Chapter 13
State-of-the-Art Pharmacometric Models in Osteoporosis

Teun M. Post, Anna Georgieva Kondic, Antonio Cabal, Ghassan N. Fayad, Khamir Mehta and Thomas Kerbusch

13.1 Introduction

Osteoporosis is a progressive degenerative bone disease associated with an increased fracture risk. Due to the related morbidity, mortality, and costs with a general increase in life expectancy, this makes osteoporosis an important worldwide health issue.

Burge et al. (2007) conducted an epidemiology study in the USA on the burden of osteoporosis-related fractures and costs in 2005, and using a state transition Markov decision model predicted how those quantities would grow for the period of 2005–2025. Starting at 2005, the actual numbers were 2 million fractures with an associated cost of $ 19 billion. Due to aging population, the numbers are predicted to increase by 50 % by 2025 with 72 % due to hip fractures (Burge et al. 2007). Similar studies have been published in other countries (Rajagopal et al. 2008).

Due to statistical requirements and the slow progression of the disease, large clinical trials with long duration are required to establish a beneficial effect of new treatments on the reduction of fracture risk. Over time, knowledge about bone physiology and the mechanisms underlying bone diseases has increased. Furthermore, various conceptual, mathematical, statistical, and epidemiological models have been established providing further insight into the biology, mechanisms, and predictive factors of osteoporosis and corresponding fracture risk (Post et al. 2010).

T. M. Post (✉) · A. Cabal · G. N. Fayad · K. Mehta · T. Kerbusch · A. Georgieva Kondic
Quantitative Pharmacology & Pharmacometrics, Merck, Sharpe & Dohme Corp., Oss, The Netherlands
e-mail: teun.post@merck.com; teunpost@gmail.com

S. Schmidt, H. Derendorf (eds.), *Applied Pharmacometrics,* AAPS Advances in the Pharmaceutical Sciences Series 14, DOI 10.1007/978-1-4939-1304-6_13

Over the past decade, different types of mechanism-based models have had an increasing impact on drug development and none more so than models of osteoporosis. There has been an increasing body of work elucidating the mechanisms behind the (patho-)physiology of osteoporosis, including the maturation and crosstalk between osteoclasts and osteoblasts and how the balance between bone resorption and bone formation changes with age and hormonal imbalance. The intrinsic nonlinearities, feedbacks, and different time-scales present in the system may lead to counter-intuitive behavior, making mathematical modeling a useful analysis tool. Various conceptual models for bone physiology and the effects of therapies have been proposed. Data included in osteoporosis models range from pharmacokinetics (PK) of (novel) drugs, pharmacodynamics (PD) biomarkers of various time scales (peptides indicative of bone-turnover, bone mineral density), bone strength, as well as the actual clinical outcome, namely, fracture rates at various sites in the skeleton (Post et al. 2010).

Published PK-PD-disease models of osteoporosis have varying degrees of biological complexity ranging from purely descriptive of disease to detailed system models spanning various spatial scales, as well as mechanistic models of bone strength. The possible identification and estimability of parameters typically decline with complexity. Deciding between the use of descriptive, semi-mechanistic, or full mechanistic models should be driven by the drug development question at hand (model fit for purpose), the availability of data, as well as whether one needs the model for extrapolation versus interpolation. These models have been used to describe data from clinical trials, simulate new trial designs with novel mechanisms of action (e.g., what doses will result in what extent of effect on biomarkers/end points, differentiation between subpopulation of patients), simulate combination treatments (e.g., what synergy—if any—should be expected?), and how these clinical trials predict for real-life settings (e.g., prevention of fractures in elderly; Post et al. 2010).

In what follows, we start by describing the main components of bone physiology and the transition to the pathophysiology of osteoporosis. Then, we provide a general description of modeling approaches to osteoporosis, followed by a series of examples of specific model applications.

13.2 Overview of Osteoporosis Components for Modeling

13.2.1 Introduction to Bone Physiology and Pathophysiology

The biology of bone formation and resorption (a process known as bone remodeling; Fig. 13.1) and how it links to the pathophysiology of osteoporosis is progressively better understood. Bone remodeling is mainly the result of the actions of two types of cells, osteoclast and osteoblasts. In the healthy state, resorption and formation are balanced and bone remodeling leads to bone renewal. The osteoclasts

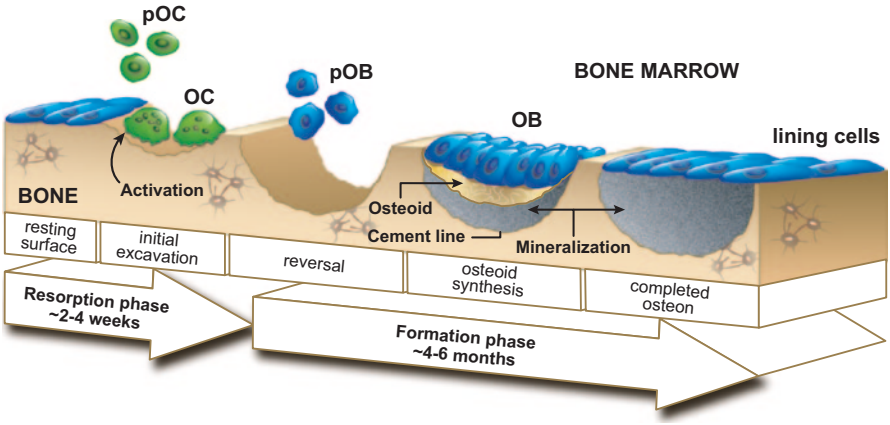

Fig. 13.1 Overview of physiological process of bone remodeling: resorption and formation

attach to the bone surface and act by removing the mineralized matrix and breaking up the organic bone component in the resorption lacuna. When resorption is complete, osteoclasts detach and die by apoptosis. In turn, the osteoblasts attach to the bone and produce a matrix of osteoid, composed predominantly of type I collagen, followed by mineralization of this matrix. The differentiation of preosteoblasts into active cells is triggered via signaling from the osteoclasts. During the mineralization process, a fraction of osteoblasts get trapped in the bone matrix, and differentiate to osteocytes (Manolagas 2000; Boyle et al. 2003).

Our current understanding is that bone remodeling is controlled through:

1. The secretion of transforming growth factor beta (TGF-β) by osteoclasts triggering the differentiation of preosteoblasts to responsive osteoblasts (early osteoblasts that are highly responsive to differentiation signals), and attenuating the differentiation of responsive osteoblasts to active osteoblasts (responsible for bone formation), controlling the build-up of a population of responsive osteoblasts that will colonize the resorption lacuna once the osteoclasts population has died out (Manolagas 2000; Boyle et al. 2003).
2. The receptor activator of nuclear factor κ B (RANK)—receptor activator of nuclear factor κ B ligand (RANKL)-osteoprotegerin (OPG) pathway, which is dedicated to the control of the osteoclasts population by osteoblasts. Schematically, active osteoblasts produce RANKL that interact with RANK located at the surface of osteoclasts precursors. Occupied RANK receptors trigger the differentiation of preosteoclasts in osteoclasts. Production of OPG that inhibits RANKL by the responsive osteoblasts ensures that the osteoclast population grows only at the end of the formation process (Aubin and Bonnelye 2000; Boyle et al. 2003).

Bone loss occurs in postmenopausal women as a result of an increase in the rate of bone remodeling and an imbalance between the activity and number of osteoclasts and osteoblasts. The bone loss occurs in two phases: (1) a rapid one, due to

predominantly estrogen deficiency and (2) a slower one, observed also in men, due to the effects of aging. While the effects of estrogen on bone are not fully understood, it is hypothesized that they may act at least partly through the osteoblasts (e.g., increased synthesis of TGF-β or decreasing OPG with decreasing estrogen), tipping the balance in bone remodeling. The effect of aging is thought to be due to a lot of factors, such as vitamin D deficiency, leading to impaired calcium absorption and increased parathyroid hormone (PTH) secretion, as well as impaired osteoblast function due to continued decline of estrogen, decreased physical activity, and decreased secretion of growth hormone (Raisz 2008).

13.2.2 Metrics of Bone Physiology

The long-term clinical end point in osteoporosis is bone fracture. Bone's material properties are assessed by a mechanical test that yields a stress–strain curve, including breaking point (Cusick et al. 2011; Lotinun et al. 2013). The linear portion of the curve, known as Young's modulus represents stiffness, while the height and inflection point are two different measures of bone strength. It has become increasingly more common to estimate bone strength through the use of finite-element analysis (FEA; Bouxsein and Seeman 2009).

Bone is categorized into two types: cortical and trabecular bone. Cortical bone, mainly the outer shell of bone, makes up about 80 % of bone mass. Trabecular bone, which accounts for only 20 % of bone mass, makes up about 80 % of bone surface. Cortical bone has a high resistance to bending and torsion and gives mechanical strength and protection. Trabecular bone is less dense than cortical bone, providing mechanical support and has a higher turnover rate than cortical bone providing a resource for calcium and phosphate for the maintenance of mineral homeostasis (Post et al. 2010).

While bone mineral density (BMD), the amount of mineral matter per square centimeter of bone is currently the best single, easy accessible, predictor of bone strength, it accounts only for 44 % of the fracture risk. Contributing to the overall bone strength are also shape, geometry, microarchitecture, bone tissue composition, mineralization, micro-damage, and the rate of bone turnover (Post et al. 2010). The most relevant areas for measuring BMD in relation to fractures are the spine (predominantly trabecular), hip (mix of trabecular and cortical), and the wrist (mainly cortical). In addition, BMD is used as a diagnostic predictor for post-menopausal osteoporosis (Melton III et al. 2003; WHO Study group 1994).

Biochemical turnover makers (BTMs) provide easily accessible information on the state of bone physiology on the shorter term. The combination of BTM and BMD has been shown to more accurately predict the risk of fracture than either marker alone, which advocates an integrated approach (Post et al. 2010). BTM can be divided into three categories: collagenous bone resorption markers, bone formation markers, and markers of osteoclast regulatory proteins (Post et al. 2010). The first are degradation products of bone collagen; most commonly used clinically are C- and N-telopeptides of collagen cross-links (CTx and NTx with existing assays in

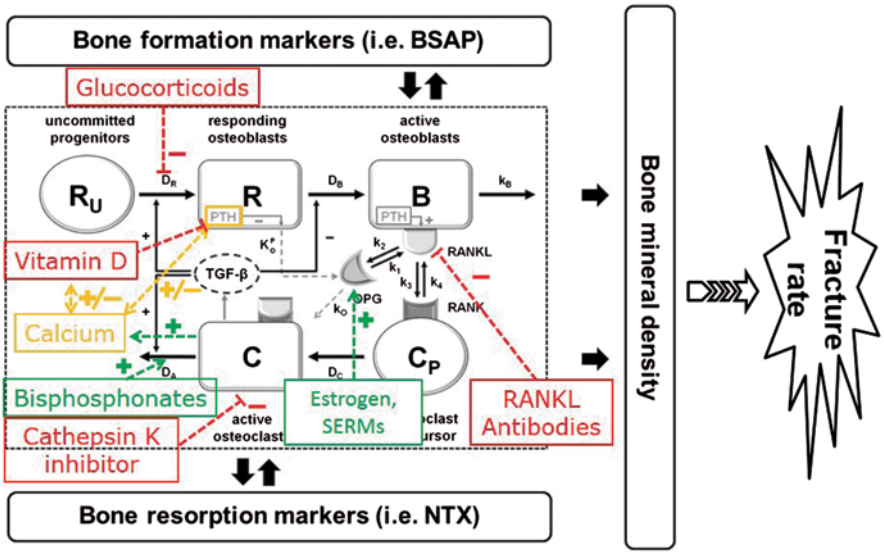

Fig. 13.2 Overview of osteoporosis mechanism of action and drug targets. (Source: Post et al. 2013, with kind permission from Springer Science+Business Media B.V.)

both serum and urine). The bone formation markers are measures of enzyme activity of osteoblasts, measures of bone protein, or measures of procollagen markers; commonly used are bone-specific alkaline phosphatase (BASP), osteocalcin (OC) and carboxy- and amino-terminal propeptide of type I collagen (procollagen type I C-terminal propeptide, PICP, and procollagen type I N-terminal propeptide, PINP). The osteoclast regulatory proteins are either markers reflecting the rate of osteoclastogenesis or the osteoclast numbers (Post et al. 2010).

13.2.3 Treatment of Osteoporosis

Various treatment paradigms have been developed that leverage the ability to influence specific components of the osteoblast–osteoclast interaction (Fig. 13.2). Treatments can be distinguished based on their differences in mechanism, site, and mode of action.

Treatments are categorized into those that (1) decrease resorption, (2) increase formation, or (3) a combination of these actions (Post et al. 2010).

13.2.3.1 Decreased Resorption

- The antiresorptive treatments include hormone replacement therapy, bisphosphonates, selective estrogen receptor modulators, and calcitonin. The bisphosphonates (e.g., alendronate, risendronate, zolendronate) act directly on the

osteoclasts' ability to resorb bone cells. This class of drugs is known to bind preferentially to calcium hydroxyapatite and can stay in the bone for years which has implications for replacement therapies.

- The RANKL inhibitor denosumab (a fully human monoclonal antibody) is a more targeted therapy, which results in osteoclast apoptosis and decreased bone resorption while avoiding some of the side effects associated with the bisphosphonate class (Baron et al. 2011). The importance of RANK–RANKL pathway was described above.
- It is recognized that estrogen, especially started soon after menopause, can maintain bone density but also leads to increased risk for blood clots, cancer, and heart disease. The selective estrogen receptor modulator (SERM) raloxifene mimics the effects of estrogen while avoiding some (but not all side effects).
- Fortical is a nasal spray that mimics the effects of calcitonin, a substance produced by the thyroid gland; it inhibits bone resorption but to a lesser degree.
- Selective and reversible inhibitors of the enzyme cathepsin K form a novel class of osteoporosis therapy. Odanacatib is currently being investigated in a phase 3 trial focused on fracture risk reduction and long-term safety. Odanacatib reduces osteoclastic bone resorption (cathepsin K-mediated) and preserves bone formation during bone remodeling (Bone et al. 2010; Langdahl et al. 2012). These actions are thought to mediate the increases in bone mineral density observed in patients with low bone mass treated with odanacatib (Bone et al. 2010; Langdahl et al. 2012). Preclinical data indicated that cathepsin K inhibition may also increase periosteal bone modeling (Cusick et al. 2011).

13.2.3.2 Increased Formation

- Injectable PTH (Forteo), which acts to preferentially increase the activity of osteoblasts. Due to the coupled mechanism of formation and resorption, the increase in formation upon continuously administered PTH leads to resorption, which presents an interesting phenomenon to be captured by modeling. The frequency of administration (daily subcutaneous injection) has largely limited the use of Forteo to severe osteoporosis patients.
- Finally, there are newer investigational medications blocking sclerostin (Amgen, Lilly, and Novartis). The full mechanism by which sclerostin causes osteoblast apoptosis is still under investigation but there is increasing evidence that sclerostin (i.e., mutations of sclerostin associated with sclerosteosis, a condition with abnormal increase in bone growth) is a promising new target for treatment of severe osteoporosis (McClung et al. 2012).

Calcium and vitamin D derivatives are important supplements that positively influence bone homeostasis and are part of the daily regimen for patients with postmenopausal osteoporosis.

Finally, there are new treatment paradigms under consideration, such as combination therapy or sequential therapy (short period with anabolic treatment, followed

by a longer maintenance with antiresorptive drug for patients with severe osteoporosis). Post et al. (2013) highlight the utility of modeling when trying to understand what the effects of such treatment regimen might be after incorporating the specific treatment effects and PK of the drug and what the effects of drug withdrawal on the bone system are.

13.3 General Pharmacometrics of Osteoporosis

Various conceptual, mathematical, statistical, and epidemiological models have been established providing insight into the biology, mechanisms, and predictive factors of osteoporosis (Post et al. 2010). In general terms, the statistical and epidemiological model provide valuable information on the correlation, predictive value, and interrelated time courses of various BTMs, BMD, and clinical outcomes and this field of research has provided valuable insight into the influences of various factors, such as age, lifestyle, and menopause, and has made it possible to evaluate, statistically confirm and compare the effects of different treatments. The conceptual mathematical models provide insight into the dynamics of the markers, the bone physiology dynamics, and are amenable for quantitative modeling purposes and are therefore the focus of this chapter. This type of modeling can be either descriptive or based on known bone physiology, i.e., more mechanistically inspired.

The benefit lies in the fact that vastly different rates of the markers or indirectly the biological system and time-variant changes in the course of the disease are incorporated. General examples of the descriptive type of modeling in osteoporosis either include single markers of bone turnover, BMD, or fracture risk or combinations of these components (Post et al. 2010).

A more integrative approach allows for a mechanism-based description of osteoporosis, and presumably other bone diseases, by explicitly including bone physiology as the underlying mechanism to which all information is linked. Various short- and long-term markers at various levels and timescales of the disease and drug action can then be combined and evaluated. The following section will describe one initial mathematical model on which two of the specific pharmacometrics of osteoporosis examples are based.

13.3.1 Bone Turnover Markers and Bone Mineral Density: Mechanism-Based Models Based on Bone Cell Interaction—Core Physiological Model

One of the first comprehensive conceptual semi-mechanistic mathematical models for bone cell interaction was published by Lemaire et al. (2004). This seminal model (Figs. 13.2 and 13.3) described pools of cells from both osteoclast and osteoblast cell lineages at different levels of maturation. Responding osteoblasts (R)

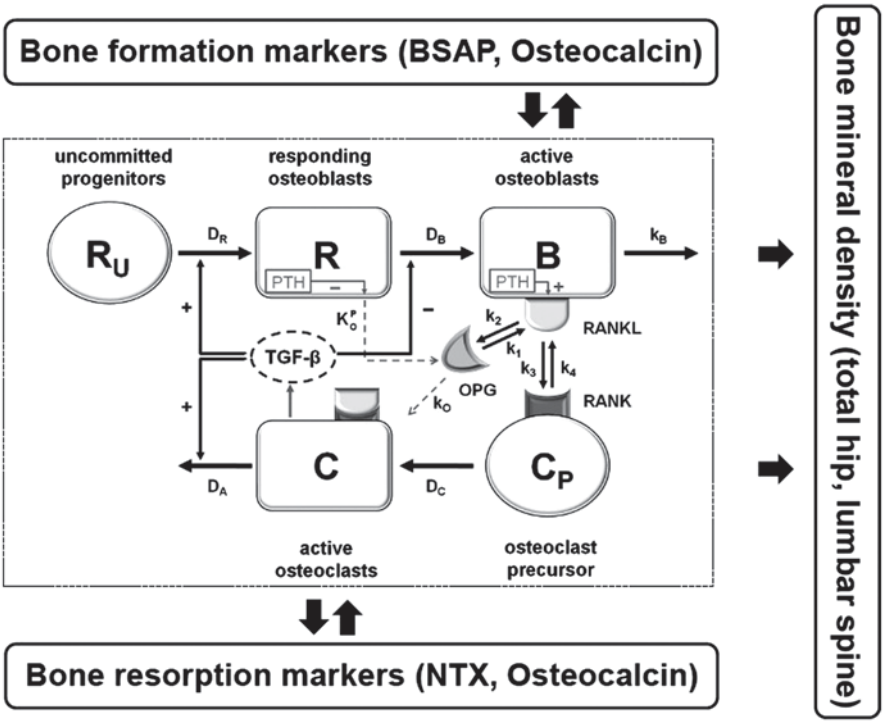

Fig. 13.3 Overview of osteoporosis mechanism of action and role of biomarkers for bone turnover and bone mineral density. (Source: Post et al. 2013, with kind permission from Springer Science+Business Media B.V.)

are recruited from a large pool of uncommitted osteoblast progenitor cells (R_u), which then differentiate into active, bone-forming osteoblasts (B). Active, bone-removing osteoclasts (C), on the other hand, are recruited from a pool of osteoclast progenitor cells (CP) upon stimulation of RANK by its ligand (RANKL). This latter process is inhibited by OPG, a soluble decoy receptor for RANKL that is formed by the responding osteoblasts. Other approaches have been published also taking into account a mathematical description of bone physiology (Komarova et al. 2003; Rattanakul et al. 2003; Moroz et al. 2006; Wimpenny and Moroz 2007; Earp et al. 2008; Pivonka et al. 2008; Peterson and Riggs 2010; Pivonka and Komarova 2010; Marathe et al. 2011; Zumsande et al. 2011; Riggs et al. 2012).

In addition, the model captures some of the postulated effects of TGF-β and PTH. In particular, TGF-β which is released from bone by active osteoclasts during bone resorption (1) stimulates the recruitment of responding osteoblasts, (2) inhibits the differentiation of responding osteoblasts into active osteoblasts, and (3) stimulates the apoptosis of active osteoclasts. On the other hand, PTH, through binding to its receptors expressed by osteoblasts, stimulates the expression of RANKL and

suppresses the secretion of OPG; we need to mention that the model by Lemaire et al. (2004) only captures the resorptive effects of PTH.

Mathematically, this translates into the following set of differential equations:

$$\begin{cases} \dfrac{dR}{dt} = D_R \pi_C - \dfrac{D_B}{\pi_C} R \\ \dfrac{dB}{dt} = \dfrac{D_B}{\pi_C} R - k_B B \\ \dfrac{dC}{dt} = D_C \pi_L(R,B) - D_A \pi_C C \end{cases}$$

in which *R, B,* and *C* denote the concentrations of responding osteoblasts, active osteoblasts, and osteoclasts, respectively, D_R, D_B, D_C represents the differentiation rates of osteoblast progenitors, responding osteoblasts, and osteoclast precursors, k_B the apoptosis rate of active osteoblasts and D_A the osteoclast apoptosis rate due to TGF-β. Finally, π_C and π_L (*R, B*) denote the TGF-β receptor occupancy and the RANK receptor occupancy. The expressions for these parameters, as well as the detailed derivations can be found in Lemaire et al. (2004).

Various extensions to the model of Lemaire were made, including explicitly incorporating calcium dynamics by Peterson and Riggs et al. and describing bone dynamics in rheumatoid arthritis by Earl et al. (Lemaire et al. 2004; Riggs et al. 2012; Earp et al. 2008; Peterson and Riggs 2010). It is worth noting that elements of this approach were also presented in Marathe et al. (2011) where the authors combined the original Lemaire et al. (2004) model and linked the number of osteoclasts to biomarkers of resorption in order to characterize the effect of the RANKL inhibitor denosumab but in multiple myeloma patients, a cancer accompanied by bone lesions. This Lemaire model forms the basis for two specific applied examples described below.

13.4 Specific Applied Examples of Pharmacometrics in Osteoporosis

13.4.1 Mechanism-Based Models of Bone Turnover Markers and Bone Mineral Density

13.4.1.1 Reduced Core Physiological Model Describing Five Biomarkers in a Population Approach

In work from Post (2009) and Schmidt et al. (2011) a way was proposed to reduce the system by Lemaire to one describing the dynamics of only osteoblasts (B) and osteoclasts (C), such that the dynamics of the system are kept and the different timescales in the system can be described as explained below:

$$\begin{cases} \dfrac{dB}{dt} = D_R \pi_C(C) - k_B B \\ \dfrac{dC}{dt} = D_C \dfrac{\alpha B}{1+\beta R} - D_A \pi_C(C) C \end{cases},$$

with the function $R = R(C)$ defined by

$$R(C) \underline{\underline{\text{def}}} \frac{D_B}{R_R} \pi_C^2(C).$$

In applying this reduced system to clinical data, Post et al. connected the dimensionless cell concentrations to the corresponding biomarkers of turnover and also to the bone mineral density measures (Post et al. 2010; Post 2009). This application of the reduced core model to clinical data was done via the population approach. To be able to include disease and treatment-related changes and to include multiple markers, the changes in B and C were related to their respective baseline values B_0 and C_0, resulting in a dimensionless system:

$$y = \frac{B}{B_0} \quad \text{and} \quad z = \frac{C}{C_0},$$

such that

$$\begin{cases} \dfrac{dy}{dt} = k_B \{\sigma(z) - y\} \\ \dfrac{dz}{dt} = D_A \pi_z(1) \left\{ \dfrac{1+b}{1+bf(t)\sigma^2(z)} y \cdot P_{Ca} \cdot E(T_i) - \sigma(z) z \right\} \end{cases},$$

$$\sigma(z) = \frac{\pi_z(z)}{\pi_z(1)},$$

where P_{Ca} and $E(T_i)$ are treatment effects of calcium and tibolone, respectively and $f(t)$ presents the disease progression related to a decline in estrogen during menopause.

This resulted in the ability to include bone turnover markers describing resorption in this system through a functional relationship of the form

$$X = X_0 p^{\rho X},$$

where the marker is linked to the dimensionless activity p, which is either z for resorption or y for formation. Biomarkers of formation (i.e., BSAP) are linked to osteoblast activity. Osteocalcin (OC) is linked to both y and z because it is produced by osteoblasts incorporated into bone and thereafter, released from bone during another resorption cycle. Markers of resorption (i.e., NTx) are linked to osteoclast activity z.

The site-specific (lumbar spine and total hip) BMD is modeled using the ratio $S = z / y$ in the following functional form:

$$\frac{dBMD}{dt} = k(1 - S^{\rho BMD}),$$

where S presents the ratio between the activities of resorption and formation, k is the turnover rate of BMD, and ρBMD is a transduction parameter relating changes in bone cells to BMD.

In this form, the reduced Lemaire model can be applied to describe the dynamics of the osteoblast/clast system under conditions of drug treatment, as it has enough granularity to capture various driving events/conditions, namely disease progression (trajectory relative to the start-of-menopause), start-of-treatment, achievement of systems (disease) steady-state, and end-of-treatment.

The system resulted in the ability to describe the effects of treatment based on clinical data within a population approach including data of NTx, BSAP, OC as bone turnover markers and lumbar spine and total hip BMD. Figure 13.4 gives the description of the model to the data by means of a predictive check (selected doses and data).

The quantitative description of the clinical biomarker data by this reduced mechanism-based core model enables the evaluation of the drug treatment effects on the various short- and long-term biomarkers. Once further developed and qualified with different biomarkers and treatments, this approach may be used to predict changes in long-term biomarkers based on short-term biomarker response. Ultimately, this model should be linked to other measures of bone strength and ultimately fracture risk (Figs. 13.2 and 13.3).

Below is an example (Fig. 13.5) which shows how to translate the estimated parameters to the course of the changes in relative osteoblasts ($z = B/B_0$) and osteoclasts ($y = C/C_0$) where the state of the RANK–RANKL–OPG system changes with each event and achieves different relative osteoblast and osteoclast turnover (z, y space) starting from healthy state (1,1). This gives a means to visualize the various changes in a two-dimensional plot. Each change in the system is defined as an orbit. The green orbit represents natural disease progression, while the blue orbit represents the addition of calcium treatment (aka placebo orbit). The solid red orbit describes the transition upon infinite tibolone treatment and the dashed red orbit represents the resetting upon treatment discontinuation after 1000 days.

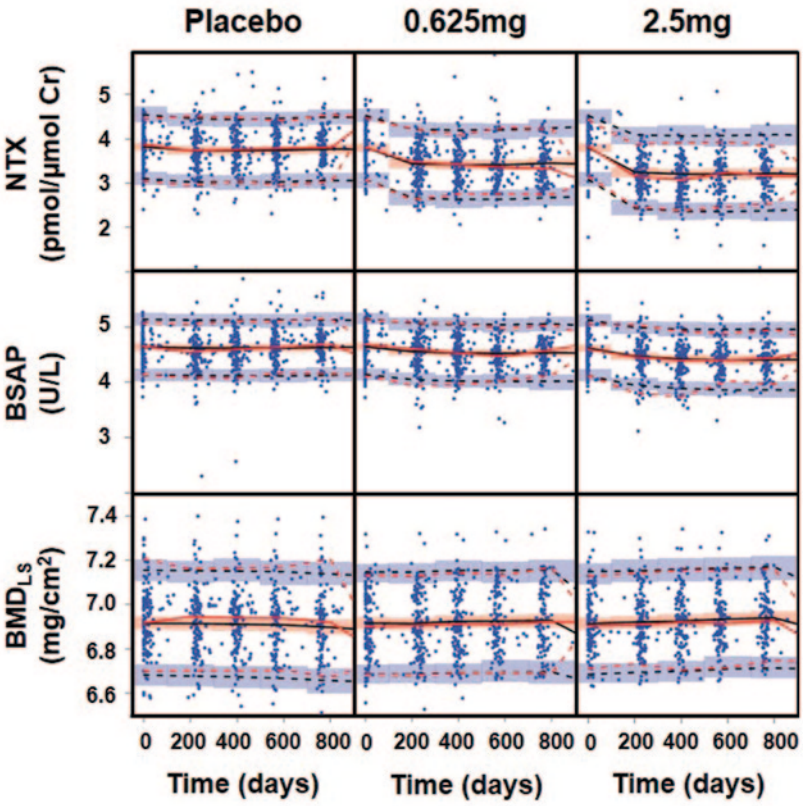

Fig. 13.4 Visual predictive check of the marker NTx, BSAP, and lumbar spine bone mineral density (*BMD*) (Post et al. 2013, with kind permission from Springer Science+Business Media B.V.). The *blue dots* represent the natural logarithms of the observations. The 5th, 50th, and 95th percentiles of the observations are presented by the *red dashed* and *red solid lines*. The 5th, 50th, and 95th percentiles of the simulated data are presented by the *black dashed* and *black solid lines.* The confidence intervals for the simulated data's 5th, 50th, and 95th percentiles are presented by the *blue, red,* and *blue area,* respectively

13.4.1.2 Extended Physiological Model in a Systems Biology Approach

Another mathematical model of dynamics of bone remodeling based on available physiological observations, specifically in the context of the mechanisms of action of available osteoporosis treatments was recently developed by Mehta et al. (2012). This work builds on prior approaches of Peterson and Riggs et al. (Peterson and Riggs 2010; Riggs et al. 2012), Marathe et al. (2008), Lemaire et al. (2004), and Komarova et al. (2003) and is novel in how it integrates known interventions in osteoporosis disease mitigation with an explicit connection to existing therapies. It also differs in the way it approaches the formulation of the model and how it retains the conceptual clarity of the relationships between the state variables and the model

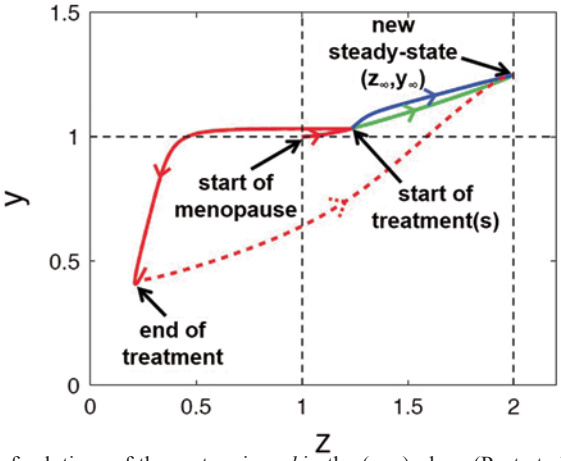

Fig. 13.5 Orbits of solutions of the system in *red* in the (z, y)-plane (Post et al. 2013, with kind permission from Springer Science+Business Media B.V.). The *green curve* is the orbit in the absence of any treatment, the *blue curve* is the orbit in the presence of calcium treatment alone and the *red curves* are orbits caused by calcium and tibolone treatment combined. The *solid red curve* is the orbit during continuous tibolone treatment, the *dashed curve* the continuation after termination and washout at $t = 1000$ days

parameters, while being parsimonious in and of itself. In contrast to the work described in the previous section, this is a deterministic and not a population model.

The extended physiological model is based on the osteoclast/osteoblast signaling model of Lemaire (Lemaire et al. 2004), the calcium sensing model of Cabal et al. (2013), a model of TGF-β signaling, and cathepsin-K (Cat K) enzymatic bone degradation, a signaling protein model of the osteoblast apoptosis regulation as suggested by Bellido (Bellido et al. 2003). The model, in the form of ordinary differential equations (ODEs), quantifies the relationships between the key molecular pathways governing bone remodeling, and links, via reasonable assumptions, the cell and molecular concentrations to the biomarkers measured in the laboratory (P1NP, CTx, and BMD). The model equations follow the interactions between the state variables of the system which are often chemical reactions following either mass-action kinetics or nonlinear hill function rates for enzymatic systems wherein the intermediate steps are excluded to preserve model simplicity.

The extended physiological model results are consistent with the known effects of PTH, bisphosphonates, and anti-RANKL treatment regimens on the bone remodeling process. Figures 13.6, 13.7 and 13.8 show the model behavior in response to the known treatment strategies for osteoporosis. Notably, it is able to predict the delicate nature of bone build up in response to PTH treatment, and the fact that the same unified model can predict treatments which differ in their mechanism of action (bisphosphonates, rPTH, and anti-RANKLs). The model allows the comparison of osteoporosis therapies already on the market and new, innovative therapies in different stages of development and lends itself as a tool to evaluate potential new therapies under various administration protocols.

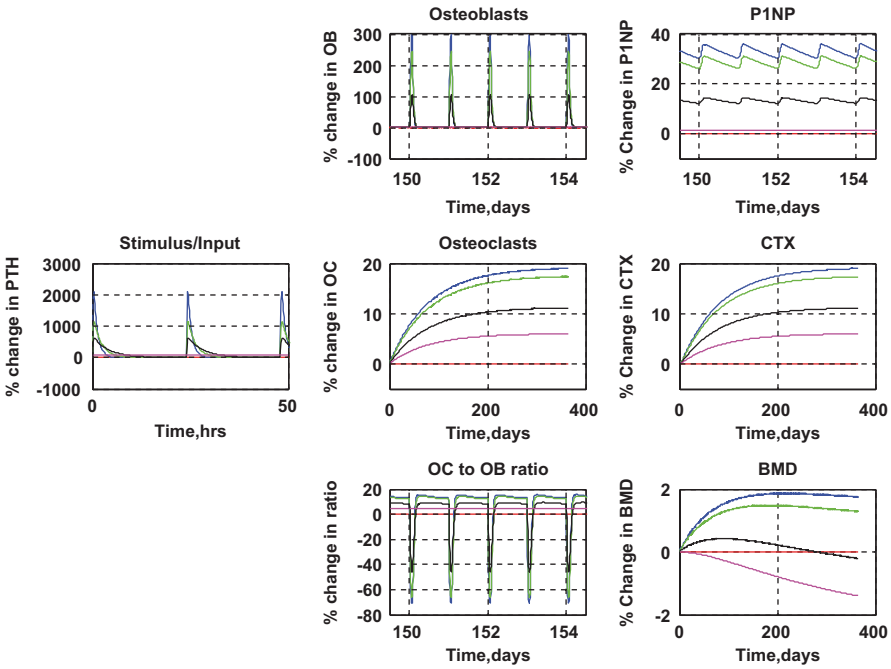

Fig. 13.6 Effect of parathyroid hormone (PTH) treatment: Simulated effect of pulse shape in the extended physiological model for five different administration profiles of PTH (*left plot:* from placebo to increasing sharper rises and declines). *Middle panel:* The shape of the PTH pulse has a nonintuitive impact on osteoblasts and progressively on bone mineral density (BMD; *right panel*). The model prediction of a sharper PTH infusion yielding improvement in BMD is consistent with results from Cosman et al. (2010). The different *colored lines* here indicate different PTH administration profiles. The total area under the curve for each of the profiles is kept similar (apart from placebo), while the pharmacokinetic profile is varied: *red*—placebo (no PTH); *magenta*—continuous administration (infusion) of PTH directly into plasma; *black*—PTH administration with slow clearance (similar to PTH secretion in response to orally administered calcilytic drugs); *green*—subcutaneous injection of PTH; and *blue*—transdermal delivery of PTH using micro-needles as per Cosman et al. (2010)

13.4.2 Finite Element Analysis

As mentioned above, the current clinical standard for diagnosing osteoporosis and assessing the risk of fracture and treatment effects is dual energy x-ray absorptiometry (DXA), which is used to measure areal BMD (aBMD) at the spine and hip. The performance of DXA-aBMD as a diagnostic, as well as a predictor of bone strength and treatment intervention are well documented (Cummings et al. 2002; Pistoia et al. 2002; Cefalu 2004; Delmas and Seeman 2004; Schuit et al. 2004; Seeman 2007). As a two-dimensional projection of three-dimensional structure, DXA-aBMD lacks the ability to interrogate the macro- and micro-architectural features of the bone that has a direct impact on its strength and ability to withstand specific

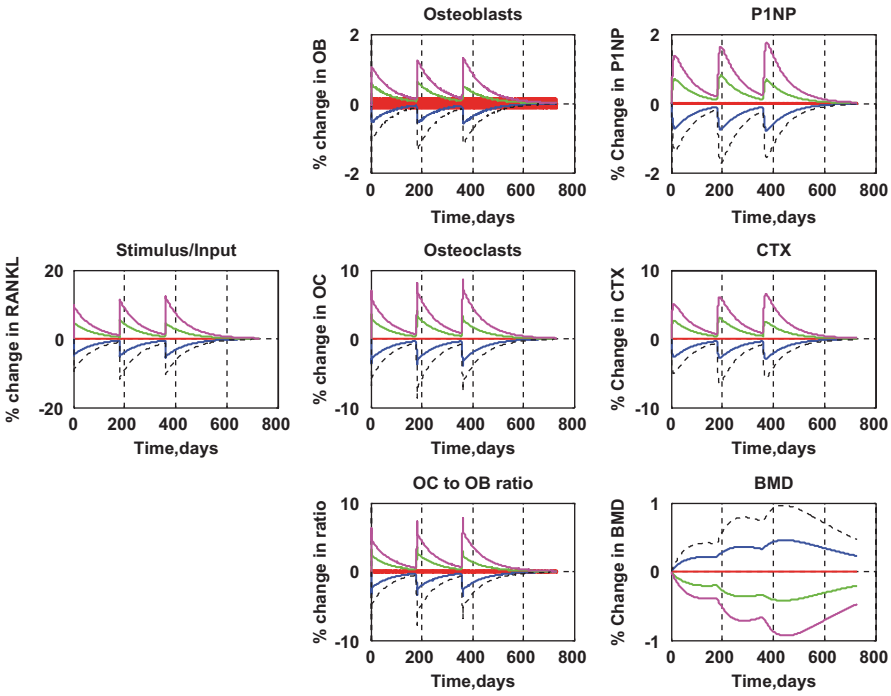

Fig. 13.7 Simulations of changing receptor activator of nuclear factor κ B ligand (RANKL) concentrations in the extended physiological model were performed as a proxy for treatment with anti-RANKL molecules like denosumab. Decreasing concentrations of RANKL resulted in a dose-dependent increase in bone mineral density (BMD), consistent with the findings of Marathe et al. (2008). The model predicts slow return to the baseline following treatment cessation after a year (three doses, at every 6 months)

loading situations. The shape and structure of bone at a macro- and micro-architectural level provide additional, independent information to better predict fracture risk, assess response to treatment, and potentially differentiate new therapies from standard of care (Homminga et al. 2002, 2004).

Computationally, this has been addressed by the use of finite element (FE) methodology, a numerical discretization procedure that has been extensively used for several decades in science and engineering to get good approximate solution of complex mathematical problems (Zienkiewicz and Taylor 2002, 2005). FEA is the most used computational analysis technique in the world today to solve solid mechanics problems and bone mechanics is no exception. Three dimensional (3D) images of bone are subdivided into a finite set of hexahedrons and tetrahedrons called elements. Applied to all nodes that form the elements, Newton's second law of motion takes the following general form:

$$\rho \frac{\partial^2 u_i}{\partial t^2} = \frac{\partial \sigma_{i,x}}{\partial x} + \frac{\partial \sigma_{i,y}}{\partial y} + \frac{\partial \sigma_{i,z}}{\partial z} + F_i$$

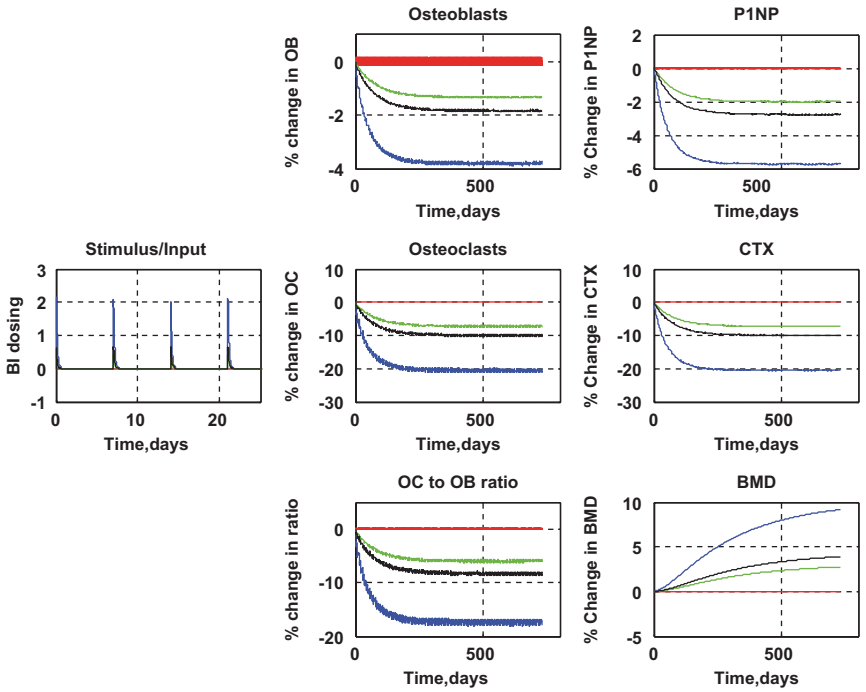

Fig. 13.8 Extended physiological model predictions of weekly dosing of bisphosphonates demonstrated a dose-dependent decrease in bone mineral density (BMD), bone biomarkers, and bone remodeling activity, which is consistent with known effects of bisphosphonates

where $i = x, y, z$ are the spatial coordinates, u_i are the coordinates of the displacement vector, F_i are the coordinates of the external body forces applied. Hooke's law yields a six-dimensional stress–strain linear relationship:

$$\begin{bmatrix} \sigma_{xx} \\ \sigma_{yy} \\ \sigma_{zz} \\ \sigma_{xy} \\ \sigma_{yz} \\ \sigma_{zx} \end{bmatrix} = \begin{pmatrix} C_{11} & C_{12} & C_{13} & C_{14} & C_{15} & C_{16} \\ C_{21} & C_{22} & C_{23} & C_{24} & C_{25} & C_{26} \\ C_{31} & C_{32} & C_{33} & C_{34} & C_{35} & C_{36} \\ C_{41} & C_{42} & C_{43} & C_{44} & C_{45} & C_{46} \\ C_{51} & C_{52} & C_{53} & C_{54} & C_{55} & C_{56} \\ C_{61} & C_{62} & C_{63} & C_{64} & C_{65} & C_{66} \end{pmatrix} \begin{bmatrix} \varepsilon_{xx} \\ \varepsilon_{yy} \\ \varepsilon_{zz} \\ \varepsilon_{xy} \\ \varepsilon_{yz} \\ \varepsilon_{zx} \end{bmatrix}$$

Various forms of this functional relationship are possible depending on the resolution of the bone 3D image available, the loads (boundary conditions) applied to the bone, and the material properties used (Young's modulus, Poisson's ratio, etc). For example, under the assumption that bone is a homogeneous isotropic material, the

above 36 coefficients are reduced to two, the Young's modulus (E) and the Poisson's ratio (g):

$$\begin{bmatrix}\sigma_{xx}\\ \sigma_{yy}\\ \sigma_{zz}\\ \sigma_{xy}\\ \sigma_{yz}\\ \sigma_{zx}\end{bmatrix} = \frac{E}{(1+\gamma)(1-2\gamma)}\begin{pmatrix}1-\gamma & \gamma & \gamma & 0 & 0 & 0\\ \gamma & 1-\gamma & \gamma & 0 & 0 & 0\\ \gamma & \gamma & 1-\gamma & 0 & 0 & 0\\ 0 & 0 & 0 & 1-2\gamma & 0 & 0\\ 0 & 0 & 0 & 0 & 1-2\gamma & 0\\ 0 & 0 & 0 & 0 & 0 & 1-2\gamma\end{pmatrix}\begin{bmatrix}\varepsilon_{xx}\\ \varepsilon_{yy}\\ \varepsilon_{zz}\\ \varepsilon_{xy}\\ \varepsilon_{yz}\\ \varepsilon_{zx}\end{bmatrix}$$

When the FE-models are generated from high-resolution micro-computed tomography (μCT) images of trabecular samples, the models accurately capture the complex morphological architecture of the structures and can be used to estimate the bone hard tissue Young's modulus (Zienkiewicz and Taylor 2002, 2005; Guo 2001). The FE model allows for computing the apparent stiffness. Physical compression experiments of trabecular bone samples provide an assessment of the experimental stiffness. The true hard tissue Young's modulus is then estimated from the ratio of the experimental and FEA-based stiffness estimates.

Animal models are a vital component of the drug discovery process and they provide an excellent opportunity to test FEA methodologies in disease models, acquiring advanced information to help design and execute clinical studies. An example of how the ovariectomized nonhuman primates osteoporosis model was used to qualify the validity of high-resolution peripheral quantitative computed tomog-

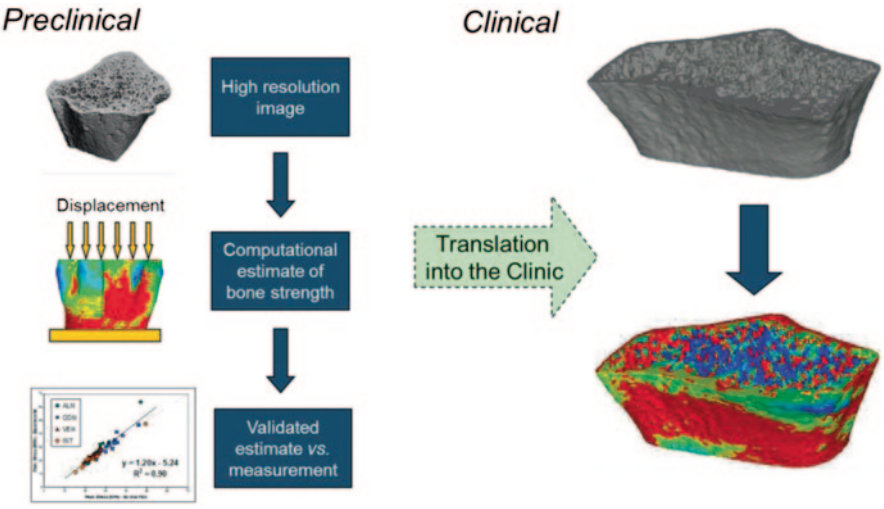

Fig. 13.9 FEA qualification and translation roadmap

raphy (HR-pQCT) based FEA estimates of bone strength was presented in (Jayakar et al. 2012). The roadmap from *ex vivo* preclinical FEA qualification to in vivo clinical translation is illustrated in Fig. 13.9.

Several clinical publications (Boutroy et al. 2008; Burghardt et al. 2010, 2011; Macdonald et al. 2011) have shown that high-resolution peripheral quantitative computer tomography (HR-pQCT)-based FEA-estimated bone strength provides information about skeletal fragility and fracture risk not assessed by BMD. A unique advantage of the preclinical FEA-estimated bone strength lies in its ability to enable in-vivo longitudinal estimates of bone strength that can be validated at the end of the study. This, in turn, provides the necessary level of confidence for the FEA predictions for the clinical estimates.

On the 3D clinical imaging technology resolution scale, the next class of lower resolution bone imaging tools is provided by QCT scanners, which can be used to scan whole bones at skeletal central sites, which are the most relevant for osteoporosis (femur and vertebra), as compared to HR-pQCT. With the lower resolution (~500 μm), the FE models generated from those images are not capable of resolving the trabecular microarchitecture at the level of a single trabecular structure. The heterogeneity of the trabecular bone is represented in the QCT-based FEA assigning different elastic properties to the different voxels of the image in correspondence to the QCT density of the given voxel (Morgan and Keaveny 2001; Crawford et al. 2003; Morgan et al. 2003).

FE models enable the testing of bone specimens in any configuration and loading conditions in silico, thus facilitating the exploration of potential treatment specific

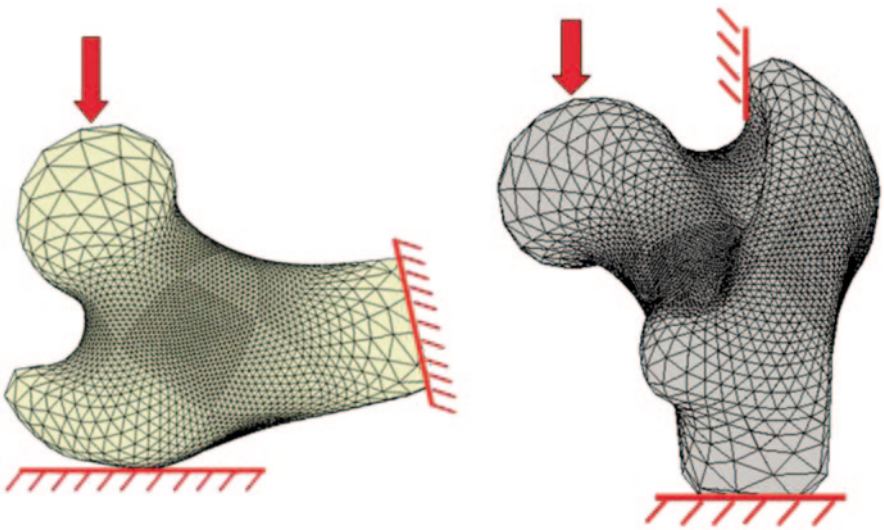

Fig. 13.10 FE-mesh of two proximal femurs under two different loading and boundary conditions

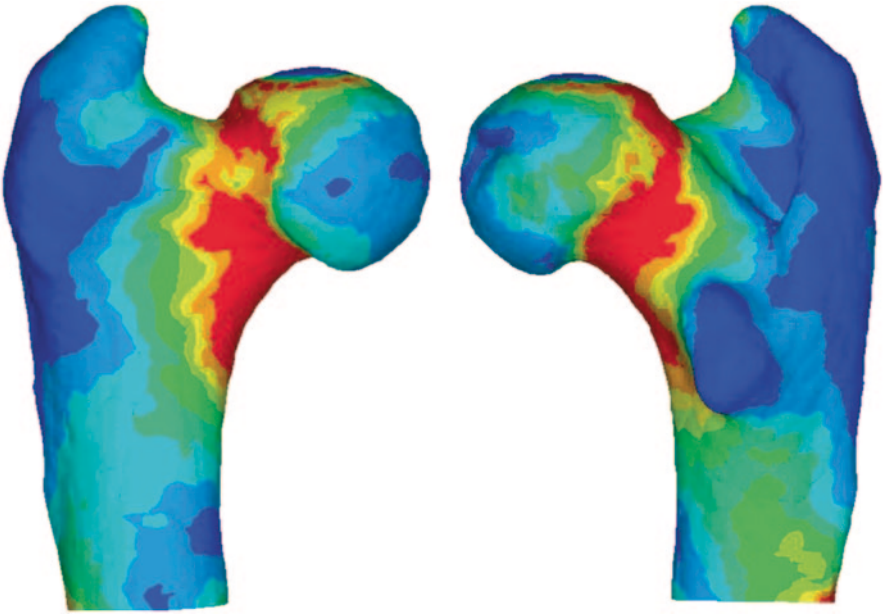

Fig. 13.11 Two different views of a rhesus monkey's right proximal femur von Mises stress spatial distribution (*red* being the highest stress and *blue* being the lowest stress)

effects. Proximal femurs could, for example, be tested in fall loading or neck shear configurations as illustrated in Fig. 13.10.

Another unique feature of the FEA is its ability to accurately estimate the spatial stress and strain distribution for any given load. This facilitates the analysis of subject specific biomechanical differences even in the cases where subjects have the same integral BMD at a given skeletal site. Some studies have been conducted to assess the ability of FE models to predict the location and type of clinically relevant fractures (Lotz et al. 1991a, b; Keyak et al. 2001). Figure 13.11 shows the spatial stress distribution on the proximal femur of a rhesus monkey subjected to a neck shear test. The location of the high stresses, in red, shows the places where the fracture is most likely to occur.

In the past decade, thanks to improvements in imaging tools and their availability, in vivo FEA have become a frequently used biomarker in phase III clinical trials for new osteoporosis therapies (Keaveny et al. 2007, 2008; Brixen et al. 2013). FEA has enabled clinical longitudinal measurements of bone strength and provided unique clinical insight into the biomechanical effects of new osteoporosis therapies.

13.5 Conclusions

During the past decade, a myriad of disease models for osteoporosis has been developed and applied to drug development questions. The current toolbox for the pharmacometricians provides state-of the-art modeling of the bone model unit including the osteoclast and osteoblast dynamics and endogenous modulator molecules, predictive biomarkers as NTx, uNTx, and bone mineral density, risk of fracture and bone strength. As a result, our understanding of mechanisms of action and (developmental) drug characteristics in osteoporosis has progressed significantly. In the future, further integration of approaches and end points will provide higher and earlier predictiveness from biomarkers to fractures and deliver on the promise of model-based drug development in osteoporosis, where the model is continuously developed in parallel with the drug. The ultimate goal is to integrate all sources of information to obtain a comprehensive description of the pathophysiology of osteoporosis, including treatment and disease. This enables the description of various treatments and their impact on clinical outcome; enabling the prediction of short-term to long-term outcome on fracture risk.

Summary and Key Aspects of the Chapter

- Due to statistical requirements and the slow progression of osteoporosis, large clinical trials with long duration are required to establish a beneficial effect of new treatments on the reduction of fracture risk.
- Over the past decade, different types of mechanism-based models have had an increasing impact on drug development.
  - Various conceptual models for bone physiology and the effects of therapies have been proposed. Data included in osteoporosis models range fromPK of (novel) drugs, PD biomarkers of various time scales (peptides indicative of bone-turnover, bone mineral density), bone strength, as well as the actual clinical outcome, namely fracture rates at various sites in the skeleton.
- Published PK–PD-disease models of osteoporosis have varying degrees of biological complexity ranging from purely descriptive of disease to detailed systems model spanning various spatial scales, as well as mechanistic models of bone strength.
  - These models have been used to describe data from clinical trials, simulate new trial designs with novel mechanisms of action, and simulate combination treatments and how these clinical trials predict for real-life settings.
- A more integrative approach allows for a mechanism-based description of osteoporosis, and presumably other bone diseases, by explicitly including bone physiology as the underlying mechanism to which all information is linked. Various short- and long-term markers at various levels and timescales of the disease and drug action can then be combined and evaluated.
- Specific applied examples of pharmacometrics in osteoporosis concern mechanism-based models based on bone cell interaction—i.e., a core physiological model.

  - Mechanism-based models of bone turnover markers and BMD.
    - Reduced core physiological model describing five biomarkers in a population approach (reduced Lemaire core model including bone turnover markers and bone mineral density).
      - Quantitative system able to describe the effects of treatment based on various short- and long-term biomarker clinical data within a population approach (treatment and disease progression).
    - Extended physiological model in a systems biology approach (extensions to the Lemaire core model).
      - Mathematical model of dynamics of bone remodeling based on available physiological observations, specifically in the context of the mechanisms of action of available osteoporosis treatments.
  - Finite element analysis
    - Describing the shape and structure of bone at a macro- and micro-architectural level provides additional, independent information to better predict fracture risk.
- The current toolbox for the pharmacometricians provides state-of the-art modeling of the bone model unit including the osteoclast and osteoblast dynamics and endogenous modulator molecules, predictive biomarkers as NTx, uNTx, and bone mineral density, risk of fracture and bone strength.
- In the future, further integration of approaches and end points will provide higher and earlier predictiveness from biomarkers to fractures and deliver on the promise of model-based drug development in osteoporosis, where the model is continuously developed in parallel with the drug. The ultimate goal is to integrate all sources of information to obtain a comprehensive description of the pathophysiology of osteoporosis, including treatment and disease. This enables the description of various treatments and their impact on clinical outcome; enabling the prediction of short-term to long-term outcome on fracture risk.

References

Aubin JE, Bonnelye E (2000) Osteoprotegerin and its ligand: a new paradigm for regulation of osteoclastogenesis and bone resorption. Osteoporos Int 11(11):905–913 (Retrieved from PM:11193242)

Baron R, Ferrari S, Russell RG (2011) Denosumab and bisphosphonates: different mechanisms of action and effects. Bone 48(4):677–692 (Retrieved from PM:21145999)

Bellido T, Ali AA, Plotkin LI, Fu Q, Gubrij I, Roberson PK et al. (2003) Proteasomal degradation of Runx2 shortens parathyroid hormone-induced anti-apoptotic signaling in osteoblasts. A putative explanation for why intermittent administration is needed for bone anabolism. J Biol Chem 278(50):50259–50272 (Retrieved from PM:14523023)

Bone HG, McClung MR, Roux C, Recker RR, Eisman JA, Verbruggen N et al (2010) Odanacatib, a cathepsin-K inhibitor for osteoporosis: a two-year study in postmenopausal women with low bone density. J Bone Miner Res 25(5):937–947 (Retrieved from PM:19874198)

Boutroy S, Van Rietbergen B, Sornay-Rendu E, Munoz F, Bouxsein ML, Delmas PD (2008) Finite element analysis based on in vivo HR-pQCT images of the distal radius is associated with

wrist fracture in postmenopausal women. J Bone Miner Res 23(3):392–399 (Retrieved from PM:17997712)
Bouxsein ML, Seeman E (2009) Quantifying the material and structural determinants of bone strength. Best Pract Res Clin Rheumatol 23(6):741–753 (Retrieved from PM:19945686)
Boyle WJ, Simonet WS, Lacey DL (2003) Osteoclast differentiation and activation. Nature 423(6937):337–342 (Retrieved from PM:12748652)
Brixen K, Chapurlat R, Cheung AM, Keaveny TM, Fuerst T, Engelke K et al (2013) Bone density, turnover, and estimated strength in postmenopausal women treated with odanacatib: a randomized trial. J Clin Endocrinol Metab 98(2):571–580 (Retrieved from PM:23337728)
Burge R, Dawson-Hughes B, Solomon DH, Wong JB, King A, Tosteson A (2007) Incidence and economic burden of osteoporosis-related fractures in the United States, 2005–2025. J Bone Miner Res 22(3):465–475 (Retrieved from PM:17144789)
Burghardt AJ, Kazakia GJ, Sode M, de Papp AE, Link TM, Majumdar S (2010) A longitudinal HR-pQCT study of alendronate treatment in postmenopausal women with low bone density: relations among density, cortical and trabecular microarchitecture, biomechanics, and bone turnover. J Bone Miner Res 25(12):2282–2295 (Retrieved from PM:20564242)
Burghardt AJ, Link TM, Majumdar S (2011) High-resolution computed tomography for clinical imaging of bone microarchitecture. Clin Orthop Relat Res 469(8):2179–2193 (Retrieved from PM:21344275)
Cabal A, Mehta K, Ross DS, Shrestha RP, Comisar W, Denker A et al (2013) A semi-mechanistic model of the time-course of release of PTH into plasma following administration of the calcilytic JTT-305/MK-5442 in humans. J Bone Miner Res. 2013 Aug;28(8):1830-6. doi: 10.1002/jbmr.1900. (Retrieved from PM:23436611)
Cefalu CA (2004) Is bone mineral density predictive of fracture risk reduction? Curr Med Res Opin 20(3):341–349 (Retrieved from PM:15025843)
Cosman F, Lane NE, Bolognese MA, Zanchetta JR, Garcia-Hernandez PA, Sees K et al (2010) Effect of transdermal teriparatide administration on bone mineral density in postmenopausal women. J Clin Endocrinol Metab 95(1):151–158 (Retrieved from PM:19858319)
Crawford RP, Cann CE, Keaveny TM (2003) Finite element models predict in vitro vertebral body compressive strength better than quantitative computed tomography. Bone 33(4):744–750 (Retrieved from PM:14555280)
Cummings SR, Karpf DB, Harris F, Genant HK, Ensrud K, LaCroix AZ et al (2002) Improvement in spine bone density and reduction in risk of vertebral fractures during treatment with antiresorptive drugs. Am J Med 112(4):281–289 (Retrieved from PM:11893367)
Cusick T, Chen CM, Pennypacker BL, Pickarski M, Kimmel D, Scott BB et al (2011) Odanacatib treatment increases hip bone mass and cortical thickness by preserving endocortical bone formation and stimulating periosteal bon formation in the ovariectomized adult rhesus monkey. J Bone Miner Res 27(3):524–537
Delmas PD, Seeman E (2004) Changes in bone mineral density explain little of the reduction in vertebral or nonvertebral fracture risk with anti-resorptive therapy. Bone 34(4):599–604 (Retrieved from PM:15050889)
Earp JC, Dubois DC, Molano DS, Pyszczynski NA, Keller CE, Almon RR et al (2008) Modeling corticosteroid effects in a rat model of rheumatoid arthritis I: mechanistic disease progression model for the time course of collagen-induced arthritis in Lewis rats. J Pharmacol Exp Ther 326(2):532–545 (Retrieved from PM:18448865)
Guo E (2001) Mechanical properties of cortical bone and cancellous bone tissue. In: Cowin S (eds) Bone biomechanics handbook, 2nd edn. CRC Press LLC, Oxford
Homminga J, McCreadie BR, Ciarelli TE, Weinans H, Goldstein SA, Huiskes R (2002) Cancellous bone mechanical properties from normals and patients with hip fractures differ on the structure level, not on the bone hard tissue level. Bone 30(5):759–764 (Retrieved from PM:11996916)
Homminga J, Van Rietbergen B, Lochmuller EM, Weinans H, Eckstein F, Huiskes, R (2004) The osteoporotic vertebral structure is well adapted to the loads of daily life, but not to infrequent "error" loads. Bone 34(3):510–516 (Retrieved from PM:15003798)
Jayakar RY, Cabal A, Szumiloski J, Sardesai S, Phillips EA, Laib A et al (2012) Evaluation of high-resolution peripheral quantitative computed tomography, finite element analysis and biome-

chanical testing in a pre-clinical model of osteoporosis: a study with odanacatib treatment in the ovariectomized adult rhesus monkey. Bone 50(6):1379–1388 (Retrieved from PM:22469953)
Keaveny TM, Donley DW, Hoffmann PF, Mitlak BH, Glass EV, San Martin JA (2007) Effects of teriparatide and alendronate on vertebral strength as assessed by finite element modeling of QCT scans in women with osteoporosis. J Bone Miner Res 22(1):149–157 (Retrieved from PM:17042738)
Keaveny TM, Hoffmann PF, Singh M, Palermo L, Bilezikian JP, Greenspan SL et al (2008) Femoral bone strength and its relation to cortical and trabecular changes after treatment with PTH, alendronate, and their combination as assessed by finite element analysis of quantitative CT scans. J Bone Miner Res 23(12):1974–1982 (Retrieved from PM:18684084)
Keyak JH, Rossi SA, Jones KA, Les CM, Skinner HB (2001) Prediction of fracture location in the proximal femur using finite element models. Med Eng Phys 23(9):657–664 (Retrieved from PM:11755810)
Komarova SV, Smith RJ, Dixon SJ, Sims SM, Wahl LM (2003) Mathematical model predicts a critical role for osteoclast autocrine regulation in the control of bone remodeling. Bone 33(2):206–215 (Retrieved from PM:14499354)
Langdahl B, Binkley N, Bone H, Gilchrist N, Resch H, Rodriguez PJ et al (2012) Odanacatib in the treatment of postmenopausal women with low bone mineral density: five years of continued therapy in a phase 2 study. J Bone Miner Res 27(11):2251–2258 (Retrieved from PM:22777865)
Lemaire V, Tobin FL, Greller LD, Cho CR, Suva LJ (2004) Modeling the interactions between osteoblast and osteoclast activities in bone remodeling. J Theor Biol 229(3):293–309 (Retrieved from PM:15234198)
Lotinun S, Kiviranta R, Matsubara T, Alzate JA, Neff L, Luth A et al (2013) Osteoclast-specific cathepsin K deletion stimulates S1P-dependent bone formation. J Clin Invest. 2013 Feb 1;123(2):666-81. doi: 10.1172/JCI64840. Epub 2013 Jan 16. (Retrieved from PM:23321671)
Lotz JC, Cheal EJ, Hayes WC (1991a) Fracture prediction for the proximal femur using finite element models: part I–linear analysis. J Biomech Eng 113(4):353–360 (Retrieved from PM:1762430)
Lotz JC, Cheal EJ, Hayes WC (1991b) Fracture prediction for the proximal femur using finite element models: part II–nonlinear analysis. J Biomech Eng 113(4):361–365 (Retrieved from PM:1762431)
Macdonald HM, Nishiyama KK, Kang J, Hanley DA, Boyd SK (2011) Age-related patterns of trabecular and cortical bone loss differ between sexes and skeletal sites: a population-based HR-pQCT study. J Bone Miner Res 26(1):50–62 (Retrieved from PM:20593413)
Manolagas SC (2000) Birth and death of bone cells: basic regulatory mechanisms and implications for the pathogenesis and treatment of osteoporosis. Endocr Rev 21(2):115–137 (Retrieved from PM:10782361)
Marathe A, Peterson MC, Mager DE (2008) Integrated cellular bone homeostasis model for denosumab pharmacodynamics in multiple myeloma patients. J Pharmacol Exp Ther 326(2):555–562 (Retrieved from PM:18460643)
Marathe DD, Marathe A, Mager DE (2011) Integrated model for denosumab and ibandronate pharmacodynamics in postmenopausal women. Biopharm Drug Dispos 32(8):471–481 (Retrieved from PM:21953540)
McClung MR, Boonen S, Brown JP, Diez-Perez A, Langdahl B, Reginster JY et al (2012) Inhibition of sclerostin with AMG 785 in postmenopausal women with low bone mineral density: phase 2 trial results. J Bone Miner Res 27(Suppl 1)
Mehta K, Cabal A, Ross SD (2012) Mathematical modeling of bone remodeling in response to osteoporosis treatments. SIAM Conference on the Life Sciences
Melton LJ III, Crowson CS, O'Fallon WM, Wahner HW, Riggs BL (2003) Relative contributions of bone density, bone turnover, and clinical risk factors to long-term fracture prediction. J Bone Miner Res 18(2):312–318 (Retrieved from PM:12568408)
Morgan EF, Keaveny TM (2001) Dependence of yield strain of human trabecular bone on anatomic site. J Biomech 34(5):569–577 (Retrieved from PM:11311697)

Morgan EF, Bayraktar HH, Keaveny TM (2003) Trabecular bone modulus-density relationships depend on anatomic site. J Biomech 36(7):897–904 (Retrieved from PM:12757797)

Moroz A, Crane MC, Smith G, Wimpenny DI (2006) Phenomenological model of bone remodeling cycle containing osteocyte regulation loop. Biosystems 84(3):183–190 (Retrieved from PM:16387419)

Peterson MC, Riggs MM (2010) A physiologically based mathematical model of integrated calcium homeostasis and bone remodeling. Bone 46(1):49–63 (Retrieved from PM:19732857)

Pistoia W, Van Rietbergen B, Lochmuller EM, Lill CA, Eckstein F, Ruegsegger P (2002) Estimation of distal radius failure load with micro-finite element analysis models based on three-dimensional peripheral quantitative computed tomography images. Bone 30(6):842–848 (Retrieved from PM:12052451)

Pivonka P, Komarova SV (2010) Mathematical modeling in bone biology: from intracellular signaling to tissue mechanics. Bone 47(2):181–189 (Retrieved from PM:20417739)

Pivonka P, Zimak J, Smith DW, Gardiner BS, Dunstan CR, Sims NA et al (2008) Model structure and control of bone remodeling: a theoretical study. Bone 43(2):249–263 (Retrieved from PM:18514606)

Post TM (2009) Disease system analysis: between complexity and (over)simplification. https://openaccess.leidenuniv.nl/handle/1887/14477. TIPharma, Division of Pharmacology, Schering-Plough, Leiden/Amsterdam Center for Drug Research, Faculty of Science, Leiden University

Post TM, Cremers SC, Kerbusch T, Danhof M (2010) Bone physiology, disease and treatment: towards disease system analysis in osteoporosis. Clin Pharmacokinet 49(2):89–118 (Retrieved from PM:20067335)

Post TM, Schmidt S, Peletier LA, de Greef R, Kerbusch T, Danhof M (2013) Application of a mechanism-based disease systems model for osteoporosis to clinical data. J Pharmacokinet Pharmacodyn. 2013 Apr;40(2):143-56. doi: 10.1007/s10928-012-9294-9. Epub 2013 Jan 12. (Retrieved from PM:23315144)

Raisz LG (2008) Overview of pathogenesis. In: Miller PD, Papapoulos S (eds) Primer on the metabolic bone disease and disorders of mineral metabolism, 7th edn. American Society for Bone and Mineral Research, Washington, DC, pp 208–212

Rajagopal V, Holroyd C, Cooper C, Dennison E (2008) Epidemiology of osteoporotic fracture. In: Clunie G, Keen RW (eds) Osteoporosis. Oxford University Press, Oxford

Rattanakul C, Lenbury Y, Krishnamara N, Wollkind DJ (2003) Modeling of bone formation and resorption mediated by parathyroid hormone: response to estrogen/PTH therapy. Biosystems 70(1):55–72 (Retrieved from PM:12753937)

Riggs MM, Peterson MC, Gastonguay MR (2012) Multiscale physiology-based modeling of mineral bone disorder in patients with impaired kidney function. J Clin Pharmacol 52(1 Suppl):45S–53S (Retrieved from PM:22232752)

Schmidt S, Post TM, Peletier LA, Boroujerdi MA, Danhof M (2011) Coping with time scales in disease systems analysis: application to bone remodeling. J Pharmacokinet Pharmacodyn 38(6):873–900 (Retrieved from PM:22028207)

Schuit SC, van der KM, Weel AE, de Laet CE, Burger H, Seeman E et al (2004) Fracture incidence and association with bone mineral density in elderly men and women: the Rotterdam study. Bone 34(1):195–202 (Retrieved from PM:14751578)

Seeman E (2007) Is a change in bone mineral density a sensitive and specific surrogate of antifracture efficacy? Bone 41(3):308–317 (Retrieved from PM:17644058)

WHO Study Group. (1994) Assessment of fracture risk and its application to screening for postmenopausal osteoporosis. Report of a WHO study group. World Health Organ Tech Rep Ser 843:1–129

Wimpenny DI, Moroz A (2007) On allosteric control model of bone turnover cycle containing osteocyte regulation loop. Biosystems 90(2):295–308 (Retrieved from PM:17070649)

Zienkiewicz OC, Taylor RL (2002) The finite element method. In: Ward JP (eds) Solid mechanics, 5th edn. Butterworth-Heinemann, Oxford

Zienkiewicz OC, Taylor RL (2005) The finite element method: its basis and fundamentals, 6th edn. Elsevier, Amsterdam

Zumsande M, Stiefs D, Siegmund S, Gross T (2011) General analysis of mathematical models for bone remodeling. Bone 48(4):910–917 (Retrieved from PM:21185412)