0.05
Logarithmic model: algorithm training (Hypothesis 3)

In the SHM 0N group SED axis crossing point with the selected logarithmic relationship was at 0.0021 MPa, compared to 0.0024 MPa derived from the in vivo experiment (Figure 5.3, Table 5.4). Maximum formation and resorption rates calculated form the experimental data were preserved. The biggest improvement with this implementation was in the dynamic morphometry, where all errors were reduced below 22%, with MAR and MRR remaining significantly different from the in vivo measurement (Figure 5.4, Table 5.3). Notably, the biggest reduction of errors was observed for the BFR and BRR. This improvement could be at least in part attributed to the shape of the log curve, which in combination with the Gaussian distribution of the SED values, comprises a more physiological response to loading. A corresponding improvement was also observed in the correlation of the static morphometric indexes, where maximum errors were reduced to less than 4.2% (Figure 5.4, Table 5.2).

The intercept of the remodeling curve for OVX 0N group was 0.0051 MPa (Figure 5.3, Table 5.4). While the maximum resorption rate was preserved for this group too, the maximum in vivo calculated formation rate was exceeded for the higher values of the SED, which was relevant for less than 1% of all SED values, and had no adverse effects on the simulation outcomes. The errors for all indexes within the dynamic morphometry were reduced to less than 24% (Figure 5.4, Table 5.3), with only MAR and MRR remaining significantly different from the experiment (p<0.05). Static morphometric evaluation showed that all parameters were simulated with error of less than 5%, which despite the slight increase in values from the linear model resulted in no significant differences from the experimental measurement (Figure 5.4, Table 5.2).

SHM 8N was the only group where a “lazy zone” was present in the linear model. In selecting an appropriate logarithmic function for this scenario, the best results were achieved if the SED intercept was set at 0.0103, which fell between the resorption and formation thresholds of the linear model. The limits of the resorption and formation saturations were preserved for the selected logarithmic
remodeling rule (Figure 5.3, Table 5.4). The outcomes of the dynamic morphometric analysis showed an improvement for BFR and BRR, where the error fell under 40%, and that of the BRR was reduced almost three-fold. Errors for other indexes showed no consistent trends; however the overall errors remained under 21% with only MAR significantly different from the experiment (p<0.05) (Figure 5.4, Table 5.3). Static morphometric indexes showed a general slight increase in errors, however still remained under 5% and showed no significant differences from the experimental measurements (Figure 5.4, Table 5.2).

Finally, visual assessment of the local remodeling sites also confirmed an improvement in the simulation quality, when comparing logarithmic to linear remodeling rules (Figures 5.5-5.7). However, because differences were mostly on a single voxel level, the formation and resorption patterns remained fairly uniform between the two implementations.

**Predictive simulation with logarithmic remodeling rules (Hypotheses 4 and 5)**

The following section discusses results of the long-term predictive simulations with individual and group settings using logarithmic remodeling rules.

In the SHM 0N group individually predicted intercept parameters ranged from 0.0191-0.0232, while the group parameter selected earlier using the training data set was 0.0217. The success of the individual outcomes was difficult to judge as there was no consistent trend for the morphometric indexes. For example, in the case of static morphometry, error from the experimental data in BV/TV evaluated with the group setting was 1.02%, compared to 2.71% calculated for the simulations with individual settings (Table 5.5). On the other hand, in the most improved sample the error in the final BV/TV measurement decreased from 11.38% to 3.44%. The outcomes were inconsistent for other indexes too, although on the group level predictions of BS/BV and Tb.Th improved, while Tb.N slightly increased, whereby no significant differences from the in vivo measurements were detected even after 8 weeks of the simulation for either group or individual outcomes. Similar results have also been found in the dynamic morphometric
analysis (Table 5.6). Errors in BFR, MAR, MRR, and MS were reduced with the individual predictions, while the remaining two indexes were predicted less accurately. In both group and individual approaches BRR remained significantly different from the in vivo outcome after 8 week predictive study (p<0.01). Overall structural changes in trabecular architecture were captured in both individual and group simulations with very minor differences between the two methods. Results were consistently successful across all time-lapsed measurements, as well as in the cumulative outcomes of the remodeling predictions both in the qualitative and quantitative domains (Figures 5.8 and 5.11).

Individual β parameters in the OVX 0N group ranged from 0.0223-0.0264, where previously selected group parameter was 0.0243. Errors in static morphometry were reduced across all evaluated indexes, and absolute mean errors from the in vivo measurements were less than 2% with the individual prediction approach (Table 5.5). Nevertheless, all but BV/TV remained significantly different from the experimental data. On the dynamic side of the evaluation, calculated errors using animal-specific approach were in line with the group-wise prediction outcomes, with slight improvements in the accuracy of MS and ES (Table 5.6). Visualization of the dynamic changes in bone architecture and total formation and resorption processes for the OVX 0N group showed that while total bone turnover was captured, changes in the trabecular structures were difficult to match due to resolution and boundary condition restrictions, which is a common drawback of the computational approach [14, 20] (Figure 5.9).

Group setting for SED intercept of the SHM 8N group was 0.0160, while animal-specific settings ranged from 0.0159-0.0175. No trends were observed in this group for changes in the prediction accuracy either in static or dynamic morphometric evaluation comparing individual and group predictions (Tables 5.5, 5.6). Errors in the static morphometric indices remained under 8% and were not significantly different from the experimental outcomes. In the dynamic assessment, final errors after 8 weeks of predictive simulations were under 35%, and only MAR and MRR significantly differed from the in vivo study.
Table 5.3. Dynamic morphometry for the training set: linear and logarithmic models

<table>
<thead>
<tr>
<th>Study</th>
<th>Remodeling rule</th>
<th>BFR [%/d]</th>
<th>BRR [%/d]</th>
<th>MAR [µm/d]</th>
<th>MRR [µm/d]</th>
<th>MS [%]</th>
<th>ES [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>SHM 0N</td>
<td>Experimental</td>
<td>0.8 ± 0.2</td>
<td>0.5 ± 0.2</td>
<td>1.2 ± 0.1</td>
<td>1.6 ± 0.3</td>
<td>49.3 ± 3.7</td>
<td>26.3 ± 3.8</td>
</tr>
<tr>
<td></td>
<td>Linear</td>
<td>1.2 ± 0.6</td>
<td>0.9 ± 0.3</td>
<td>1.2 ± 0.2</td>
<td>1.5 ± 0.1</td>
<td>48.9 ± 9.8</td>
<td>27.2 ± 7.7</td>
</tr>
<tr>
<td></td>
<td>Logarithmic</td>
<td>0.9 ± 0.4</td>
<td>0.5 ± 0.2</td>
<td>1.0 ± 0.1</td>
<td>1.3 ± 0.2</td>
<td>49.6 ± 10</td>
<td>24.0 ± 7.2</td>
</tr>
<tr>
<td>O VX 0N</td>
<td>Experimental</td>
<td>1.3 ± 0.3</td>
<td>0.9 ± 0.5</td>
<td>1.3 ± 0.1</td>
<td>1.8 ± 0.4</td>
<td>47.2 ± 5.2</td>
<td>22.9 ± 3.8</td>
</tr>
<tr>
<td></td>
<td>Linear</td>
<td>1.7 ± 0.6</td>
<td>1.4 ± 0.4</td>
<td>1.3 ± 0.1</td>
<td>1.8 ± 0.1</td>
<td>46.6 ± 8.5</td>
<td>21.8 ± 4.2</td>
</tr>
<tr>
<td></td>
<td>Logarithmic</td>
<td>1.3 ± 0.5</td>
<td>1.1 ± 0.3</td>
<td>1.1 ± 0.1</td>
<td>1.5 ± 0.1</td>
<td>41.8 ± 8.7</td>
<td>26.2 ± 5.1</td>
</tr>
<tr>
<td>SHM 8N</td>
<td>Experimental</td>
<td>0.7 ± 0.3</td>
<td>0.3 ± 0.1</td>
<td>1.1 ± 0.1</td>
<td>1.2 ± 0.3</td>
<td>46.8 ± 7.8</td>
<td>23.8 ± 2.3</td>
</tr>
<tr>
<td></td>
<td>Linear</td>
<td>1.1 ± 0.5</td>
<td>0.7 ± 0.2</td>
<td>1.2 ± 0.1</td>
<td>1.3 ± 0.1</td>
<td>47.2 ± 9.8</td>
<td>23.1 ± 7.9</td>
</tr>
<tr>
<td></td>
<td>Logarithmic</td>
<td>1.0 ± 0.4</td>
<td>0.4 ± 0.1</td>
<td>0.9 ± 0.1</td>
<td>1.2 ± 0.2</td>
<td>54.6 ± 9.1</td>
<td>18.9 ± 6.9</td>
</tr>
</tbody>
</table>

* p<0.05 and ** p<0.01 from paired Student’s t-test corrected for multiple comparison considered significant

Visual examination of the local remodeling for SHM 8N group showed close matches of the formation and resorption sites (Figure 5.10), however some structural distinctions on the single trabecula level were present similarly to the O VX 0N group. Quantitative analysis of the serial comparison with the in vivo data revealed initial challenge of the simulation in matching experimental data, however after the third iteration the model fully captured morphometric changes with no significant differences between the in vivo and in silico outcomes.
In conclusion, while it is remarkable, that model parameters could be successfully calculated without having any *a priori* knowledge of the individual and using only statistical modeling, these results showed no convincing evidence that animal-specific approach should be preferred over group-wise simulations.

*Table 5.4. Control parameters for the logarithmic remodeling rules*

<table>
<thead>
<tr>
<th>Parameters for ( u = \alpha \ln(\text{SED}) + \beta )</th>
<th>Unit</th>
<th>Symbol</th>
<th>Settings</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>SHM 0N</td>
</tr>
<tr>
<td>Gradient</td>
<td>mm/week</td>
<td>( \alpha )</td>
<td>0.0035</td>
</tr>
<tr>
<td>Intercept</td>
<td>mm/week</td>
<td>( \beta )</td>
<td>0.0217</td>
</tr>
</tbody>
</table>

**Discussion**

The work in this project has been structured on the basis of the logical hypothesis flow, where the results from the first investigation were used as motivation for the following hypothesis. First, we were able to show that separate control functions for bone formation and resorption processes of the *in silico* model performed better than previously implemented symmetric settings [14].

On the other hand, such algorithm expansion further increased the complexity of the control parameters. Consequently, we were able to link *in silico* setting to the *in vivo* measurements. Establishing a connection between experimental measurements and computational algorithm has not only simplified the process of choosing the right parameters, but also brought a degree of biological relevance to the *in silico* model for the first time. Notably, the parameters that have been derived from the experimental data, and resulted in the best simulation outcomes, lead to the remodeling curves where the “lazy zone” was absent for the
experimental groups without the loading regime, thus contradicting the assumption of the Mechanostat Theory.

Figure 5.5. Visualization of the local remodeling sites for SHM 0N group. From left to right the images show a coronal cross sectional view of the 6th caudal vertebral for the in vivo experiment, in silico simulation with a linear model, and in silico simulation with the logarithmic model. Color code refers to the total formation, resorption, and quiescence of bone over the period of 4 weeks of the study. Fine details of the linear and logarithmic implementations of the computational remodeling algorithm can be better assessed in the 4X zoom in view.
Table 5.5: Static morphometry for the predictive study: long-term group and individual outcomes

<table>
<thead>
<tr>
<th>Study</th>
<th>Remodeling Rule</th>
<th>Static Morphometric Parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td>SHM 0N</td>
<td>Experimental</td>
<td>17.4 ± 2.2</td>
</tr>
<tr>
<td></td>
<td>Group</td>
<td>17.3 ± 0.7</td>
</tr>
<tr>
<td></td>
<td>Individual</td>
<td>16.9 ± 3.5</td>
</tr>
<tr>
<td>OVX 0N</td>
<td>Experimental</td>
<td>9.5 ± 1.6</td>
</tr>
<tr>
<td></td>
<td>Group</td>
<td>9.0 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>Individual</td>
<td>9.2 ± 1.0</td>
</tr>
<tr>
<td>SHM 8N</td>
<td>Experimental</td>
<td>14.7 ± 1.9</td>
</tr>
<tr>
<td></td>
<td>Group</td>
<td>14.4 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>Individual</td>
<td>15.1 ± 1.8</td>
</tr>
</tbody>
</table>

* p<0.05 and ** p<0.01 from ANOVA corrected for multiple comparison considered significant
Table 5.6: Dynamic morphometry for the predictive study: long-term group and individual outcomes

<table>
<thead>
<tr>
<th>Study</th>
<th>Remodeling Rule</th>
<th>Dynamic Morphometric Parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>BFR [%/d]</td>
</tr>
<tr>
<td>SHM 0N</td>
<td>Experimental</td>
<td>0.2 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>Group</td>
<td>0.4 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>Individual</td>
<td>0.3 ± 0.2</td>
</tr>
<tr>
<td>OVX 0N</td>
<td>Experimental</td>
<td>0.2 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>Group</td>
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</tr>
<tr>
<td></td>
<td>Individual</td>
<td>0.3 ± 0.2</td>
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<tr>
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<td>Experimental</td>
<td>1.2 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>Group</td>
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</tr>
<tr>
<td></td>
<td>Individual</td>
<td>1.0 ± 0.4</td>
</tr>
</tbody>
</table>

* p<0.05 and ** p<0.01 from paired Student’s t-test corrected for multiple comparison considered significant
Figure 5.6. Visualization of the local remodeling sites for OVX 0N group. From left to right the images show a coronal cross sectional view of the 6th caudal vertebra for the experiment, simulation with a linear model, and simulation with the logarithmic model. Color code refers to the total formation, resorption, and quiescence of bone over the period of 4 weeks of the study. Fine details of the linear and logarithmic implementations of the computational remodeling algorithm can be better assessed in the 4X zoom in view.

For the group that was undergoing mechanical loading, the separation between bone formation and resorption thresholds was present, although in a minimal representation, comprising less than 1% of the total SED range. The insignificance of the “lazy zone” phenomenon in load-driven bone remodeling is in agreement with a number of other recent investigations both in animal and human bone [12, 21-23].
Figure 5.7. Visualization of the local remodeling sites for SHM 8N group. From left to right the images show a coronal cross sectional view of the 6th caudale vertebra for the experiment, simulation with a linear model, and simulation with the logarithmic model. Color code refers to the total formation, resorption, and quiescence of bone over the period of 4 weeks of the study. Fine details of the linear and logarithmic implementations of the computational remodeling algorithm can be better assessed in the 4X zoom in view.

Interestingly, supporting evidence for this conclusion was also found in an earlier animal study [12], which considered linear rather than logarithmic remodeling relationship between bone remodeling and mechanical strains. Combined, these findings provide justification for implementing a bone remodeling function, such as a natural logarithm, which does not accommodate “lazy zone”.

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Therefore, we have been able to simplify the model with \textit{in vivo} derived parameters further by replacing the piece-wise linear remodeling rules with continuous analytical relationships of the logarithmic form. Natural logarithm functions were given consideration due to their resemblance of the shapes of the linear remodeling rules, as well as their ubiquity in the biological world [24-27]. The relationship between resorption and formation and their rate of change with respect to the local mechanical strains could also be regarded as an underlying principle for selecting logarithmic functions. For example, \textit{in vivo} derived linear remodeling rules suggest that bone is more sensitive to changes in mechanical environment in the regulation of resorptive processes, while the rate of formation is more stable once the corresponding mechanical threshold is reached. A natural logarithmic function represents a derivative of such a response rate, and therefore appears to be an appropriate selection for reflecting such differences in bone formation and resorption processes.

In addition, in the transfer from linear to logarithmic rules we observed that the major differences between the SHM 0N and OVX 0N groups was in the gradient of the SED curve, while the intercept of the functions remained almost identical (\textit{Figure 5.3}). This change resulted in the reduction of maximum formation rate for the osteopenic animals, while resorption controls remained very close to those of the healthy group, an effect reported in many biological investigations across species [28, 29]. In the SHM 8N group, on the other hand, the gradient remained similar to that of the control group, while the intercept shifted to reflect the effect of changes in the mechanical state of the system. As follows, both formation and resorption processes were reduced. While this is in agreement with literature reports of decreased bone resorption under mechanical loading conditions [30, 31], the finding disagrees with the earlier computational study on the lack of effect of mechanical loading in bone formation [7]. Additionally, the relation between SED and bone formation differed between loaded and control groups, which could lead to two conclusions: first, the sensitivity of bone to mechanical stimulus is reduced in the elevated strain scenarios, and second, it is the ratio of resorption and formation, and not a single process, that leads to the net bone gain
in the loading scenarios. While easily observable in the logarithmic functions, these biologically relevant conclusions are not evident from the analysis of the linear relationships. Stable outcomes in the static morphometry and improved results of the dynamic morphometric evaluation further justified the selection of the logarithmic remodeling rules for bone remodeling \((Tables\ 5.3, 5.4)\). Close visual examination of the local remodeling sites was also in agreement with other measurements: while the locations of the formation and resorption have hardly shifted between the linear and logarithmic models, the amount of bone added and removed appeared to resemble biological patterns closer in simulations with the logarithmic models \((Figures\ 5.5-5.7)\). In conclusion, the replacement of the linear relationship with the logarithmic functions improved the quality of the simulation outcomes and particularly correlations of the dynamic morphometry between the \textit{in silico} and \textit{in vivo} outcomes.

Another confirmation of the advantage of logarithmic rules and decoupling of bone formation and resorption was that long-term (8 week) studies resulted in accurate \textit{in silico} predictions of \textit{in vivo} outcomes using both animal and group-specific control settings. As part of this approach control settings for the algorithm were calculated on the individual basis using newly developed statistical tools. Our original hypothesis was that such single-animal predictions could improve not only individual outcomes, but also those of the entire group. While the approach is noteworthy in that it is the first true attempt of long-term prediction of the bone remodeling without any \textit{a priori} knowledge about the subject, the variable success of the outcomes suggests that the statistical model for the calculation of individual parameters still lacks the precision and accuracy to confirm our hypothesis. The lack of improvement in the individual outcomes can also be partially explained by the genetic homogeneity of the animal pool, which was already well captured in the group-wise simulations. While not sensitive enough for applications in inbred animals, this approach can lead to more successful outcomes in the clinical setting where the variability between subjects is much higher.

In conclusion, we have developed a new implementation of the load-driven algorithm for \textit{in silico} prediction of bone remodeling. Using a mouse model, bone
remodeling rules were established for healthy, osteopenic, and mechanically loaded animals. Our results not only contradicted the existence of the “lazy zone” in the mechanical control of bone remodeling, but also provided strong evidence of logarithmic relationship between local mechanical strains and bone formation and resorption processes. These results provided new insights into the effects of mechanical loading on bone remodeling that improve in silico predictions and contributed to better understanding of the underlying biological processes. The outcomes of the first fully predictive simulations and their validation against in vivo study were reported. Finally, we have attempted to classify individual animals and predict their long-term remodeling outcomes without previous knowledge of their bone health in an effort to imitate clinical setting. While no distinct advantages were seen in the inbred animal pool, the technique opens a door of possibility for further development of the model and its applications in the medical realm.

Materials and Methods

Ethics Statement

All animal studies have been approved by the veterinary authority of the canton of Zurich, license number 117/2008 (Kantonales Veterinaeramt Zurich, Zurich, Switzerland). Experiments have been carried out under veterinary supervision, with an extra effort to minimize animal suffering.

Animal and visualization procedure

For the purposes of algorithm training and validation, we have relied on two separately planned and executed animal studies. For the training setup a shorter experiment was used in which female C57Bl/6 (B6) mice were either ovariectomized or sham operated at 15 weeks of age [20, 32]. The procedure of removing the ovaries leads to the state of estrogen depletion, and has been established as a valid model for osteopenia, and eventually osteoporosis in animals. The sham operation, in which animals undergo similar procedure, but all internal structures remain intact, served as a control measure in the experiment.
The lag of 11 weeks was allowed for animal maturation and development of changes in the bone structure. At week 26 mechanical loading of the 6th caudal vertebra (CV6) was started for the animals assigned to the loading treatment group [33]. This setup was designed to test the effect of controlled loading on bone adaptation and architecture, which is highly relevant in the context of Mechanostat Theory.

Briefly, the experimental procedure involved insertion of metal pins into the 5th and 7th caudal vertebrae of the animals and application of a sinusoidal force of 8N at the frequency of 10Hz for 5 min/day, three times a week. All experimental procedures were carried out under general anesthesia over the period of 4 weeks. A total of three experimental scenarios have been investigated: sham operated (SHM 0N; n=8) or healthy group, sham operated and mechanically loaded group (SHM 8N; n=8), and ovariectomized group (OVX 0N; n=9). Time-lapsed bone adaptation was monitored on the fortnightly basis for a period of 4 weeks, resulting in a total of 3 measurements. In vivo micro computed tomography (μCT) scans were performed with the isotropic voxel resolution of 10.5 µm (vivaCT 40, Scanco Medical, Brüttisellen, Switzerland).

In the validation setup experimental data was taken from two previously published longitudinal studies. Where experimental procedures for SHM 0N (n=7) and OVX 0N (n=9) followed a similar protocol as described for the training set but for a period of 12 instead of 4 weeks [34]. The SHM 8N (n=5) group used in the validation study has not undergone surgery, but otherwise has been subjected to the similar treatment, loading, and monitoring procedures as previously described [35]. Consistently with the other two groups in the validation setup SHM 8N animals have been subject to biweekly μCT scanning for a total of 7 measurements.

In the validation study the first three measurements were not used in the simulations due to the rapid bone changes associated with the effect of the ovariectomy in a juvenile animal for the OVX 0N group [34], and for consistency reasons in the other two groups.
Figure 5.8. Serial and cumulative visualization of bone remodeling for the SHM ON group. (Top row) Time-lapsed changes in the trabecular bone architecture of the in vivo experiment over the period of 8 weeks, where the final image shows cumulative formation, resorption, and quiescence patterns. (Bottom row) Corresponding steps in structural changes in the outcomes of the in silico simulation with the logarithmic implementation of the model.
Figure 5. Serial and cumulative visualization of bone remodeling for the OVX 0N group.

(Top row) Time-lapsed changes in the trabecular bone architecture of the in vivo experiment over the period of 8 weeks, where the final image shows cumulative formation, resorption, and quiescence patterns. (Bottom row) Corresponding steps in structural changes in the trabecular bone architecture of the in silico experiment with the logarithmic implementation of the model.

Outcomes of the in silico simulation with the logarithmic implementation of the model.
Figure 5.10. Serial and cumulative visualization of the bone remodeling for the SHM 8N group. (Top row) Time-lapsed changes in the trabecular bone architecture of the in vivo experiment over the period of 8 weeks, where the final image shows cumulative formation, resorption, and quiescence patterns. (Bottom row) Corresponding steps in structural changes in the outcomes of the in silico simulation with the logarithmic implementation of the model.
Figure 5.11. Time course of static morphometric parameters over all time points for SHM 0N. The solid black line represents the experimental group, while grey dashed line corresponds to the simulation. The error bars describe the standard deviation calculated for all animals. Stars denote statistical significance assessed with ANOVA corrected for repeated measurements, where p values below 0.05 were considered significant.

All post-processing and image registration procedures, as well as resulting differentiation of the sites of bone formation and resorption have followed previously published protocols [21, 36].

Finite Element Calculation

Based on the existing literature and previous implementation of the bone remodeling algorithm [14, 37], strain energy density (SED) was chosen as a mechanical signal in the remodeling simulations. Individual voxels in the μCT
scans were converted to 8 node hexahedral elements. Tissue properties were carried over from literature, where Young’s modulus was set to 14.8 GPa, and Poisson’s ratio to 0.3 [33].

Figure 5.12. Time course of static morphometric parameters over all time points for OVX 0N. The solid black line represents the experimental group, while grey dashed line corresponds to the simulation. The error bars describe the standard deviation calculated for all animals. Stars denote statistical significance assessed with ANOVA corrected for repeated measurements, where p values below 0.05 were considered significant.

All models were assumed to be linear elastic. Circular intervertebral discs were applied on the top and bottom surfaces of the vertebrae to ensure even force distribution. To prevent numerical issues during solving, intervertebral discs were assigned same tissue properties as the rest of the model. SED distributions were derived from applying uniaxial compressive forces of 4N for the non-loaded
groups [38], and 8N for the animals undergoing mechanical loading in vivo. FE analysis was performed on a reference input image, as well as on follow-up simulation outputs in an iterative manner. All calculations were carried out at the Swiss National Supercomputing Center (CSCS, Lugano, Switzerland) using ParFE solver [39] with 128 CPUs and solution time of less than 60 seconds for a model of approximately 1.8 million elements.

**Bone remodeling algorithm**

To test the first hypothesis of whether decoupling the control parameters for bone resorption and formation events would improve the outcomes of the simulations the original algorithm for bone adaptation had to be modified respectively (Figure 5.2A-B). Briefly, the framework was structured in a strain-adaptive feedback structure, where bone surface was modified by adding or removing voxels in response to the mechanical stimuli in an iterative manner [1].

This version of the algorithm was based on the symmetric interpretation of the Mechanostat Theory, where both formation and resorption were controlled by four variable parameters, according to the following formula (adapted from [1]):

\[
\begin{align*}
\text{u}(x) &= \begin{cases} 
-\frac{u_{\text{max}} \cdot SED(x)}{\tau} < SED_{\text{res}} - \frac{u_{\text{max}}}{\tau} < SED(x) < SED_{\text{res}} \\
(SED_{\text{res}} \cdot \tau) + \tau \cdot SED(x), SED_{\text{res}} - \frac{u_{\text{max}}}{\tau} < SED(x) < SED_{\text{res}} \leq SED(x) < SED_{\text{for}} \\
0, SED_{\text{res}} < SED(x) < SED_{\text{for}} \\
(SED_{\text{for}} \cdot \tau) + \tau \cdot SED(x), SED_{\text{for}} < SED(x) < SED_{\text{for}} + \frac{u_{\text{max}}}{\tau} \\
u_{\text{max}}, SED_{\text{for}} + \frac{u_{\text{max}}}{\tau} < SED(x)
\end{cases}
\end{align*}
\]

Where \(u(x)\) is bone remodeling rate in mm/week, \(\tau\) is bone formation and resorption rate per SED in (mm/week)/MPa, \(SED_{\text{res}}\) and \(SED_{\text{for}}\) are resorption and formation thresholds respectively in MPa, and \(u_{\text{max}}\) is a maximum remodeling velocity in mm/week. The first modification of the algorithm comprised decoupling \(\tau\) and \(u_{\text{max}}\) into formation and resorption components. The corresponding remodeling equation is as follows:
In the second modification, several non-linear functions (exponential and logarithmic) were considered as a replacement to the linear implementation. The major motivation in testing this hypothesis was reduction of the model complexity, as well as improvement of the biological relevance of the model. Natural logarithm implementation was found to be the closest fit to the existing remodeling curves, and produced better or similar simulation outcomes than linear models. Logarithmic approach thus was chosen as the preferred form of the bone remodeling rules.

Selection of parameters for the linear model

Following the decoupling of formation and resorption controls, the number of variable parameters in the algorithm increased from four to six. In order to reduce the complexity of the model, we have attempted to derive all of the control parameters from the corresponding in vivo measurements, and thus test the second hypothesis (Table 5.1). Therefore, $u_{res}$ and $u_{for}$ were calculated directly from the maximum detected formation and resorption rates in the experimental data. This was achieved through using registered time-lapsed in vivo μCT scans to detect formation and resorption pits [36], measuring their maximum depth and converting the values to the appropriate time scale. The extracted maximum values were then averaged over all animals within each group, resulting in the maximum formation and resorption velocities for each experimental scenario. To derive the settings for formation and resorption thresholds we have relied on the previously introduced method of remodeling probability curves [21]. Briefly,
according to the technique, there exists a number of voxels that will be formed, removed, or remain quiescent for each SED value. When incorporating time into this method, probability of each remodeling event can be calculated using non-linear regression analysis. To make results comparable across individual animals and groups SED values were normalized by the maximum value calculated for each animal. Due to the uncertainty factor associated with the probability calculation, direct transfer of the outcomes into the model parameters was less successful than expected, however SED intersection points between resorption and quiescence, and quiescence and formation derived from the remodeling probability curves could be used as reliable reference values for SED$_{res}$ and SED$_{for}$ settings. Similarly, due to the lack of direct links between experimental measurements and formation and resorption rates ($\tau_{res}$ and $\tau_{for}$) with respect to measured SED value this parameter has to be selected iteratively. However, because of the strong interplay between the SED thresholds and remodeling rates and derivation of the former from $in$ $vivo$ data, the number of available settings for $\tau_{res}$ and $\tau_{for}$ was reduced from the previous algorithm implementation. Therefore, this approach not only improved biological relevance of the model, but also established reasonable basis for the parameter selection, reducing the optimization time.

While the expansion of the algorithm improved simulation capacity for formation and resorption, as well as allowed direct incorporation of the $in$ $vivo$ measured parameters into the model, it also made controlling the model more challenging. In an attempt to simplify the extended remodeling rules, we have hypothesized that by replacing linear curves governed by 6 parameters with non-linear functions the model could be simplified without compromising the quality of the simulations. In the process of selecting appropriate non-linear functions focus was placed on exponential and logarithmic relationships that resembled the shapes of the existing $in$ $vivo$ derived linear relationships ($Figure$ $5.2C$-$D$). Logarithmic curves proved to be the best fit for the linear functions and produced improved outcomes when compared to $in$ $vivo$ data. Selection of the group-wise logarithmic functions was optimized around SED thresholds derived from the
experimental data, as well as maximum formation and resorption rates, calculated directly from the in vivo measurements (Figure 5.3, Table 5.4). SED values of 0 were inherently excluded from the evaluation, eliminating the need for the cut off values on the resorption sides. Excessive remodeling at either end of the logarithmic curve was not relevant due to the Gaussian distribution of the SED values. In addition, concave down shape of the logarithmic functions reduced the number of available parametric option for the gradient (α) and intercept (β) terms, thus simplifying the optimization of the group remodeling rules [20]. In summary, in selecting two parameters of the logarithmic remodeling rules interplay between the two variables was a deciding factor, at the same time proximity to the values of the linear relationships was favored whenever possible (Figure 5.3, Table 5.2).

Statistical analysis for the calculation of individual remodeling rules

The fourth hypothesis we have posed explored the potential of predicting animal-specific model parameters for the logarithmic relationships from the experimental data. To be able to verify whether exists a relationship between in vivo measured parameters and control setting of the algorithm, individual simulation for each animal in the training data set were carried out. Within this process, distinct sets of α and β values were derived for each animal. From the resulting remodeling rules it became apparent that α parameter is related to the experimental treatment of the group, where the value for the parameter was identical for both SHM groups and distinct for the OVX group, while β was strictly individual.

Therefore, to predict α term of the logarithmic function, a logistic regression, or in the case of binary coefficients, conditional probability approach was chosen to differentiate between the treatment groups [40]. Within this approach a ratio of probabilities of the individual belonging to either group is calculated. In the case of the training set there was a clear separation between SHM and OVX groups, where one of the probability terms was always 0, leading us to consider the classification approach instead [41].
Figure 5.13. Time course of static morphometric parameters over all time points for SHM 8N. The solid black line represents the experimental group, while grey dashed line corresponds to the simulation. The error bars describe the standard deviation calculated for all animals. Stars denote statistical significance assessed with ANOVA corrected for repeated measurements, where p values below 0.05 were considered significant.

Selection of logarithmic remodeling rules

This method allowed establishing a rule for the separation of all animals into the respective treatment groups, using three parameters from the *in vivo* measurement, namely, the starting bone volume fraction (BV/TV), the final BV/TV measurement, and the overall bone resorption rate (BRR).

Linear models allowing complex interactions between the predictor variables were then used for each group to calculate individual $\beta$ terms [41]. For SHM 0N group the model was based on the cubic interaction between the starting BV/TV,
and a combination of BFR and BRR ($r^2>0.61$). The predictive model for SHM 8N group comprised a quadratic interaction of BFR and BRR ($r^2>0.60$). Finally, for the OVX 0N, similarly to the SHM 0N group, the prediction was based on the cubic interaction of the starting BV/TV values and BFR and BRR ($r^2>0.99$). Simulations for the long-term data set were then performed on the group-wise basis, using earlier selected logarithmic remodeling curves, as well as individually, with the animal-specific determination of the $\alpha$ and $\beta$ terms of based on the predictive models established with the training set. The certainty of the differentiation of $\alpha$ parameter was 100% without any a priori knowledge of the group classification. In calculating the individual $\beta$ terms, the predicted fit value was considered in the first simulation attempt, and then modified within the range of the prediction to maximally improve the outcomes. Statistical analysis was performed with the software package R 3.1.2 (www.r-project.org/) and SPSS (IBM SPSS Statistics, V20.0).

**Comparison of static and dynamic morphometry**

Static and dynamic morphometry was assessed for both in vivo and in silico data sets according to the previously established protocols [20, 36]. Bone volume fraction (BV/TV), specific bone surface (BS/BV), trabecular thickness (Tb.Th), and trabecular number (Tb.N) were included for the static evaluation. Dynamic morphometry was derived from the registration of the last measurement onto the first one, and included 3D bone formation rate (BFR), mineral apposition rate (MAR), mineralizing surface (MS), as well as bone resorption rate (BRR), mineral resorption rate (MRR) and eroded surface (ES).

For static morphometric evaluation residual plots, Q-Q plots, histograms, and Levine’s tests for homogeneity of variances were used to test model assumptions prior to comparing group means using one-way ANOVA analysis with post hoc Bonferroni correction for multiple comparisons. Kolmogorov-Smirnof test was used to test for equal variances prior to applying a paired two-sample Student’s t-test corrected for multiple comparisons for the dynamic evaluation. $p$ values of 0.05 or less were considered significant for all test.
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References:


Chapter 6
Synthesis

Trabecular bone tissue is fascinating in its adaptive capacity. It is known to react to physiological stresses and strains by changing its architectural orientation, and adding or removing material as needed. At the same time it is an integral part of the body, and is thus governed by systemic endocrine and molecular processes. When the homeostatic state of bone is disrupted, the trabecular structures remodel in a manner that compromises their physiological function. One of the most common diseases of bone in humans is osteoporosis. While there are a number of pathways that lead to this condition, its effect on bone tissue is predictable: the remodeling processes become impaired and a net loss of bone is sustained. In its early stages the condition is referred to as osteopenia and it is characterized by moderate bone loss, which is relatively benign in its consequences. If left undetected and untreated, the disease often develops into osteoporosis, which is characterized by significant bone loss, and a corresponding increased risk of developing fractures. While regular clinical screening with dual X-ray absorptiometry (DXA) are recommended for women over the age of 65, the individual course of the disease is difficult to predict, and early fractures often go unnoticed. Once the first fracture takes place, the quality of life of a patient will be irreversibly decreased. Severe cases can even result in life-threatening complications. The best way to deal with the effects of osteoporosis is by preventing fractures and stopping the progression of bone loss. While a number of pharmaceutical treatments to counter the effects of the disease are available in clinics, computational approaches provide a promising auxiliary tool to the medical practice in individual prediction and treatment planning of bone diseases.

Similar to the drug development process, the development of in silico tools for clinical integration is an extensive and time-intensive process comprising concept development, programming and implementation of the idea, and stage-wise testing and validation procedures. Before the functional framework can be used in practice, accuracy and reproducibility studies should be carried out in animals, and if possible validated against in vivo data.
A number of promising computational models for bone remodeling were brought to the attention of the scientific community when the associated computational tools became available in the early 2000’s [1-3]. Yet, none has advanced through all the stages of clinical integration due to large scale of required resources, or limited validation tools. In addition, poor understanding of the underlying biological processes, and the difficulty of translating patient-specific bone remodeling processed into a controlled algorithm framework, have further reduced relevance of such in silico models in the clinical setting.

The work presented in this thesis aimed to address a number of the above outlined limitations of the current computational approaches in bone research. First, previously developed simulated bone atrophy (SIBA) algorithm was tested in its correlative modeling capacity. Cases of age-related bone loss, as well as preventive and curative treatments with anti-resorptive and anabolic agents were simulated. High-resolution three dimensional geometries of human vertebrae and femora were used as input for the model. Population- and literature-based validations were a final objective of this study.

Additionally, a more biologically integrative algorithm for bone remodeling was considered for further development [4]. Briefly, this model was developed on the principles of the Mechanostat Theory of bone modeling and remodeling [5]. The workflow was based on the iterative strain-feedback loop, in which an output of the first step served as an input for the next step. The model was developed using in vivo animal data collected as part of ovariectomy and mechanical loading experiments. The aim within this thesis was to perform a comprehensive validation of the framework using a database of 240 animals. The validation addressed not only bone loss and mechanical loading scenarios, but encompassed preventive and curative stages of treatment with anabolic and anti-resorptive therapies. This was the first case where the algorithm was subjected to such an exhaustive validation process, performed not only on the population level, but rather in the animal-specific format using both quantitative and qualitative assessment techniques.
Several of the algorithm limitations became apparent during the large-scale simulations of catabolic and anabolic processes in bone. While some of the limitations, such as simplified tissue properties and boundary conditions, seemed to be inherent to the current state of computational capabilities, the restrictive capacities of the model in controlling bone adaptation processes became a major focus of the thesis. Within this part of the study, the control parameters of the *in silico* algorithm were decoupled to allow independent control of formation and resorption. In addition, the original iterative process of parameter matching and selection was abandoned in favor of deriving the settings directly from the available *in vivo* data. Briefly, the maximum formation and resorption rates were calculated directly from the experimental measurements, while strain thresholds for both formation and resorption were derived using a sophisticated remodeling probability technique [6]. The remaining formation and resorption rate per SED could also be integrated with the other settings, leaving very little room for iterative selection. One of the observations that resulted from this development process was that the previously linear relationships between bone remodeling and strain resembled a logarithmic curve once the *in vivo* settings were incorporated. This led to the hypothesis that using a single logarithmic curve to model bone remodeling with respect to mechanical signal would allow simplification of the algorithm controls without compromising the quality of the simulation outcomes. This indeed proved to be correct when verified in a correlative study with the experimental animal data. A reduction in the number of model controls by a factor of three allowed construction of a statistical model of manageable complexity for the prediction of individual parametric settings. This was a novel and clinically relevant advance. Both earlier selected group-specific parameters and individually predicted model controls were then verified in a truly predictive investigation. In this most advanced validation to date, an independent animal *in vivo* datasets of healthy, osteopenic, and pharmaceutically- and mechanically-treated bone conditions were used for quantitative and qualitative verification of the *in silico* outputs. The result of this hypothesis-driven algorithm development was a new set of biological relationships between bone remodeling and local strain environment for healthy, mechanically loaded, and osteopenic conditions; a statistical model
for the prediction of patient-specific simulation parameters; and finally, predictive validation outcomes that exceeded accuracy of any previously reported simulation.

The main outcome of this thesis was the development of an *in silico* model for prediction of bone adaptation in clinically relevant scenarios. Additionally, previously unexplored computational representations bone remodeling processes were investigated and integrated into the *in silico* algorithm.

**Simulations of bone remodeling in whole human bone in health and disease**

The main objective of this study was simulation of the microstructural changes in high resolution three dimensional samples of whole human bone. An additional novelty to this work was brought in through expanding the focus from osteoporotic effects alone to incorporation the effects of the clinically relevant treatments, such as bisphosphonates and parathyroid hormone.

The SIBA algorithm was previously shown to be a suitable tool for the simulation of bone loss in human samples [7] and bone gain in an animal model [8]. The present work in effect combines both developments into a comprehensive study of catabolic, anabolic, and anti-resorptive transformations in bone. One of the major outcomes of the investigation was the development of a sample database, which allowed juxtaposition of individual bone architecture with the clinical measure of bone quality. It has been shown that degree of anisotropy (DA) can provide valuable additional information regarding the structural integrity of trabecular bone [9], however, due to the extensive analysis required for the measurement of this parameter, the practice is currently not included in the clinical evaluation of bone quality. A sample database would allow indirect incorporation of this metric into patient assessment through a reference system.

With respect to the simulation outcomes, the data is in agreement with previous literature reports. For example, Müller reported an average decrease of 38% in vertebral BV/TV as a result of 30 years of bone loss [7]. This result was confirmed and repeated in the current study, supporting the consistency of the
approach. Clinical reports of changes due to PTH and BIS treatments could also be captured in the *in silico* measurements, despite the fact that the initial year of therapy required a separate set of control parameters to reflect the striking effect of 6% increase in bone mass \([10\text{-}12]\). The algorithm was also able to replicate the subsequent plateau of the treatment effect, as well as match the iteration lengths reported in literature.

In conclusion, *in silico* tools can offer not only a powerful model of biological events, but also serve as the reinforcement to the established clinical approaches. Current application of the SIBA algorithm for the simulation of microstructural changes in whole three-dimensional human vertebrae and femora resulted in accurate predictions of bone formation and resorption processes in response to osteoporosis and associated treatment therapies. Nevertheless, further development of the model to increase its biological relevance was required to maximize its clinical applicability and predictive potential.

**In vivo validation of the mechanical feedback algorithm**

The objective of the second study was to carry out a validation of a novel strain-adaptive *in silico* algorithm for simulation of trabecular bone adaptation. The comprehensive validation process of the model was facilitated through direct comparison of the simulation results with the *in vivo* \(\mu\)CT data. The experimental study was based on a mouse model for estrogen-depletion induced osteopenia at an early and advanced age, as well as interventions in the form of mechanical loading, bisphosphonate and parathyroid hormone treatments. Unlike many of the previously introduced algorithms that underwent population-based validation \([1\text{-}3]\), including the one discussed in the previous section, this study provided encompassing validation according to the proposed gold standard \([13]\), and included static and dynamic morphometric assessments \([14]\), as well as comparison of the local bone architecture.

With this application of the mechanical-feedback algorithm we were able to reproduce changes in BV/TV with a maximum error of 4.5% for all investigated scenarios. This result is an improvement on the previously reported error of group
means of 12.1% [4]. The overall agreement of other morphometric indexes between *in vivo* and *in silico* results was also improved, with the exception of Tb.Th, which remained a challenging target. Dynamic morphometry, evaluated in terms of bone formation and resorption rates, and mineral apposition and resorption rates, and surfaces, was also captured well in the simulations; however closer matches were compromised in favor of better static morphometric and local architectural agreements. For example, due to the symmetric implementation of the remodeling algorithm for formation and resorption, and the major focus on the bone volume fraction, the best results were achieved in predictions of trabecular spacing and number, along with bone formation rates, while mineralizing and eroding surfaces, trabecular thickness, and bone resorption rate were more difficult to match.

Among many other promising models for bone adaptation that have been previously published [1, 2, 4, 15]; none have undergone such an extensive validation based not only on direct comparison with *in vivo* data, but also inclusive of the effects of bone loss and therapeutic strategies with distinct mechanisms of action. These developments increase the degree of biological validity of the model, and grant it further relevance on the way to clinical implementation.

Nevertheless, there is a number of limitations that must be discussed and addressed with the proposed model. The first is the assumption of the tissue homogeneity, which can only be addressed through adapting higher resolution tissue visualization and finite element analysis methods, both of which are currently technologically unavailable. The second limitation of the presented model, which became apparent in the validation process, was the coupling of the control parameters for formation and resorption, as well as for the remodeling saturation. Separate definition of those parameters would allow independent control of the two processes, at the same time preserving the mechanical-feedback principle of the model. Finally, linking the parameters to the *in vivo* measurements could improve model outcomes, at the same time simplifying the simulation process and adding a more realistic dimension to the outcomes.
In conclusion, a comprehensive *in vivo* validation of the predictive strain-adaptive computational model was presented. The validation was performed for hormone-related bone loss, as well as for anabolic and anti-resorptive therapies. The simulation errors for the static and dynamic morphometric indices were evaluated along with accompanying visual comparison of the local remodeling sites for 181 animals.

**Predictive simulations of bone remodeling**

The first objective of this section of the thesis was driven by the main limitation observed in the validation study, namely that formation and resorption could not be adequately simulated with a single set of parameters. Therefore, the first aim was to decouple the control parameters for bone formation and resorption. While this modification increased the overall complexity of the algorithm, the outcomes of the simulations confirmed that this was indeed a necessary step. The biological basis for this modification were also undeniable, with numerous studies [16-18] demonstrating distinct pathways for the two remodeling processes.

In an effort to simplify parametric selection for the model, as well as to add biological relevance to the implementation, control settings for the expanded algorithm were derived from the *in vivo* data. A large dataset of experimental outcomes from the mouse model for osteoporosis and related treatments was utilized to directly calculate or indirectly derive all six control setting. Previous computational algorithms have mostly relied on literature and/or assumptions from the experimental studies to choose control settings, therefore, this effort denotes the first attempt to run an algorithm fully based on the *in vivo* measurements. Improved simulation outcomes in terms of quantitative and qualitative comparison with experimental data confirmed the advantages of the chosen strategy.

Further analysis of the *in vivo* derived settings with respect to the relationship between bone remodeling and local mechanical signal, revealed not only a distinct similarity of the resultant piece-wise linear functions to the shape of the natural logarithm curve, but also the absence of the so called “lazy zone”. This is not an
entirely new finding, as several recent studies reported results contradicting this previously widely accepted concept [19-21]; however, the results of this study provide valuable evidence that further supports this paradigm shift.

The following conversion of the remodeling equations into natural logarithm format allowed reduction in the complexity of the model, and facilitated some further discoveries of bone remodeling processes. For example, we observed that thresholds for formation and resorption between ovariectomized and healthy animals were very similar, while the sensitivity to the local strains was increased, and the overall maximum formation rate decreased. In the mechanically loaded group, on the other hand, the maximum remodeling rates remained the same, while the threshold shifted to reflect the effect of changes in the mechanical state of the system, thus reducing both formation and resorption processes. This led to two possible interpretations: first, that the sensitivity of bone to mechanical stimulus is reduced if systemic strain rates are increased, and second, that it is the ratio of resorption to formation, rather than a change on either side, that resulted in the net bone change. These conclusions, while apparent in the logarithmic implementation, were not evident in the linear model. The outcomes of the simulations were also further improved, particularly with respect to the dynamic morphometric parameters.

In the last two objectives of the study a novel method to predict individual remodeling settings was introduced. Additionally, group-wise logarithmic relationships and animal-specific predictions were validated using a separate in vivo dataset. The results of these truly prognostic simulations were encouraging in their accuracy, as both quantitative and qualitative outcomes were better than previously reported with the original model [22, 23].

**Limitations and future research**

The thesis was structured in a way that facilitated each objective to address the main limitation of the previous study with the focus on improving the capabilities of bone remodeling simulations. Nevertheless, there remain many drawbacks of the available tools and selected implementations. Following is the discussion of
the major limitations of the work along with the suggestions for potential improvements.

The SIBA algorithm in itself is a useful tool for simulating changes in bone; however, due to its lack of mechanical or endocrine feedbacks, the outcomes have very limited level of biological relevance. This results in a global simulation of bone changes, where local environment is given no consideration. The second algorithm used in this thesis addressed both of those limitations by incorporating the strain energy density signal calculated for each individual voxel. On the other hand, this new implementation required the use of Finite Element analysis, a ubiquitous computational tool, subject to many criticisms of its own. Thus, the main concerns in the analysis were the use of homogeneous tissue properties for the entire bone, as well as simplified boundary condition assumptions. On the side of the model controls for the bone remodeling, the first and foremost drawback was a combined implementation of formation and resorption controls. This issue was addressed in the last objective of the thesis, along with a better system for parametric selection. With respect to the current status of bone remodeling frameworks, there remain a number of limitations to be addressed. First, the statistical tool developed for the prediction of individual parameters did not prove to be as useful as expected in the validation with genetically controlled population of animals. A verification of the model potential with human data, or a diverse animal pool could show a direction for further development. The algorithm could also benefit from converting micro-CT grey scale values directly into the tissue properties. Additionally, using more realistic loading scenarios, such as those proposed by Christen et al. [24] could lead to more accurate strain maps, and thus better matches in the remodeled surfaces. Finally, since the model has undergone exhaustive validation with the animal data, transfer into the human domain would be the next logical step on the way to clinical applications.

Conclusions

In summary, the work carried out within this thesis led to relevant developments of computational application for bone remodeling and made a contribution to the
current understanding and in silico implementation of the underlying biological processes. The thesis commenced with the phenomenological simulations of clinically relevant cases in human vertebrae and femora. The second achievement was a large-scale validation of the mechanical-feedback algorithm according to the current gold standard. Third, the in vivo derivation of the relationships between bone remodeling and local mechanical environment was carried out. Fourth, conversion of the linear model into a logarithmic format accompanied by the introduction of the statistical model for the prediction of algorithm setting was performed. Finally, the predictive power and validity of the developed algorithm were established.

These achievements correspond to the goals of the thesis to develop an in silico framework for the prediction of bone adaptation in clinically relevant scenarios, and brings the algorithm for bone remodeling one step closer to clinical applications.

References


Curriculum Vitae

Alina Levchuk

Born on 28th May, 1986

Citizen of the United States of America

Education

2011 – 2015 Ph.D., ETH Zurich, Institute for Biomechanics, Zurich, Switzerland

2009 – 2010 M.S., Specialization in Biomechanics, ETH Zurich, Zurich, Switzerland

2004 – 2008 B.S., Biomedical Engineering, Macaulay Honors College, The City College Of New York, New York, NY

Awards and honors (since 2008)

2014 Deutsche Gesellschaft für Biomechanik Award Finalist, World Congress of Biomechanics (WCB)

2013 Student Competition Finalist, Dreiländertagung der Deutschen, Schweizerischen und Österreichischen Gesellschaft für Biomedizinische Technik (BMT)

2012 ESB Clinical Biomechanics Award Winner, European Society of Biomechanics (ESB)

2009 Extension to the Whitaker International Fellowship

2008 – 2009 Whitaker International Fellowship
Publications


