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A Theoretical Model for Simulating Effect of Parathyroid Hormone on Bone Metabolism at Cellular Level

Yanan Wang*, Qing-Hua Qin* and Shankar Kalyanasundaram*

Abstract: A mathematical model is developed for simulating anabolic behaviour of bone affected by Parathyroid Hormone (PTH) in this paper. The model incorporates a new understanding on the interaction of PTH and other factors with the RANK-RANKL-OPG pathway into bone remodelling, which is able to simulate anabolic actions of bone induced by PTH at cellular level. The RANK-RANKL-OPG pathway together with the dual action of TGF-β, which represent the core of coupling behaviour between osteoblasts and osteoclasts which are two cell types specialising in the maintenance of bone integrity, are widely considered essential for the regulation of bone remodelling at cellular level. Moreover, the anabolic effect of PTH on bone remodelling (mainly causing bone gain) is significant for therapies of bone disease such as osteoporosis. Although the Food and Drug Administration of United States has approved PTH as an anabolic treatment for osteoporosis, the corresponding underlying mechanism of bone anabolism remains elusive. The proposed mathematical model provides a detailed biological description of bone remodelling using the latest experimental findings and can explain the mechanism of bone anabolic action by PTH that is administered intermittently as well as catabolic effect when applied continuously. The development of such a model provides a rational basis for developing more biologically extensive models that may support the design of optimal dosing strategies for different therapies such as PTH-based anti-osteoporosis treatments.

Keyword: theoretical model, bone remodelling, parathyroid hormone, anabolic effect

* Department of Engineering, Australian National University, Canberra, ACT 0200, Australia

1 Introduction

Bone is a living organ that undergoes remodelling throughout life. Remodelling results from the action of osteoblasts and osteoclasts which are two principal cell types found in bone, and defects such as microfractures are repaired by their coupling reaction. The osteoblast produces the matrix which becomes mineralized in well regulated manner. This mineralized matrix can be removed by the activity of the osteoclast when activated. In a homeostatic equilibrium resorption and formation are balanced so that old bone is continuously replaced by new tissue, which regulated by a variety of biochemical and mechanical factors. In 1963 Frost defined this phenomenon as bone remodelling [1].

For normal adults, there is a balance between the amount of bone resorbed by osteoclasts and the amount of bone formed by osteoblasts [2]. In this complex process, bone is remodelled by groups of cells derived from different sources, which are usually called the basic multicellular units (BMUs) [3] that follow an activation-resorption-formation sequence event. The BMU is a mediator mechanism bridging individual cellular activity to whole bone morphology [4].

The remodelling cycle consists of three consecutive phases [5]: resorption, reversal, and formation. Resorption begins with the migration of partially differentiated mononuclear preosteoclasts to the bone surface where they form multinucleated osteoclasts. After the completion of osteoclastic resorption, there is a reversal phase when mononuclear cells appear on the bone surface. These cells prepare the surface for new osteoblasts to begin bone formation and provide signals for osteoblast differentiation and migration. The formation phase follows with osteoblasts lay-

ing down bone until the resorbed bone is completely replaced by the new one. When this phase is completed, the surface is covered with flattened lining cells and a prolonged resting period begins until a new remodelling cycle is initiated. The stages of the remodelling cycle have different lengths. Resorption probably continues for about 2 weeks, the reversal phase may last up to 4 or 5 weeks, while formation can continue for 4 months until the new bone structural unit is completely created.

The assumption that a coupling mechanism must exist between bone formation and resorption was first reported in 1964 [6]. However, the exact molecular mechanism that describes the interaction between cells of the osteoblastic and osteoclastic lineages was only recently identified [7]. Recent breakthrough in our understanding of osteoclast differentiation and activation have come from the analysis of a family of biologically related tumour necrosis factor (TNF) receptor (TNFR)/TNF-like proteins: Osteoprotegerin (OPG), receptor activator of nuclear factor (NF)-kB (RANK) and RANK ligand (RANKL), which together regulate osteoclast function [2, 8, 9]. With the discovery of RANK, RANKL and OPG, a revolutionary understanding of osteoclastogenesis was born.

Bone, a major reservoir of body calcium, is under the hormonal control of PTH [10]. Bone remodelling plays an important role in mineral homeostasis by providing access to stores of calcium and phosphate [11, 12]. PTH is secreted in response to a drop in plasma Ca^{2+} levels. With the goal of maintaining plasma Ca^{2+}, PTH increases bone resorption to release Ca^{2+} stored in bone. Interestingly, PTH mainly stimulates osteoclasts indirectly, first affecting osteoblasts that have receptors for PTH [13, 14]. Acting on osteoblasts, PTH alters expression of RANKL and OPG, leading to a large increase in the RANKL/OPG ratio, thus stimulating osteoclastogenesis and bone resorption [15, 16]. Most intriguingly, the overall effect of PTH on bone mass depends primarily on its mode of administration. Whereas a continuous increase in PTH levels decreases bone mass, intermittent PTH administration has an anabolic action on bone [13, 17-20].

Mathematical modelling provides a powerful tool to predict the outcome of multiple, simultaneous actions of autocrine, paracrine and endocrine factors on bone remodelling [21]. Kroll [10] and Rattanakul et al [22] proposed a mathematical model respectively, accounting for the differential activity of PTH administration on bone accumulation. Komarova et al [23] presented a theoretical model of autocrine and paracrine interactions among osteoblasts and osteoclasts. Komarova et al [24] developed a mathematical model that describes the actions of PTH at single site of bone remodelling, where osteoblasts and osteoclasts are regulated by local autocrine and paracrine factors. Potter et al [25] proposed a mathematical model for PTH receptor (PTH1R) kinetics, focusing on the receptor's response to PTH dosing to discern bone formation responses from bone resorption. Lemaire et al [26] incorporated detailed biological information and RANK-RANKL-OPG pathway into remodelling cycle of the model that includes the catabolic effect of PTH on bone, but the PTH's anabolic effect was not designed to described.

In this paper we propose a mathematical model of bone remodelling at cellular level which incorporates a different understanding of RANK-RANKL-OPG pathway into bone remodelling model, which is able to simulate PTH's anabolic effect observed in clinical trials. The model has taken the latest progress in bone biology of remodelling into consideration using appropriate parameters and equations in order to provide the underlying mechanism of a variety of complex experimental observations. Importantly the simulation results of this model are consistent with all the experimental findings that we simulated. Particularly, the tight coupling behaviour between osteoblasts and osteoclasts which is the key in bone remodelling is well explained by the model, also PTH therapy, which is the only anabolic agent for osteoporosis approved by FDA by now, is in harmony with the theoretical outcome of the model. It is expected that the model concerning systemic and local regulation of bone remodelling will lead to new approaches in the diagnosis and treatment of skeletal disorders. In particular, this model

will help to develop new therapeutic approaches at molecular and cellular level based on definition of the abnormalities of the osteoblastic and osteoclastic lineage that lead to bone diseases such as ostoporosis.

2 Model development

The overall integrity of bone appears to be controlled by hormones and many other proteins secreted by both hemopoietic bone marrow cells and bone cells. There is both systemic and local regulation of bone cell function. PTH is the most important regulator of calcium homeostasis, which can stimulate bone formation when it is given intermittently and bone resorption when it is secreted continuously [27]. Moreover, PTH is currently involved in numerous clinical trials as an anabolic agent for the treatment of low bone mass in osteoporosis and Forteo (PTH 1-34) was recently approved as an anabolic therapy by the Food and Drug Administration [28, 29]. The insulin-like growth factor (IGFs) system is also important for skeleton growth, they are among the major determinants of adult bone mass through their effect on regulation of both bone formation and resorption [30]. IGF-1 promotes chondrocyte and osteoblast differentiation and growth. It is also a pivotal factor in the coupling of bone turnover because it is stored in the skeletal matrix and released during bone resorption [31] and stimulates bone formation directly.

PTH receptors are largely expressed on the osteoblastic surface [32, 33]. Quasi-steady state levels of plasma PTH, by binding these receptors, stimulates the production of RANKL and inhibits the production of OPG by osteoblasts [33-35], which causes an increase in AOC numbers. Direct effect of PTH on osteoblasts that is anti-apoptosis has also been experimentally observed [32, 36].

As far as the local regulation of bone cell function is concerned, after the recent discovery of the RANK-RANKL-OPG system, there is a clearer picture regarding the control of osteoclastogenesis and bone remodelling in general. The main switch for osteoclastic bone resorption is the RANKL [37], a cytokine that is released by activated osteoblasts. Its action on the RANK receptor is regulated by OPG, a decoy receptor, which is also derived from osteoblastic lineage-preosteoblasts. Osteoclast-to-osteoblast cross-talk occurs mostly through growth factors, such as transforming growth factor-β (TGF-β), which are released from the bone matrix during resorption.

The opposite phenotypes of OPG over expression or with RANKL deletion mice (osteopetrosis) and OPG-deficient or with RANKL over expression (osteoporosis) have led to the hypothesis that OPG and RANKL can be the mediators for the stimulatory or inhibitory effects of a variety of systemic hormones, growth factors, and cytokines on osteoclastogenesis [5]. This is recently referred to as "the convergence hypothesis" in that the activity of the resorptive and antiresorptive agents "converges" at the level of these two mediators, whose final ratio controls the degree of osteoclast differentiation, activation, and apoptosis [38].

The logical structure of the model is presented in Fig. 1 which shows the simplified lineages of osteoblasts, osteoclasts and their interactions.

The previously described BMU comprises a collection of different cell types with different origins. The osteoclast teams that line the cutting cone are derived from hematopoetic stem cells residing mainly in the marrow and spleen. Osteoclastogenesis begins when a hematopoetic stem cell is stimulated to generate mononuclear cells, which then become committed preosteoclasts and are introduced into the blood stream. This step requires expression of the Ets family transcription factor PU.1 and macrophage colony stimulating factor (M-CSF) [39, 40]. The circulating precursors exit the peripheral circulation at or near the site to be resorbed, and fuse with one another to form a multinucleated immature osteoclast. Fusion of the mononuclear cells into a immature osteoclast requires the presence of M-CSF and RANKL, a tumor-necrosis factor family member [41]. RANKL interacts with a receptor on osteoclast precurosor called RANK. Further differentiation of the immature osteoclast into mature and activated osteoclasts (AOC) occurs only under the continued presence of RANKL [42]. The

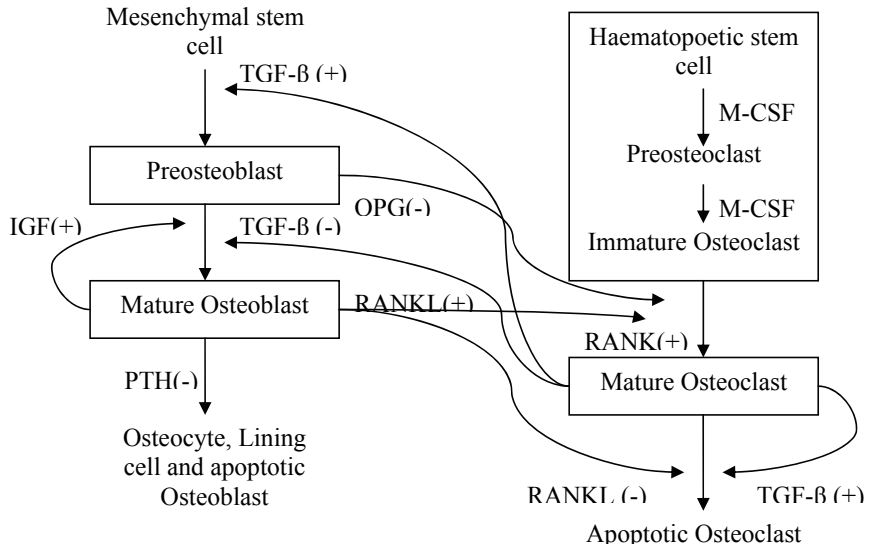

Figure 1: Schematic representation of structure of the model. The solid arrow with a (+) or (-) next to them represent a stimulatory or inhibitory action by the factor. The thin frame squares indicate types of cells that are included in this model.

RANK/RANKL interaction results in activation, differentiation and fusion of hematopoietic cells of the osteoclast lineage so that they begin the process of resorption. Furthermore, it also prolongs osteoclast survival by suppressing apoptosis [43].

Osteoblast development follows a different course, beginning with the local proliferation of mesenchymal stem cells residing in the marrow, which can also give rise to other types of cells such as myocytes, chondrocytes and adipocytes [44]. Proliferating precursors are pushed toward the preosteoblasts - responding osteoblasts (ROB) under the complex effects of specific factors such as PTH and TGF-β [44]. After further differentiation, responding osteoblasts mature to active osteoblasts (AOB) which are responsible for bone formation. Eventually osteoblasts either die or transform to either lining cells or osteocytes [45].

Bone matrix is the largest source of TGF-β in the body [46]. TGF-β, as well as growth factors and specific components embedded in the bone matrix, are released by osteoclasts during bone resorption [47]. TGF-β's effect on osteoblasts is bi-directional depending upon the state of maturation of the osteoblasts [26]. On one hand, TGF-β has the potential to stimulate osteoblast recruitment, migration and proliferation of osteoblast precursors (meaning ROBs in our model) [47]. On the other, TGF-β inhibits terminal osteoblastic differentiation into AOBs [48]. TGF-β is also known to induce osteoclast apoptosis [46, 49].

3 Mathematical formulation

In the model shown in Fig. 1, cellular interactions are carried out via activation of cell receptors. The receptors either bind molecules secreted by other cell types called paracrine, or with molecules secreted by the same cell called autocrine, or with other transmembrance molecules via direct cell-to-cell contacts. The different cell types represented in the model respond to the activation of their receptors by producing new molecules, differentiating or dying [26]. The mathematical formulation of the model is primarily influenced by physiological events involving receptor binding and intracellular signalling modelling [50, 51]. Applying the law of mass action [26] that used to describe the reactions of receptors and corresponding ligands including PTH (P) with its receptor (P_r,) RANKL (L) with OPG (O), RANKL (L) with RANK (K) and IGF (I) with its receptor

(*IR*).

$$\frac{dP}{dt} = S_p + I_P + \left(k_6 \cdot P_r * P - k_5 \left(R_T^P - P_r * P\right) \cdot P\right) \cdot (B+R) - k_P \cdot P \quad (1)$$

$$\frac{dP_r * P}{dt} = k_5 \left(R_T^P - P_r * P\right) - k_6 \cdot P_r * P \quad (2)$$

$$\frac{dO}{dt} = p_O - k_1 \cdot O \cdot L + k_2 \cdot O * L - d_O \quad (3)$$

$$\frac{dO * L}{dt} = k_1 \cdot O \cdot L - k_2 \cdot O * L \quad (4)$$

$$\frac{dL}{dt} = p_L - k_1 \cdot O \cdot L + k_2 \cdot O * L - k_3 \cdot K \cdot L + k_4 \cdot K * L - d_L \quad (5)$$

$$\frac{dK * L}{dt} = k_3 \cdot K \cdot L - k_4 \cdot K * L \quad (6)$$

where $X * Y$ stands for one substance. In this paper we propose that the cell proliferation rate is proportional to the receptor occupancy [26] and we apply this rule to other types of cell responses besides cell proliferation. While the anti-proliferative cell responses are inversely proportional to the receptor occupancy. Consequently the production rate of OPG (P_O) is down regulated by PTH and up regulated by TGF-β, the expression of P_O is:

$$p_O = K_O^P \cdot \left(\frac{1}{\pi_P} + \pi_C\right) R + I_O \quad (7)$$

where the proportion of occupied PTH receptor is:

$$\pi_P = \sqrt{\frac{P_r * P}{R_T^P}} = \sqrt{\frac{\overline{P} + P^0}{\overline{P} + P^s}} = \sqrt{\frac{I_P/k_P + S_P/k_P}{I_P/k_P + k_6/k_5}} \quad (8)$$

Applying the same rule as obtaining P_O, we can deduce [26]:

$$p_L - d_L = r_L \cdot \left(1 - \frac{L + O * L + K * L}{K_L^P \cdot \pi_P \cdot B}\right) + I_L \quad (9)$$

$$L = \frac{K_L^P \cdot \pi_P \cdot B}{1 + k_3 K/k_4 + k_1 O/k_2} \cdot \left(1 + \frac{I_L}{r_L}\right) \quad (10)$$

$$O = \frac{K_O^P}{k_O \pi_P} R + \frac{I_O}{k_O} \quad (11)$$

The entering flow into the ROB compartment depends on the mesenchymal stem cells response to c binding. This response is represented by a proportionality relationship with the TGF-β receptor occupancy π_C:

$$D_R \cdot \pi_C = D_R \cdot \sqrt{\frac{C + C^0}{C + C^S}} \quad (12)$$

The outgoing flow of the ROB compartment is also the feeding flow to the AOB compartment. Under the influence of TGF-β and IGF, which inhibit and stimulate AOB production respectively:

$$D_B \cdot R \cdot \left(\frac{1}{\pi_C} + \pi_I\right) \quad (13)$$

RANK-RANKL binding promotes the differentiation of mesenchymal stem cells into AOC [26], the differentiation rate is proportional to the RANK occupancy ratio π_L:

$$D_C \cdot \pi_L = D_C \cdot \frac{K * L}{K} \quad (14)$$

TGF-β induces osteoclast apoptosis via binding to specific receptors and also under the influence of RANKL, this phenomenon is then represented as:

$$D_A \cdot (\pi_C - \pi_L) \cdot C = D_A \cdot \frac{C + C^0}{C + C^S} \cdot C \quad (15)$$

The equations governing the evolution of the number of cells in each compartment are simply balance equations [26], which means each cell compartment is fed by an entering flow and is emptied by the outgoing flow of differentiated or apoptotic cells:

$$\frac{dR}{dt} = D_R \cdot \pi_C - D_B \cdot R \cdot \left(\frac{1}{\pi_C} + \pi_I\right) \quad (16)$$

$$\frac{dB}{dt} = D_B \cdot R \cdot \left(\frac{1}{\pi_C} + \pi_I\right) - (k_B - \pi_P) \cdot B \quad (17)$$

$$\frac{dC}{dt} = D_C \cdot \pi_L - D_A \cdot (\pi_C - \pi_L) \cdot C \quad (18)$$

The rate of bone resorption and formation is assumed to be proportional to the numbers of osteoclasts and osteoblasts respectively,

$$\frac{dZ}{dt} = -m_1 \cdot C + m_2 \cdot B \tag{19}$$

where Z is total bone mass. Model (16)-(19) is analysed using numerical integration by a fourth Runge-Kutta algorithm using Matlab.

4 Results and Discussion

To demonstrate the tight coupling between osteoblast and osteoclast, we computationally perturbed this system by adding or removing specific cells. The results are displayed from Fig. 2∼Fig. 7.

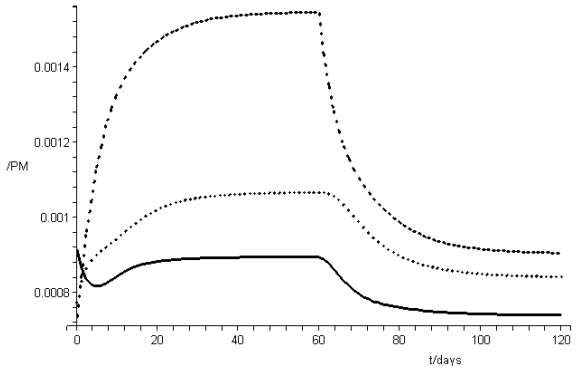

Figure 2: The AOBs are added at a constant (0.0001pM/day) rate for 60 days from start. From top to bottom, dash curves, dot curves and solid curves represent AOB, ROB and AOC respectively.

It is evident from Figs. 2 and 3 that adding AOBs can initiate a remodelling cycle from initial stable state as shown in Fig. 2. It also shows that the number of AOC decreases in the first around 7 days and then increases to initial value, while the number of AOB increases as expected, which means direct administration of AOB does not have a strong stimulatory effect on AOC, which is consistent with experimental observation [41]. Fig. 3 clearly displays that bone mass increases as administration of AOB and will rise a little slowly after the stop of injection of AOBs.

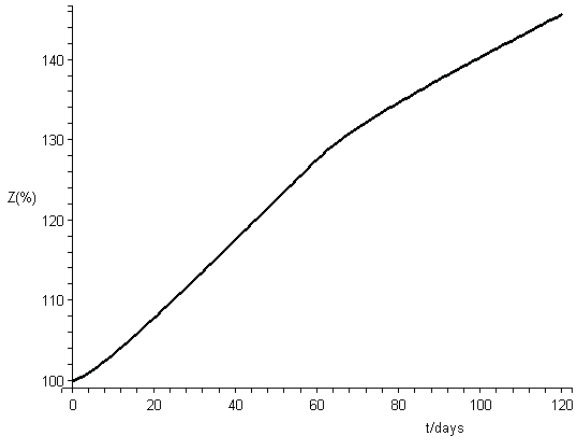

Figure 3: The effect of adding AOBs at a constant rate 0.0001pM/day) on bone mass. Changes in bone mass are expressed as a percentage of initial bone mass (100%)

Fig. 4 shows that administration of AOCs initiates a remodelling cycle and its number keeps almost unchanged from about the 7^{th} day to the 60^{th} day. It also exhibits a strong and immediate stimulatory effect on ROBs (top dash curve), which means that the number of ROBs increases immediately as the injection of AOCs and decreases to initial level after stoping injection of AOCs. Whereas the response of AOBs to the administration of AOCs is slow down and delayed until the stoping injection of AOCs. It can be seen from Fig. 5 that the amount of AOBs that are responsible for producing bone mass begins to increase fast, which accounts for the increase of bone mass after the 60^{th} day.

An interesting phenomenon was observed when ROBs are administered to the system. That is, the AOBs increase in their number along with an increase in ROB, whereas the number of AOCs keep, after a decrease for about the first 7 days, to unchanged at a particular level which is lower than the initial state until the 60^{th} day. After 60^{th} days, it equilibrates to an even smaller value as shown in Fig. 6. Consequently it is reasonable that the bone mass keeps rising as shown in Fig. 7. This novel observation may have the potential to be exploited as a therapeutic target for metabolic

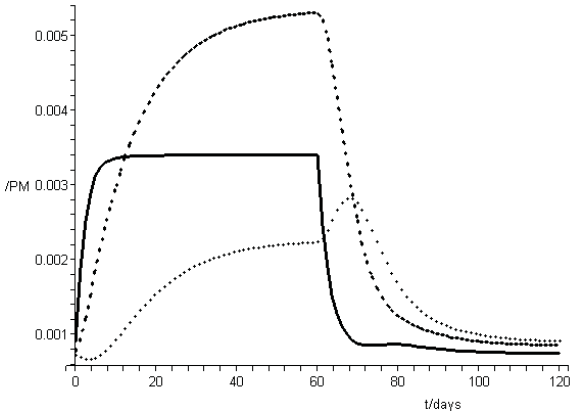

Figure 4: The AOCs are added at a constant rate (0.001pM/day) for 60 days from start

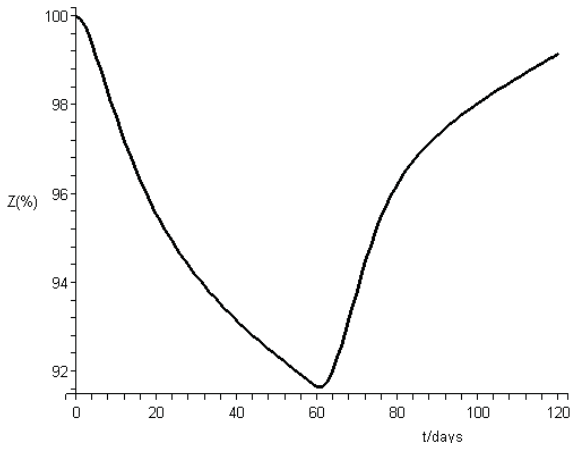

Figure 5: The effect of adding AOCs at a constant rate (0.001pM/day) on bone mass

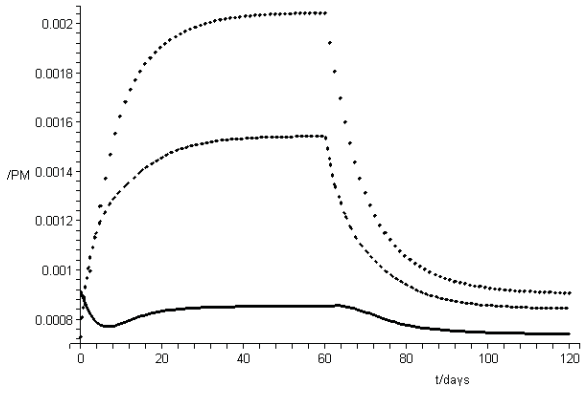

Figure 6: The ROBs are added at a constant rate (0.0001pM/day) for 60 days from start

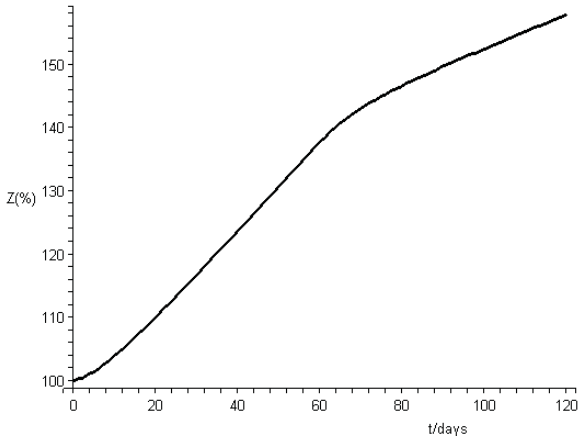

Figure 7: The effect of adding ROBs at a constant rate (0.0001pM/day) on bone mass

diseases.

The only systemic hormone considered in the model is PTH. As mentioned earlier, intermittent infusion of PTH has potent anabolic effects on bone mass. To test the anabolic action of PTH in the model, the hormone is delivered at a steady rate of 3000 pM/day for 60 days. As can be seen from Fig. 8, the number of AOC bursts as the infusion of PTH, and drops quickly in response to the stop administration of PTH. The response of AOB to intermittent injection of PTH is, however, relatively slow, the number of AOB keeps going up even after the PTH administration which is the key to final increase of bone mass as shown in Fig. 9, which is in agreement with the experimental observation [28].

As expected under the continuous administration of PTH, bone mass keeps decreasing as shown in Fig. 11. This simulation is in good agreement with the experimental results [52, 53]. It can be seen from Fig. 10 that the response of AOC, ROB and AOB affected by the PTH. particularly, the number of AOC increases promptly as PTH is injected, followed by a minor drop and then keeps rising at a lower rate. Whereas the number of AOB increases only a little bit at a very low rate over the first 120 days. Through the direct stimu-

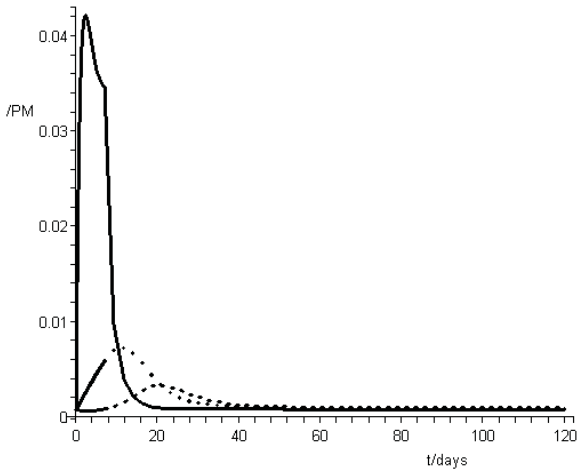

Figure 8: The responses of AOC, AOB and ROB to the intermittent administration of PTH for the first 7 days

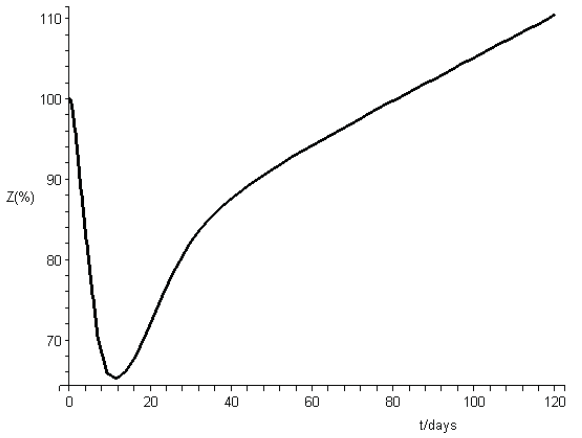

Figure 9: The effect of intermittent administration of PTH for the first 7 days on bone mass

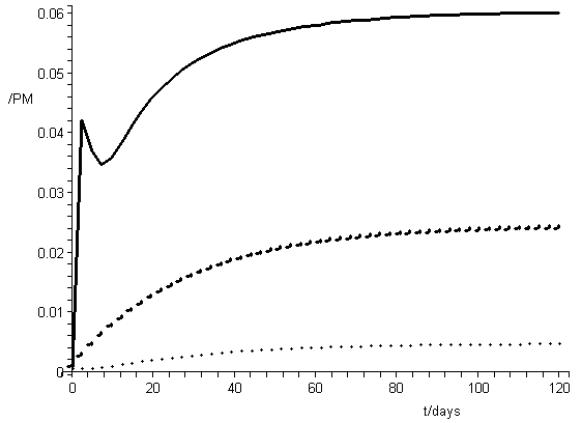

Figure 10: The responses of AOC, AOB and ROB to the continuous administration of PTH for 120 days

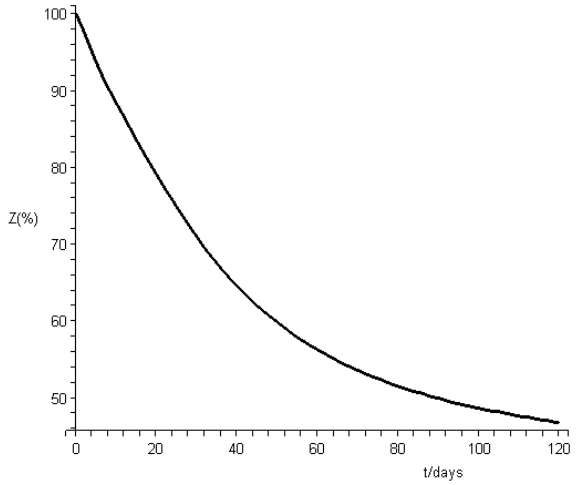

Figure 11: The effect of continuous administration of PTH for 120 days on bone mass

latory effect of TGF-β released by AOC, the number of ROB increases at a higher rate than that of AOB.

5 Conclusion

In this paper, a relatively complete bone remodelling model which incorporates the latest findings such as RANK-RANKL-OPG pathway in bone biology at cellular level and is able to corroborate all behaviours of bone remodelling system that we have simulated, including the tight coupling between osteoblasts and osteoclasts, and the anabolic therapeutic interventions such as the most promising treatment that emerges when PTH is administered intermittently.

Appendix. A

5.1 A.1 model's parameters [26]

See Table 1.

Table 1

Symbol	Unit	Valve	Description
C^S	pM	5×10^{-3}	Value of C to get half differentiation flux
D_A	Day^{-1}	0.7	Rate of osteoclast apoptosis caused by TGF-β
d_B	Day^{-1}	0.7	Differentiation rate of responding osteoblasts
D_C	pM Day^{-1}	2.1×10^{-3}	Differentiation rate of osteoclast precursor
D_R	pM Day^{-1}	7×10^{-4}	Differentiation rate osteoblast progenitors
f_0	No dimension	0.05	Fixed proportion
I_L	pM Day^{-1}	$0-10^6$	Rate of administration of RANKL
I_O	pM Day^{-1}	$0-10^6$	Rate of administration of OPG
I_P	pM Day^{-1}	$0-10^6$	Rate of administration of PTH
K	pM	10	Fixed concentration of RANK
k_1	$\text{pM}^{-1}\ \text{Day}^{-1}$	10^{-2}	Rate of OPG-RANKL binding
k_2	Day^{-1}	10	Rate of OPG-RANKL unbinding
k_3	$\text{pM}^{-1}\ \text{Day}^{-1}$	5.8×10^{-4}	Rate of RANK-RANKL binding
k_4	Day^{-1}	1.7×10^{-2}	Rate of RANK-RANKL unbinding
k_5	$\text{pM}^{-1}\ \text{Day}^{-1}$	0.02	Rate of PTH binding with its receptor
k_6	Day^{-1}	3	Rate of PTH unbinding
k_B	Day^{-1}	0.189	Rate of elimination of AOB
K_L^P	pmol/pmol cells	3×10^6	Maximum number of RANKL attached on each cell surface
k_O	Day^{-1}	0.35	Rate of elimination of OPG
K_O^P	pmol day^{-1}/pmol cells	2×10^5	Minimal rate of production of OPG per cell
k_P	day^{-1}	86	Rate of elimination of PTH
r_L	pM day^{-1}	10^3	Rate of RANKL production and elimination
S_P	pM day^{-1}	250	Rate of synthesis of systemic PTH
R^S	pM	5×10^{-2}	Value of R to get half differentiation flux
m_1	$\%\ \text{cell}^{-1}\ \text{d}^{-1}$	122	Average rate of bone resorbed per day per AOC
m_2	$\%\ \text{cell}^{-1}\ \text{d}^{-1}$	195	Average rate of bone formed per day per AOB

5.2 A.2 Model's variables and initial values

Noting that, at equilibrium, the numbers of AOB, AOC and ROB do not change with time, solving the following three equations can determine the initial values of B, C and R.

$$0 = D_R\cdot\pi_C - D_B\cdot R\cdot\left(\frac{1}{\pi_C}+\pi_I\right) \tag{A.1}$$

$$0 = D_B\cdot R\cdot\left(\frac{1}{\pi_C}+\pi_I\right) - (k_B-\pi_P)\cdot B \tag{A.2}$$

$$0 = D_C\cdot\pi_L - D_A\cdot(\pi_C-\pi_L)\cdot C \tag{A.3}$$

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