A Model of Calcium Homeostasis in the Rat.

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David Granjon\textsuperscript{1,2}, Olivier Bonny\textsuperscript{2}, and Aurélie Edwards\textsuperscript{1}

\textsuperscript{1}Sorbonne Universités, UPMC Univ Paris 06, Université Paris Descartes, Sorbonne Paris Cité, INSERM UMRS 1138, CNRS ERL 8228, Centre de Recherche des Cordeliers, Paris, France;

\textsuperscript{2}Department of Pharmacology and Toxicology, University of Lausanne, and Service of Nephrology, Lausanne University Hospital, Lausanne, Switzerland.

Direct editorial correspondence to

Aurélie Edwards

Centre de Recherche des Cordeliers \cdot ERL 8228, UMRS 1138 \cdot 15 rue de l’école de Médecine \cdot 75006 Paris, France

Phone: (33) 144 275 099 \cdot E-mail: aurelie.edwards@crc.jussieu.fr
We developed a model of calcium homeostasis in the rat in order to better understand the impact of dysfunctions such as primary hyperparathyroidism and vitamin D deficiency on calcium balance. The model accounts for the regulation of calcium intestinal uptake, bone resorption, and renal reabsorption by parathyroid hormone (PTH), vitamin D3, and Ca^{2+} itself. It is the first such model to incorporate recent findings regarding the role of the calcium sensing receptor (CaSR) in the kidney, the presence of a rapidly exchangeable pool in bone, and the delayed response of vitamin D3 synthesis. Accounting for two (fast and slow) calcium storage compartments in bone allows the model to properly predict the effects of bisphosphonates on the plasma levels of Ca^{2+} ([Ca^{2+}]_p), PTH, and vitamin D3. Our model also suggests that Ca^{2+} exchange rates between plasma and the fast pool vary with both sex and age, allowing [Ca^{2+}]_p to remain constant in spite of sex- and age-based hormonal and other differences. Our results suggest that the inconstant hypercalciuria that is observed in primary hyperparathyroidism can be attributed in part to counterbalancing effects of PTH and CaSR in the kidney. Our model also correctly predicts that calcimimetic agents such as cinacalcet bring down [Ca^{2+}]_p to within its normal range in primary hyperparathyroidism. In addition, the model provides a simulation of CYP24A1 inactivation that leads to a situation reminiscent of infantile hypercalcemia. In summary, our model of calcium handling can be used to decipher the complex regulation of calcium homeostasis.

Key words: calcium, PTH, vitamin D3, homeostasis, mathematical model.
1 Introduction

Calcium plays an essential role in organisms as it is involved in many biological processes such as cardiac activity regulation, muscle contraction, blood clotting, and bone formation. More than 99% of total body calcium is found in bones, only 1% of which can be readily exchanged with the extracellular fluid. The latter contains \( \approx 0.1\% \) of total body calcium, approximately 60% of which is ionized; the remainder is bound to albumin or other ions such as phosphate, bicarbonate, and citrate [42, 90]. In rats, the plasma concentration of total calcium is normally comprised between 2.2 and 2.6 mM, and that of ionized calcium between 1.1 and 1.5 mM [74, 90].

Given the key biological roles of calcium, its levels in plasma need to be tightly regulated. The main organs involved in calcium homeostasis are the intestine, bones, and kidneys, all of which are linked via the plasma compartment. The intestinal epithelium absorbs 20-60% of calcium intake [21, 35]. The bones constitute a reservoir that can either accumulate calcium (by accretion) or release it (by resorption), as required. The kidneys freely filter \( \text{Ca}^{2+} \) and subsequently reabsorb about 98% of the filtered load; thus fractional \( \text{Ca}^{2+} \) urinary excretion is 1-2%.

Calcium fluxes between these compartments are regulated by several hormones, principally parathyroid hormone (PTH) and calcitriol, i.e., the active form of vitamin \( \text{D}_3 \left(1,25(\text{OH})_2\text{vitamin D}\right) \). In response to a decrease in \( \text{Ca}^{2+} \) plasma levels, PTH is secreted by the parathyroid glands; it then binds to its receptor on bone and kidney cells, where it respectively acts to increase bone resorption and renal calcium reabsorption [26, 74, 90]. In bone, PTH acts on osteoblasts, thereby stimulating the synthesis of receptor activator of nuclear factor kappa-B
ligand (RANK-L), which is the primary mediator of osteoclast differentiation and activation [11]. PTH also inhibits the production of osteoprotegerin; the latter acts as a decoy receptor that binds to RANKL and prevents it from interacting with its cellular receptor RANK. In the kidney, PTH elicits an increase in the paracellular permeability of the thick ascending limb to Ca\(^{2+}\); it also enhances the expression of calcium transport proteins in the distal convoluted and connecting tubules, such as the transient receptor potential vanilloid type 5 (TRPV5) and the sodium-calcium exchanger 1 (NCX1) [27].

PTH also triggers the conversion of the inactive form of vitamin D\(_3\) (i.e., 25-hydroxyvitamin D) to its active form, calcitriol, in the proximal tubule [47]. Calcitriol binds to the vitamin D receptor (VDR) in the intestine, bones, kidneys, and parathyroid glands. In the intestine, calcitriol (thereafter denoted vitamin D\(_3\)) acts to increase intestinal calcium absorption by increasing the permeability of tight junctions as well as upregulating the activity and expression of calcium transport proteins such as the transient receptor potential vanilloid type 6 (TRPV6), calbindin D9k, and plasma membrane Ca\(^{2+}\) pumps (PMCA) [37]. Vitamin D\(_3\) also enhances Ca\(^{2+}\) reabsorption in the kidney by upregulating the expression of TRPV5, NCX1 and calbindin D28k [9]. Lastly, in bone, under a low calcium diet, vitamin D\(_3\) steps up the production of RANK-L by osteoblasts to accelerate the maturation of osteoclasts, thereby favoring bone resorption [87, 96].

In turn, the synthesis and secretion of PTH are regulated by the calcium sensing receptor (CaSR). The binding of Ca\(^{2+}\) to parathyroid gland CaSR triggers an intracellular signaling cascade that results in inhibition of PTH secretion and that ultimately cleaves intravesicular PTH into inactive forms [18, 76]. CaSR is also present in other tissues such as bone, brain and intestine [62]. In the kidney, CaSR has been shown to enhance urinary Ca\(^{2+}\) excretion
via a PTH-independent pathway [48, 61].

As summarized in Figure 1, the mechanisms that regulate plasma Ca\(^{2+}\) levels are highly coupled and can be hard to decipher. The major objective of this study was to develop a mathematical model of calcium homeostasis in the rat so as to better understand the impact of dysfunctions (such as primary hyperparathyroidism) on calcium balance and concentration. Several models in human have been developed [24, 57, 69, 74], however they do not incorporate several findings which the present study seeks to account for: the role of the renal CaSR, the delayed response of vitamin D\(_3\) synthesis to changes in PTH levels, and the rapidly exchangeable pool in bone (with the exception, for the latter, of the model of Peterson and Riggs [69]).

2 Mathematical Model

Our model of calcium homeostasis is based on conservation equations that yield the plasma concentration of Ca\(^{2+}\), PTH, and vitamin D\(_3\) in rats, as well as Ca\(^{2+}\) fluxes between plasma, bone, intestine, and kidneys. Model parameters apply to a rat weighing about 300 g (i.e., ~2 month old).

2.1 PTH synthesis and secretion

PTH is the main hormone involved in the short term regulation of plasma calcium levels. The synthesis of PTH in parathyroid glands is downregulated by vitamin D\(_3\) [81, 82], and its secretion is regulated by the calcium sensing receptor (CaSR), which can detect minute variations in [Ca\(^{2+}\)]\(_p\), on the order of 0.1 mM (i.e., 4% of its equilibrium value). The half-life
of PTH is about 4-6 minutes, and its plasma concentration ranges between 1 and 8 pM [22, 39].

We respectively denote the concentration of PTH within parathyroid cells and in plasma by \([\text{PTH}]_g\) and \([\text{PTH}]_p\). Assuming a constant cell volume, changes in \([\text{PTH}]_g\) with time are given by:

\[
\frac{d[\text{PTH}]_g}{dt} = \frac{k_{\text{prod}}^{\text{PTH}g}}{1 + \gamma_{\text{prod}}^D[D_3]_p} - \left( k_{\text{deg}}^{\text{PTH}g} + F([Ca^{2+}]_p) \right)[\text{PTH}]_g
\]  

(1)

The first term on the right-hand-side represents the synthesis of PTH, at a basal rate \(k_{\text{prod}}^{\text{PTH}g}\) that can be modulated by vitamin D\(_3\). The repression factor \(\gamma_{\text{prod}}^D\) is adjusted so that at equilibrium, the synthesis of PTH is reduced by 10\%. The second term on the left hand side characterizes PTH elimination from the cell, either by degradation (with a first-order rate constant \(k_{\text{deg}}^{\text{PTH}g}\)) or by exocytosis. The exocytosis function is defined as:

\[
F([Ca^{2+}]_p) = \beta_{\text{exo}}^{\text{PTH}g} - \frac{\gamma_{\text{exo}}^{\text{PTH}g} [Ca^{2+}]_p^{n(Ca)}}{[Ca^{2+}]_p^{n(Ca)} + K_{Ca}^{n(Ca)}}
\]  

(2)

This function accounts for the inhibition of PTH secretion by CaSR [73]: \(\beta_{\text{exo}}^{\text{PTH}g}\) is the maximal rate of PTH secretion from the gland, \(\gamma_{\text{exo}}^{\text{PTH}g}\) characterizes the degree to which CaSR can maximally inhibit secretion, \(K_{Ca}\) is the binding affinity of Ca\(^{2+}\) to CaSR, and \(n(Ca)\) is a steepness factor. As discussed by Shresta et al. [80], to properly account for the effects of both hypercalcemia and hypocalcemia on PTH secretion, it is necessary to use an adaptive \(n\), which can be calculated as:

\[
n(Ca) = \frac{n_1^{\text{exo}}}{1 + e^{-p_{\text{exo}}(R-[Ca^{2+}]_p)}} + n_2^{\text{exo}}
\]  

(3)

\(n_1^{\text{exo}}\) and \(n_2^{\text{exo}}\) respectively characterize the sensitivity of the receptor under hypocalcemia
and hypercalcemia, R is the threshold between these two states, and $\rho_{exo}$ is an amplification term. When $[Ca^{2+}]_p$ is well below R, $n(Ca)$ tends towards high values (>100), as commonly used to fit hypocalcemia profiles [1, 73, 80]. Conversely, when $[Ca^{2+}]_p$ rises above R, $n(Ca)$ takes on a lower value.

Assuming a constant plasma volume ($V_p$), changes in $[PTH]_p$ with time are given by:

$$\frac{d[PTH]_p}{dt} = \left( \beta_{exo}^{PTH} - \frac{\gamma_{exo}^{PTH} [Ca^{2+}]^{n(Ca)}_p}{[Ca^{2+}]^{n(Ca)}_p + K_C^{n(Ca)}} \right) \frac{V_c}{V_p} [PTH]_g - k_{deg}^{PTH} [PTH]_p$$  \hspace{1cm} (4)

where $V_c$ is the parathyroid gland volume. The PTH degradation rate in plasma ($k_{deg}^{PTH}$) is chosen so that the half-life of PTH in plasma fits the experimental data of Lewin et al. [60].

### 2.2 Vitamin D$_3$

The biosynthesis pathway of vitamin D$_3$ is complex [28]. Its precursor 25-hydroxyvitamin D (denoted D$_3^{inact}$ below) is converted to vitamin D$_3$ (that is, 1,25(OH)$_2$D$_3$, or calcitriol) in the proximal tubule by the enzyme CYP27B1, also known as 1-\(\alpha\)(OH)-ase. For simplicity, we assume that the level of D$_3^{inact}$ remains constant. The equilibrium plasma concentration of vitamin D$_3$ is $\sim$ 200 pm in a 200-250 g rat [43, 89], and $\sim$ 80 pM in older rats (300-700 g) [39, 64]. In parathyroidectomized (PTX) rats, the level of vitamin D$_3$ falls sharply, by a factor of at least 2 [89]. The level of D$_3^{inact}$ also appears to decrease with age; its baseline value is taken as 25 nM [39].

The dynamic evolution of the plasma concentration of vitamin D$_3$ (denoted $[D_3]_p$) is determined as:
\[
\frac{d[D_3]_p}{dt} = \left[ k^{\text{min}}_{\text{conv}} + \delta^{\text{max}}_{\text{conv}}[\text{PTH}(t - \tau)]^{p_{\text{conv}}}_p \right] [D_3^{\text{inactive}}]_p \\
	imes \left( [\text{PTH}(t - \tau)]^{p_{\text{conv}}}_p + K^{p_{\text{conv}}}_{\text{conv}} \right) \left( 1 + \gamma^{Ca}_{p_{\text{conv}}}[Ca^{2+}]_p \right) \left( 1 + \gamma^{D_3}_{p_{\text{conv}}}[D_3]_p \right) \\
- \frac{k^{D_3}_{\text{deg}}[D_3]_p}{1 + \gamma^{PTH}_{p_{\text{deg}}}[PTH]_p}
\] (5)

The first term on the right-hand-side represents the conversion of $D_3^{\text{inactive}}$ to vitamin $D_3$, which is catalyzed by 1-$\alpha$(OH)-ase. This conversion occurs at a minimum rate $k^{\text{min}}_{\text{conv}}$, and we assume that it is activated by PTH with a delay $\tau$ of 4 hours, and a maximum gain $\delta^{\text{max}}_{\text{conv}}$ [50, 63]. The factors $\gamma^{Ca}_{\text{conv}}$ and $\gamma^{D_3}_{\text{conv}}$ respectively account for the repression of 1-$\alpha$(OH)-ase by calcium and vitamin $D_3$ [20]. The second term represents the degradation rate of vitamin $D_3$ in plasma, which is catalyzed by CYP24A1 and regulated by calcitriol and PTH (among other factors). The constant $k^{D_3}_{\text{deg}}$ is adjusted so that the half life of vitamin $D_3$ in plasma is 5-8 hours, and $\gamma^{PTH}_{\text{deg}}$ accounts for PTH-induced repression of CYP24A1.

### 2.3 Calcium exchanges between organs

#### 2.3.1 The intestinal compartment

Calcium crosses the intestinal barrier via both paracellular and transcellular routes [53]. The paracellular pathway, across which transport is driven by the luminal-to-plasma calcium concentration gradient, is not saturable and prevails under a high calcium diet. In contrast, the transcellular pathway predominates when calcium intake is low. $Ca^{2+}$ entry into the cell is mediated by TRPV6 (note that the concentration of $Ca^{2+}$ is several orders of magnitude lower in the cytosol than in the lumen). Within the cytosol, $Ca^{2+}$ binds to calbindin D9k, which facilitates its transfer to the basolateral side, where $Ca^{2+}$ is extruded mostly (\sim 90%)
by PMCA, with a small contribution from Na\(^+\)/Ca\(^{2+}\) (NCX1) exchangers. There may also be other Ca\(^{2+}\) transporters, yet to be identified, in the gut [3, 7]. Vitamin D\(_3\) regulates intestinal Ca\(^{2+}\) transport via both the transcellular and paracellular routes [53]. Across the paracellular pathway, it is thought to modulate the expression of claudins 2 and 12 [41].

We assume that there is no net accumulation of calcium in the intestinal compartment over time, so that intestinal calcium excretion (\(E_{Ca}\)) is the difference between calcium intake (\(I_{Ca}\)) and net intestinal absorption (\(\Gamma_{abs}(D_3)\)):

\[
E_{Ca} = I_{Ca} - \Gamma_{abs}(D_3)
\]  

Experimental findings suggest that at most, 70 of % ingested calcium is absorbed into the bloodstream [21], and that two thirds of intestinal calcium transport are modulated by vitamin D\(_3\) [16]. The rate of net intestinal calcium absorption is thus determined as:

\[
\Gamma_{abs}(D_3) = I_{Ca} \left( 0.25 + \frac{0.45[D_3]^2_{\beta}}{[D_3]^2_{\beta} + (K_{abs}D_3)^2} \right)
\]  

The first term within parentheses on the right-hand-side represents the fraction of absorbed calcium that is vitamin D\(_3\)-independent, and the second term represents the fraction that is regulated by vitamin D\(_3\).

### 2.3.2 The bone compartment

The bone compartment can be divided into a rapidly exchangeable pool and a slowly exchangeable pool; the amount of Ca\(^{2+}\) they respectively contain is denoted as \(N_{Ca_t}\) and \(N_{Ca_s}\). The rapidly exchangeable (or "fast") pool is thought to mediate the short-term regulation
of calcium homeostasis, but it remains poorly characterized [17, 70]. We assume that the amount of Ca$^{2+}$ in the fast pool is given by:

$$\frac{dN_{Ca_f}}{dt} = k_{p-f}^{Ca} [Ca^{2+}]_p V_p - k_{f-p}^{Ca} N_{Ca_f} - \Gamma_{ac} N_{Ca_f}$$  \hspace{1cm} (8)$$

The first two terms on the right-hand-side represent the flow of Ca$^{2+}$ from the plasma to the rapidly exchangeable pool and vice-versa, which we assume obey first-order kinetics. Since it remains unclear whether PTH and vitamin D$_3$ act directly on the rapidly exchangeable pool, we assume no such regulation. The last term accounts for Ca$^{2+}$ accretion from the fast to the slow pool, at a rate $\Gamma_{ac}$.

The amount of Ca$^{2+}$ in the slow bone pool varies with time as:

$$\frac{dN_{Ca_s}}{dt} = \Gamma_{ac} N_{Ca_f} - \Gamma_{res}(PTH, D_3)$$ \hspace{1cm} (9)$$

where the Ca$^{2+}$ resorption rate, $\Gamma_{res}(PTH, D_3)$, is determined as:

$$\Gamma_{res}(PTH, D_3) = \Gamma_{res}^{min} + \delta_{res}^{max} \left(0.2 \times \frac{[PTH]_p^2}{[PTH]_p^2 + (K_{res}^{PTH})^2} + 0.8 \times \frac{[D_3]_p^2}{[D_3]_p^2 + (K_{res}^{D_3})^2}\right)$$ \hspace{1cm} (10)$$

We assume that resorption is activated by (saturating levels of) PTH and D$_3$ [42, 54], and that resorption increases 6-fold when stimulation by PTH and D$_3$ is maximal; that is, $\Gamma_{res}^{min} + \delta_{res}^{max} = 6\Gamma_{res}^{min}$.

### 2.3.3 The kidney compartment

The fraction of calcium that is not bound to plasma proteins is filtered non-selectively in proportion to the glomerular filtration rate (GFR). Most of the filtered load (about 98 %) is
then reabsorbed: roughly two thirds across the tight junctions of the proximal tubule, ~ 25% via the paracellular route in the thick ascending limb (TAL), and ~ 10% in distal convoluted (DCT) and connecting (CNT) tubules via transcellular pathways [40]. The contribution and mechanisms of Ca$^{2+}$ transport in the collecting duct remain poorly understood, and are not explicitly considered here.

As in the intestine, we assume that there is no accumulation of calcium in the kidney over time, so that the fractions of the filtered load that are reabsorbed ($\lambda_{\text{reab}}$) and excreted ($\lambda_u$) sum to unity. In other words:

$$\lambda_{\text{reab}} + \lambda_u = 1 \quad (11)$$

Ca$^{2+}$ reabsorption in the kidney (relative to its filtered load) is given by the sum of fractional Ca$^{2+}$ reabsorption in the proximal tubule ($\lambda_{PT}$), the thick ascending limb ($\lambda_{TAL}$), and the DCT-CNT ($\lambda_{DCT}$):

$$\lambda_{\text{reab}} = \lambda_{PT} + \lambda_{TAL} + \lambda_{DCT} \quad (12)$$

PTH inhibits Ca$^{2+}$ reabsorption in the proximal tubule indirectly: it inhibits the activity of the sodium/proton exchanger NHE3, thereby reducing the transmembrane electric potential difference that drives the paracellular reabsorption of Ca$^{2+}$ [4, 67]. We assume that $\lambda_{PT}$ is given by:

$$\lambda_{PT} = \lambda_{PT}^0 + \frac{\delta_{PT}^{\text{max}}}{1 + \left(\frac{[\text{PTH}]_p}{\text{PTH}_{\text{ref}}}\right)^{n_{PT}}} \quad (13)$$

where $\lambda_{PT}^0$ is taken as 0.60, $\delta_{PT}^{\text{max}}$ as 0.05, PTH$_{\text{ref}}$ as 15 pM, and $n_{PT}$ as 5.
Besides, $\lambda_{TAL}$ is modulated by $\text{Ca}^{2+}$ (via CaSR) and PTH, and $\lambda_{DCT}$ by PTH and vitamin D$_3$. In the thick ascending limb, we assume that:

$$\lambda_{TAL} = \lambda^0_{TAL} + \delta_{TAL}(Ca) + \delta_{TAL}(PTH)$$

(14)

where

$$\delta_{TAL}(Ca) = \frac{\delta_{\text{CASR}}^{\max}}{1 + \left(\frac{[Ca^{2+}]_{p}}{C^{ref}_{\text{TAL}}} \right)^{n_{TAL}}}$$

(15)

$$\delta_{TAL}(PTH) = \frac{\delta_{\text{PTH}}^{\max} [PTH]_{p}}{[PTH]_{p} + K_{\text{PTH}}^{TAL}}$$

(16)

The maximum value of $\lambda_{TAL}$ is set to 0.250; $\lambda^0_{TAL}$ is taken as 0.225, $\delta_{\text{CASR}}^{\max}$ as 0.0175, and $\delta_{\text{PTH}}^{\max}$ as 0.0075. We assume that $C^{ref}$ equals 1.33 mM and $n_{TAL}$ equals 4, so that the regulation by CaSR is very sensitive to small changes in $\text{Ca}^{2+}$ levels.

Similarly, fractional $\text{Ca}^{2+}$ reabsorption in the DCT-CNT is calculated as:

$$\lambda_{DCT} = \lambda^0_{DCT} + \delta_{DCT}(PTH, D_3)$$

(17)

where

$$\delta_{DCT}(PTH, D_3) = \delta_{\text{DCT}}^{\max}\left(0.8 \times \frac{[PTH]_{p}}{[PTH]_{p} + K_{\text{PTH}}^{DCT}} + 0.2 \times \frac{[D_3]_{p}}{[D_3]_{p} + K_{\text{DCT}}^{D_3}} \right)$$

(18)

The maximum value of $\lambda_{DCT}$ equals 0.10, with $\lambda^0_{DCT}$ set to 0.08 and $\delta_{\text{DCT}}^{\max}$ to 0.02. Equation 18 assumes that the contribution of PTH is greater than that of vitamin D$_3$ [91].

### 2.3.4 The plasma compartment

Net changes in the total amount of calcium in plasma result from the combined effect of intestinal calcium absorption, $\text{Ca}^{2+}$ resorption from the slow bone pool, $\text{Ca}^{2+}$ exchanges
with the fast pool, and urinary Ca\(^{2+}\) excretion, such that:

\[
V_p \frac{d[Ca^{tot}]_p}{dt} = \left\{ \Gamma_{abs}(D_3) + \Gamma_{res}(PTH, D_3) + k_f^{Ca}[Ca^{2+}]_f V_f - k_{p-f}^{Ca}[Ca^{2+}]_p V_p \right.  \\
- \lambda_u GFR[Ca^{2+}]_p \right\}
\]

(19)

where \([Ca^{tot}]_p\) is the plasma concentration of total calcium. As described above, a fraction \(\kappa_b\) of calcium in plasma is bound to proteins such as albumin. We assume that \(\kappa_b\) remains fixed (and equal to 0.4) such that:

\[
\frac{d[Ca^{tot}]_p}{dt} = \frac{d[Ca^{2+}]_p}{dt} + \frac{d[Ca^{bound}]_p}{dt},
\]

\[
= \frac{1}{1 - \kappa_b} \frac{d[Ca^{2+}]_p}{dt},
\]

(20)

where \([Ca^{bound}]_p\) is the plasma concentration of bound calcium. Combining the previous two equations, we obtain:

\[
\frac{d[Ca^{2+}]_p}{dt} = \frac{1 - \kappa_b}{V_p} \left\{ \Gamma_{abs}(D_3) + \Gamma_{res}(PTH, D_3) + k_f^{Ca}[Ca^{2+}]_f V_f - k_{p-f}^{Ca}[Ca^{2+}]_p V_p \right.  \\
- \lambda_u GFR[Ca^{2+}]_p \right\}
\]

(21)

Parameters related to the metabolism of PTH, vitamin D\(_3\), and calcium are respectively listed in Tables 1, 2 and 3.

### 2.4 Numerical methods

The differential equations were integrated using the MATLAB solver dde23. Simulations were performed on a personal computer with an Intel-based processor.
3 Experimental Measurements

All experiments were conducted in accordance with the regulations of the Veterinarian office of the Canton de Vaud. Five male and five female mice aged 55 days and weighing ~ 20g were sacrificed, and their skeleton was prepared following the general approach of Pramod and coworkers [72]. Their skin was removed, as well as much of the viscera and muscles. The uncleaned skeletons were washed with water, immersed in a 95% ethanol solution during 2-4 days, and then in a 2% KOH solution. The KOH bath was repeated during 3 extra days (to remove remaining parts of skin, gristles). The skeletons were then cleaned with hot water in a fine-mesh strainer to prevent small bones from being lost, and then weighed. Two samples of final KOH solutions from each mice were examined to ensure that no calcium was dissolved in the bath. Skeletons were dried into a stove during 1 day at 37°C. Finally, they were reduced to ashes in ceramic cups in an oven at 800°C during 4 days (60h heating + cooling time). Ashes were collected, weighed and ultimately dissolved in a 6 mol/L HCl solution (4mL). Their calcium content was assessed by the NM-BAPTA method on a Cobas analyzer.

4 Results

4.1 Experimental Measurements

Most of the variables used for the model were obtained from the literature. However, bone calcium content was measured directly in mice and extrapolated to a 300 g rat. The calcium content of the whole mouse skeleton was 6.02 +/- 0.57 mmol per animal (n = 10) with a
mean weight of 20 g, or 0.28 g of calcium per gram body weight. To extrapolate these data to a 300 g rat, we assumed that the ratio of bone calcium to body weight is the same in rats and mice. With this hypothesis, the total amount of calcium in the rat skeleton was estimated as 105 mmol. Based upon this value and other model parameters, the amount of calcium in the rapidly exchangeable pool was estimated as 1.6 mmol, or 1.5% of the total amount in bone.

4.2 Model Validation

Predicted values of Ca\(^{2+}\), PTH, and vitamin D\(_3\) plasma concentrations at steady-state, summarized in Table 4, all fall within the experimental range. The plasma concentration of Ca\(^{2+}\) (denoted \([\text{Ca}^{2+}]_p\)) is predicted as 1.21 mM. Intestinal Ca\(^{2+}\) absorption is computed as 1.06 \(\mu\)mol/min, urinary excretion is 0.047 \(\mu\)mol/min, and the balance (1.02 \(\mu\)mol/min) is the net flux of Ca\(^{2+}\) into bone (i.e., the difference between accretion and resorption). We also validated our model by comparing the predicted and measured profiles of \([\text{Ca}^{2+}]_p\) as a function of time under different scenarios.

4.2.1 Acutely induced hypocalcemia

Lewin and coworkers infused the chelating agent EGTA to normal and parathyroidectomized rats during two hours [60]. They observed a steep decrease in \([\text{Ca}^{2+}]_p\) during the first 20 minutes, followed by a two-phase return to equilibrium over the next 3 hours. We simulated this experiment by accounting for the reaction between calcium and EGTA in the plasma compartment, as described in the Appendix. As shown in Figure 2, the dynamic behavior of \([\text{Ca}^{2+}]_p\) (normalized by its initial, pre-injection value) that our model predicts closely
matches the experimental data in normal rats.

We also compared model predictions of the plasma concentration of PTH (denoted $[\text{PTH}]_p$) with measured values reported in another study. As observed by Fox [39], when EGTA was infused at a exponentially decreasing rate during 2 hours, $[\text{PTH}]_p$ exhibited a peak-and-plateau behavior: $[\text{PTH}]_p$ reached its peak value in less than 10 minutes, and its plateau value was twice as high as its steady-state value. As illustrated in Figure 2, the predicted and experimental time courses of $[\text{PTH}]_p$ are in good agreement.

4.2.2 Acutely induced hypercalcemia

Finally, we simulated the experiment performed by Bronner and Stein, in which rats received an intravenous calcium gluconate bolus [17]. As shown in Figure 2, predicted values of $[\text{Ca}^{2+}]_p$ fit the experimental data well in the initial phase, but the model predicts too rapid a return to steady-state. Part of this discrepancy may stem from the fact that the rats in these experiments weighed significantly less than our model rats (180 vs. 300 g), such that there must have existed important differences in plasma volume, $[\text{PTH}]_p$ and/or calcium bone content between the two populations.

4.3 Model Predictions

We then used the model to better understand how the plasma concentration of $\text{Ca}^{2+}$ is regulated following various metabolic perturbations.

Primary hyperparathyroidism

Primary hyperparathyroidism was simulated by increasing the base-case PTH synthesis
rate \( k_{\text{prod}}^{\text{PTH}_g} \) by a factor ranging from 1 to 100. Shown in Figure 3 are the predicted steady-state values of \([\text{PTH}]_p\), \([\text{Ca}^{2+}]_p\), and \([\text{D}_3]_p\), as well as intestinal calcium absorption, urinary excretion, resorption, and accretion, as a function of \( k_{\text{prod}}^{\text{PTH}_g} \). The focus of these simulations is not on transient (dynamic) profiles, but on concentrations and fluxes once equilibrium is reached.

Our model suggests that \([\text{PTH}]_p\) increases approximately linearly with \( k_{\text{prod}}^{\text{PTH}_g} \). Since PTH stimulates bone \( \text{Ca}^{2+} \) resorption and reabsorption, as well as the synthesis of vitamin \( \text{D}_3 \) (which in turn enhances intestinal calcium absorption), \([\text{Ca}^{2+}]_p\) and \([\text{D}_3]_p\) rise in parallel with \([\text{PTH}]_p\). Given that the effects of PTH on vitamin \( \text{D}_3 \) production and calcium exchanges between compartments are modeled as saturable processes, the rate at which \([\text{Ca}^{2+}]_p\) and \([\text{D}_3]_p\) increase with PTH synthesis diminishes as \( k_{\text{prod}}^{\text{PTH}_g} \) is elevated beyond 20 times its baseline value.

Note that the rise in \([\text{Ca}^{2+}]_p\) leads to a partial, CaSR-mediated inhibition of PTH exocytosis. This, combined with the inhibitory effects of vitamin \( \text{D}_3 \) on PTH synthesis, explains why the rate at which \([\text{PTH}]_p\) varies with \( k_{\text{prod}}^{\text{PTH}_g} \) is slow at first; the slope increases once \( k_{\text{prod}}^{\text{PTH}_g} \) reaches 10 times its baseline value (Figure 3).

Interestingly, our results suggest that fractional urinary calcium excretion and total urinary calcium excretion \( (U_{\text{Ca}}) \) may not vary monotonically with \( k_{\text{prod}}^{\text{PTH}_g} \). Indeed, urinary \( \text{Ca}^{2+} \) excretion is regulated by counterbalancing mechanisms: whereas an increase in vitamin \( \text{D}_3 \) favors enhanced \( \text{Ca}^{2+} \) reabsorption, increases in \([\text{Ca}^{2+}]_p\), via the renal calcium-sensing receptor (CaSR), act instead to inhibit renal calcium reabsorption; moreover, PTH inhibits and stimulates \( \text{Ca}^{2+} \) transport, respectively, in the proximal tubule and in the distal nephron. Our model predicts that urinary calcium excretion first increases rapidly with increasing
\( k_{\text{PTH}}^{\text{prod}} \), as CaSR-mediated effects prevail. As \( k_{\text{PTH}}^{\text{prod}} \) is elevated above 20 times its baseline value, \([\text{PTH}]_p \) increases at a much greater rate than \([\text{Ca}^{2+}]_p \) and PTH-mediated effects thus become dominant; given the counteracting effects of PTH along the nephron, \( U_{\text{Ca}} \) is predict to decrease slightly before rising steeply (Figure 3). It is equal to 2.4 times its base-case value when \( k_{\text{PTH}}^{\text{prod}} \) is multiplied by 100, and 4.7 times at its plateau level (Figure 4).

These results were obtained assuming a constant GFR. There is evidence that GFR decreases under hypercalcemic conditions; a large enough GFR reduction could abolish the CaSR-induced \( U_{\text{Ca}} \) increase. According to our simulations, a 30% GFR decrease, as reported in Ref. [59], lowers urinary \( \text{Ca}^{2+} \) excretion below its base-case value if \( k_{\text{PTH}}^{\text{prod}} \) is increased by a factor < 60 above its baseline value (and PTH levels by a factor < 4.5); above that, urinary \( \text{Ca}^{2+} \) excretion is predicted to be higher than in the base case.

The extent to which PTH modulates \( \text{Ca}^{2+} \) transport in the proximal tubule remains to be determined. To evaluate the impact of this uncertainty on model results, we varied the PTH-dependent component of \( \text{Ca}^{2+} \) reabsorption in the PT (i.e., the parameter \( \delta_{\text{PT}}^{\max} \) in Eq. 13) between 0 and 0.10; its baseline value is 0.05. In these simulations, the total percentage of \( \text{Ca}^{2+} \) reabsorbed by the PT was kept fixed at 65%, and the PTH synthesis rate \( (k_{\text{PTH}}^{\text{prod}}) \) was increased by a factor ranging from 1 to 300.

In the absence of PTH effects on proximal tubule \( \text{Ca}^{2+} \) transport (i.e., \( \delta_{\text{PT}}^{\max} = 0 \)), urinary calcium excretion fluctuates less as \( k_{\text{PTH}}^{\text{prod}} \) is varied. As \( k_{\text{PTH}}^{\text{prod}} \) is increased above 20 times its baseline value, PTH-mediated stimulation of \( \text{Ca}^{2+} \) reabsorption in the TAL and DCT/CNT predominates and \( U_{\text{Ca}} \) decreases (Figure 4). Conversely, when the (inhibitory) effects of PTH in the proximal tubule are enhanced (i.e., \( \delta_{\text{PT}}^{\max} = 0.10 \)), urinary calcium excretion varies much more significantly with \( k_{\text{PTH}}^{\text{prod}} \). Assuming a constant GFR, absolute urinary
calcium excretion (which equals 0.047 μmol/min in the base case) is predicted to reach a maximal value of 0.06 μmol/min for $\delta_{PT}^{\text{max}} = 0$, 0.22 μmol/min for $\delta_{PT}^{\text{max}} = 0.05$, and 0.37 μmol/min for $\delta_{PT}^{\text{max}} = 0.10$ (Figure 4). In the remainder of the study, $\delta_{PT}^{\text{max}}$ is fixed at 0.05.

Experimental evidence suggests that CaSR and PTH act via independent pathways in the TAL [61]. However, their respective contribution to Ca$^{2+}$ handling in that segment also remains unclear. To probe the impact of this uncertainty, we conducted simulations in which we enhanced the contribution of CaSR relative to that of PTH in the TAL (while keeping the total contribution fixed), and vice-versa. More specifically, we first increased $\delta_{CaSR}^{\text{max}}$ from 0.0175 to 0.0200, while decreasing $\delta_{PTH}^{\text{max}}$ from 0.0075 to 0.0050 (Eq. 14). Conversely, we then raised $\delta_{PTH}^{\text{max}}$ from 0.0075 to 0.0200 while reducing $\delta_{CaSR}^{\text{max}}$ from 0.0175 to 0.0050.

As expected, when the maximal amplitude of CaSR-mediated inhibition is enhanced and that of PTH-mediated stimulation is reduced as described above, the model predicts that total urinary calcium excretion is higher than with base-case parameters, by about 0.006 μmol/min at a given value of $k_{prod}^{PTH}$ (Figure 5). Conversely, when the maximal amplitude of CaSR-mediated inhibition is reduced and that of PTH-mediated stimulation is augmented as described above, $U_{Ca}$ is about 0.03 μmol/min lower than with base-case parameters at a given value of $k_{prod}^{PTH}$ (Figure 5).

Altogether, these results suggest that the extent to which primary hyperparathyroidism affects urinary Ca$^{2+}$ excretion is strongly dependent on the balance between PTH- and CaSR-mediated effects along the nephron. The influence of other factors, such as the hydration status and vitamin D levels, was not considered here.
Primary hypoparathyroidism

We then simulated the opposite scenario, hypoparathyroidism, by decreasing $k_{prod}^{PTH}$ from its base-case value to zero. Depicted in Figure 6 are the steady-state values of $[PTH]_p$, $[Ca^{2+}]_p$, $[D_3]_p$ as well as $Ca^{2+}$ fluxes between compartments. As shown, the plasma concentration of PTH decreases slowly for small reductions in $k_{prod}^{PTH}$, and faster as $k_{prod}^{PTH}$ nears zero; indeed, the compensatory effects exerted by vitamin D$_3$ and $Ca^{2+}$ diminish as their own concentration decreases.

In the absence of PTH, $[D_3]_p$ and $[Ca^{2+}]_p$ are respectively 59% and 41% lower than in the base case: whereas vitamin D$_3$ synthesis isn’t stimulated by PTH any more, it is also less inhibited by calcium under these hypocalcemic conditions. In the absence of PTH, calcium bone resorption is reduced by 55% (relative to the base case) as a result of the $[PTH]_p$ and $[D_3]_p$ decrease, while calcium bone accretion is reduced by 40% owing to the $[Ca^{2+}]_p$ decrease. Intestinal absorption is 33% lower than in the base case.

Urinary $Ca^{2+}$ excretion was computed assuming a constant GFR, as suggested by studies in hypocalcemic dogs [77]. $U_{Ca}$ is determined by the balance between counteracting effects: whereas the reduced levels of PTH and vitamin D$_3$ act to lower $Ca^{2+}$ reabsorption, lower plasma $Ca^{2+}$ levels decrease the filtered load. As shown in Figure 6, the latter effects predominate (i.e., $U_{Ca}$ decreases) until PTH synthesis is almost fully abolished.

Vitamin D$_3$ deficiency

To investigate the effects of vitamin D$_3$ deficiency, we decreased the concentration of its precursor ($[D_3^{inact}]$) by a factor ranging from 1 to 100. In these simulations too, we focused on steady-state profiles. As shown in Figure 7, diminishing $[D_3^{inact}]$ reduces the rate of vitamin
D₃ synthesis, which subsequently decreases the rate of intestinal Ca²⁺ absorption and bone resorption, thereby lowering [Ca²⁺]ₚ. In parallel, diminishing [D₃ inact] reduces the inhibitory effects of vitamin D₃ on PTH production, thereby elevating [PTH]ₚ. Together, the [Ca²⁺]ₚ decrease and [PTH]ₚ increase accelerate the conversion of D₃ inact to vitamin D₃ (i.e., by stimulating 1-α(OH)-ase), therefore mitigating the effects of decreasing the concentration of its precursor. Owing to these feedback mechanisms, when [D₃ inact] is halved, [D₃]ₚ is predicted to decrease by 10% only. Intestinal Ca²⁺ absorption concomitantly decreases from 1.06 to 1.01 μmol/min.

Relative to its base-case value, [PTH]ₚ is 28% higher when [D₃ inact] is halved. The increase in PTH levels favors enhanced Ca²⁺ reabsorption along the nephron, but the [D₃]ₚ decrease acts in the opposite direction. The net result is a decrease in fractional Ca²⁺ excretion (from 1.95 to 1.75%), i.e., an increase in fractional reabsorption. Note that since the filtered load of Ca²⁺ is lower, absolute Ca²⁺ reabsorption is nevertheless slightly reduced (from 2.37 to 2.28 μmol/min).

Similarly, the [PTH]ₚ increase stimulates Ca²⁺ resorption whereas the [D₃]ₚ decrease has opposite effects. There is near compensation; i.e., bone resorption diminishes slightly, from 0.48 (base case) to 0.47 μmol/min when [D₃ inact] is halved. The overall effect of the reduction in calcium absorption, resorption, and urinary excretion is a 4% decrease in [Ca²⁺]ₚ (from 1.21 to 1.16 mM).

As [D₃ inact] is decreased by more than a factor of 10, vitamin D₃ concentrations are predicted to fall more rapidly: the counterbalancing effects of Ca²⁺ and PTH on its conversion by 1-α(OH)-ase (see above) are less potent at very low [D₃ inact] values. Concomitantly, intestinal calcium absorption, bone resorption, and urinary Ca²⁺ excretion decrease more rapidly,
and variations in \([\text{Ca}^{2+}]_p\) and \([\text{PTH}]_p\) with \([\text{D}_3^{\text{act}}] \) become more significant (Figure 7). Since bone accretion decreases at a faster rate than bone resorption, calcium retention diminishes, but it remains positive even under vitamin D\(_3\) depletion.

**CYP24A1 inhibition**

To mimic the effects of an inactivating Cyp24A1 mutation, we set the degradation rate constant \(k_{\text{deg}}^{\text{D}_3}\) to zero. The model then predicts a 8-fold increase in \([\text{D}_3]_p\), a 95 % reduction in \([\text{PTH}]_p\) (since vitamin D\(_3\) inhibits PTH synthesis), and a 44% increase in \([\text{Ca}^{2+}]_p\). Calcium intestinal absorption and calcium bone resorption respectively increase by 49% and 42%. The \([\text{PTH}]_p\) reduction, in combination with the increase in the filtered load of Ca\(^{2+}\), enhances total calcium excretion by a factor of 2.6. In comparison, in Cyp24A1 knock-out mice, \([\text{D}_3]_p\) and \([\text{Ca}^{2+}]_p\) were respectively increased by a factor of 9 and 2 [86].

**Inhibition of resorption**

Bisphosphonates, which inhibit bone resorption, are used to treat several bone diseases, such as osteoporosis and Paget’s disease. To investigate the effects of bisphosphonates on calcium metabolism, we performed simulations in which the maximal resorption rate (the sum of \(\Gamma_{\text{res}}^{\text{min}}\) and \(\delta_{\text{res}}^{\text{max}}\) in Eq. 10) was progressively reduced to zero. We first examined the dynamic effects of halving the maximal rate of resorption on the dynamic evolution of plasma Ca\(^{2+}\), PTH, and vitamin D\(_3\) levels. As depicted in Figure 8, diminishing the resorption rate leads to a sudden decrease in \([\text{Ca}^{2+}]_p\), which in turn triggers a rapid increase in PTH levels, and parallel reductions in accretion and urinary Ca\(^{2+}\) excretion. Since the effects of PTH on vitamin D\(_3\) production occur with a time delay (taken as 4 hours in our model), \([\text{D}_3]_p\)
varies slowly over the first 4 hours. When it starts to rise significantly, intestinal calcium absorption is augmented, which in turn elevates \([\text{Ca}^{2+}]_p\) and conversely lowers \([\text{PTH}]_p\). Due to these feedback mechanisms, oscillations ensue, but they dampen rapidly. At equilibrium, the predicted value of \([\text{Ca}^{2+}]_p\) is 5% lower than its basal value, which is similar to the values reported by Fleisch [38]. The equilibrium values of \([\text{PTH}]_p\) and \([\text{D}_{3}]_p\) are respectively, 17% and 30% higher than in the base case, which is also comparable to experimental measurements [34].

We then varied the resorption rate over a large range, from its base-case value to zero. Shown in Figure 9 are predicted concentrations and fluxes at steady-state (i.e., once equilibrium has been reached) as a function of the resorption rate. As described above, inhibiting resorption lowers the plasma level of \(\text{Ca}^{2+}\), raises that of \(\text{PTH}\) and vitamin \(\text{D}_{3}\), enhances \(\text{Ca}^{2+}\) absorption, decreases \(\text{Ca}^{2+}\) accretion, and reduces both absolute and fractional urinary \(\text{Ca}^{2+}\) excretion.

Our results suggest that the (steady-state) plasma concentration of \(\text{PTH}\) increases with decreasing resorption, before reaching saturation (Figure 9): as \([\text{Ca}^{2+}]_p\) decreases, CaSR-mediated inhibition of \(\text{PTH}\) exocytosis is reduced. Since \(\text{PTH}\) stimulates the synthesis of vitamin \(\text{D}_{3}\) and inhibits its degradation, \([\text{D}_{3}]_p\) increases in parallel with \(\text{PTH}\) (Figure 9). In counterbalance, the increase in vitamin \(\text{D}_{3}\) levels lowers the rate of \([\text{PTH}]\) synthesis.

Intestinal calcium absorption, which is stimulated by vitamin \(\text{D}_{3}\), increases as the resorption rate is lowered, whereas \(\text{Ca}^{2+}\) accretion decreases (Figure 9). The model predicts that the rate of accretion falls more slowly than that of resorption, so that \(\text{Ca}^{2+}\) retention increases, as observed experimentally [38, 75]. Urinary \(\text{Ca}^{2+}\) excretion decreases with the resorption rate, which indicates that the reabsorption-enhancing effects of \(\text{PTH}\) and vitamin
D₃ (as a result of PTH and vitamin D₃ elevation) are greater than the excretion-enhancing effects of Ca²⁺ (via CaSR).

**Cinacalcet treatment**

Cinacalcet, a calcimimetic agent acting on the calcium sensing receptor by increasing its sensitivity to calcium is commonly used to treat secondary hyperparathyroidism in chronic renal failure or, more rarely, primary hyperparathyroidism. Cinacalcet decreases PTH exocytosis from parathyroid glands, and decreases Ca²⁺ reabsorption in the TAL [61].

To assess the theoretical impact of Cinacalcet on rats with hyperparathyroidism, we performed the following simulations. To mimic primary hyperparathyroidism, we increased the rate of PTH synthesis by a factor of 185, so that the plasma concentration of PTH is 10-fold its basal value. To mimic the effects of Cinacalcet, we fixed the rate of PTH exocytosis to its minimal value, and maximized the inhibitory effects of CaSR on Ca²⁺ reabsorption in the TAL. As illustrated in Table 5, Cinacalcet is predicted to return both [PTH]ₚ and [D₃]ₚ in rats with hyperparathyroidism to their levels in control animals; the plasma calcium concentration is thus rescued, i.e., its predicted value (1.24 mM) falls within the normal range. The rates of calcium exchanges between compartments are also very close to their base-case values (i.e., without primary hyperparathyroidism).

**Effects of age and sex on calcium homeostasis**

Maintenance of the calcium balance varies between the two sexes; it also evolves with age. In particular, the plasma concentration of vitamin D₃ is about 50% lower in female rats than in age-matched male rats [65]. Vitamin D₃ levels (and intestinal calcium absorption) also
decrease with age [95]. Conversely PTH levels increase with age, but do not vary significantly between age-matched female and male rats [39, 44, 65]. Bone turnover is also widely affected by aging [13, 31]. Despite these differences, plasma calcium concentration varies very little as a function of either age or sex, under physiological conditions.

We used the model to examine how \([\text{Ca}^{2+}]_p\) can remain stable given these documented variations in hormonal levels. We considered 2- and 8-month old, male and female rats, and accounted for differences in PTH and vitamin D$_3$ synthesis, as described above. We also accounted for sex- and age-based differences in plasma volume (\(V_p\)), glomerular filtration rate (GFR), calcium intake (\(I_{Ca}\)), the sensitivity of intestinal absorption to vitamin D$_3$ (\(\Gamma_{abs}^{D_3}\)), and the rate of bone resorption (i.e., the parameters \(\Gamma_{\text{res}}^{\min}\) and \(\delta_{\text{res}}^{\max}\)), as summarized in Table 6. The model predicts that to maintain \([\text{Ca}^{2+}]_p\) around 1.2 mM in all 4 rat groups, it is also necessary to posit sex- and age-based differences in the rate of calcium exchanges between plasma and the rapidly exchangeable bone pool fluxes (i.e., \(k_{fp}^{Ca}\) and \(k_{pf}^{Ca}\)), as also suggested by one study [12]. However, other model parameters need not change (Table 6).

Intestinal absorption is predicted to be halved in 2-month old females compared to young males, mainly because vitamin D$_3$ levels are 50% lower in females. Bone resorption follows the same trend. Bone accretion is decreased in females because they have less calcium in the rapidly exchangeable bone pool than males. This results in a lower bone turnover in females and a net calcium flux into the deep bone that is halved. Urinary calcium excretion is predicted to be reduced in females, as a result of the lower GFR, even though fractional calcium excretion is higher in females (owing to lower levels of vitamin D$_3$). In 8-month old animals, the intestinal absorption and bone accretion of calcium are substantially reduced (\(\sim -50\%\) in both sexes). Given that PTH levels increase with age, urinary calcium excretion
is predicted to be lower (-15% and -17%, respectively, in 8-month old male and female rats).

5 Discussion

Scope of model

We have developed a mathematical model that describes Ca\(^{2+}\) exchanges between the intestine, plasma, slowly and rapidly exchangeable bone pools, and the kidneys, as well as their regulation by PTH and vitamin D\(_3\). To our knowledge, this is the first such model of Ca\(^{2+}\) homeostasis that applies to the rat, accounts for the rapidly exchangeable pool in bone, and considers the impact of the renal CaSR. As shown in Figure 2, the model adequately reproduces experimental findings in different scenarios.

Model limitations

We should nevertheless acknowledge a number of limitations. Model parameters apply to \(~300\)g rats, a weight at which rats are usually used for physiological experiments but for which experimental calcium measurements are limited. We thus extrapolated measured values of calcium bone content in mice. The model also assumes fixed values for plasma volume and GFR, whereas these may vary in tandem with Ca\(^{2+}\) and/or PTH under certain conditions. Taking into consideration the numerous factors that regulate plasma volume and GFR is beyond the scope of this model. Instead, we accounted for observed changes in GFR in specific simulations.

In addition, our model does not consider the interactions between calcium and phosphate, which are particularly relevant in bone. Accounting for this coupling is challenging because
the regulation of phosphate homeostasis has not been fully characterized. Fibroblast growth factor 23 (FGF23), a bone-derived hormone that regulates systemic phosphate homeostasis, has recently been recognized as an important component of the bone-parathyroid-kidney axis; in particular, FGF23 inhibits the synthesis of vitamin D₃ and the secretion of PTH [8]. Of note, FGF23 may have also a direct effect on calcium transport in the kidney [5]. A better quantitative understanding of phosphate metabolism is needed before it can be added to the model.

Since we do not represent the metabolism of compounds other than Ca²⁺, PTH, and vitamin D₃, we cannot account for the allosteric factors that modulate the binding affinity of CaSR to Ca²⁺, such as sodium and ionic strength. Nor can we account for the effects of plasma volume, sodium reabsorption, and thiazides on the fraction of Ca²⁺ that is reabsorbed in the proximal tubule (λₚₜ). Similarly, the model does not consider other known regulators of calcium homeostasis, including klotho, calcitonin, and steroids. Sex hormones are also known to regulate calcium homeostasis by modifying calcium absorption in the intestine, calcium bone metabolism, and renal calcium reabsorption. The model does not explicitly represent the mechanisms underlying these regulations and takes into account only some aspects of sex-based differences (body size, calcium intake, as well as PTH and vitamin D₃ levels) [30, 49, 66].

Finally, the model is hampered by its description of organs as black boxes, meaning that transport and regulation mechanisms at the molecular and cellular scales are described instead by simplified relationships, such as first-order kinetics and Michaelis-Menten equations. There exists detailed, cellular-based models of calcium homeostasis in kidney [32], and intestine [33, 46, 83]; these could be integrated in the present mathematical model in
the future. Similarly, models of bone remodeling could be incorporated to describe more accurately the dynamics of bone resorption and formation [58, 71].

**PTH-related disorders and Ca$^{2+}$ metabolism**

Our results suggest that the effects of primary hyperparathyroidism (HPTH) on urinary Ca$^{2+}$ excretion (and thus hypercalciuria) depend in part on the degree to which PTH levels are increased. PTH favors the release of Ca$^{2+}$ from bone and therefore enhances its filtered load, but it also stimulates Ca$^{2+}$ reabsorption along the nephron. Moreover, PTH and Ca$^{2+}$ exert opposite effects on Ca$^{2+}$ fluxes in the thick ascending limb. Whereas an increase in PTH stimulates reabsorption therein, increased binding of Ca$^{2+}$ to calcium-sensing receptors expressed by that segment inhibit reabsorption. Thus, our model predicts that whether urinary Ca$^{2+}$ excretion increases or decreases varies according to the balance between PTH and Ca$^{2+}$, i.e., their respective contribution to Ca$^{2+}$ fluxes along the nephron. The inconstant presence of hypercalciuria in primary hyperparathyroidism may also be due to variations in vitamin D levels. Broadus et al. observed a strong positive correlation between plasma levels of vitamin D and calcium excretion in humans with primary hyperparathyroidism [14]. Altogether these results suggest that the high variability of hypercalciuria in primary hyperparathyroidism may be related to differences in PTH-CaSR and/or vitamin D levels.

Patients with hypoparathyroidism are treated with vitamin D supplements that restore their plasma Ca$^{2+}$ levels but promote hypercalciuria, as intestinal calcium reabsorption is stimulated while PTH-enhancing effects on renal Ca$^{2+}$ reabsorption are significantly reduced [93, 94]. It has been suggested that CaSR inhibitors may be used to treat disorders related to impaired PTH secretion [61], and a recent model of Ca$^{2+}$ transport along the nephron
suggests that blocking the renal CaSR could significantly reduce urinary Ca\(^{2+}\) excretion in the absence of PTH secretion [32]. In the present study, fractional Ca\(^{2+}\) reabsorption along the entire nephron equals 88.2 % in parathyroidectomized rats. Inhibition of the renal CaSR would raise that fraction to 92.4 %, without a noticeable impact on \([\text{Ca}^{2+}]_p\) or on calcium exchanges between other compartments. However, given that concentration of Ca\(^{2+}\) in the tubular fluid increases approximately 10-fold along the collecting duct, a small increase in reabsorption in the TAL can have a significant impact on urinary concentration [32]. The current model does not track tubular and urinary Ca\(^{2+}\) concentrations, however.

**Other regulations of Ca\(^{2+}\) metabolism**

According to our simulations, when PTH synthesis is raised so that \([\text{PTH}]_p\) is doubled, \([\text{Ca}^{2+}]_p\) increases by 44%. In contrast, when \([\text{D}_3^{\text{inact}}]\) is raised so that \([\text{D}_3]\) is doubled, \([\text{Ca}^{2+}]_p\) increases by 26%. However, we cannot conclude that plasma Ca\(^{2+}\) levels are more sensitive to changes in PTH levels than in vitamin D\(_3\) levels, since raising \([\text{PTH}]_p\) stimulates calcitriol production, whereas raising \([\text{D}_3]_p\) represses PTH synthesis. Given the combination of both negative and positive feedback mechanisms, the interactions between Ca\(^{2+}\), PTH, and vitamin D\(_3\) are highly non-linear, as illustrated in Figures 3, 5, and 7.

We investigated the effects of bisphosphonates on calcium metabolism in rats by lowering the maximal resorption rate over the entire duration of the simulation. Thus our results should be compared with experimental studies in which bisphosphonates were administered to rats chronically. In accordance with these studies, our model predicts that inhibiting resorption reduces plasma Ca\(^{2+}\) concentrations and increases Ca\(^{2+}\) retention [75]. The model also predicts that the secondary hyperparathyroidism of bisphosphonate treatment reduces
urinary Ca\(^{2+}\) excretion, as observed by Bushinsky et al. [19]; other studies, however, have found that at high doses, bisphosphonates may raise U\(_{\text{Ca}}\), for reasons that remain unclear [75]. It has been suggested that some bisphosphonates may exert direct effects on tubular calcium transport in the kidney [75].

Overall, the effect of vitamin D\(_3\) on intestinal calcium absorption is predicted to be limited. In our simulations, primary hyperparathyroidism resulted in only a 1.5-fold increase in intestinal calcium reabsorption despite a 7-fold increase in vitamin D\(_3\) levels. During profound vitamin D\(_3\) deficiency, however, intestinal calcium absorption decreased significantly. The proposed model for calcium homeostasis takes into account the role of the two CYP450 enzymes involved in the tight control of vitamin D\(_3\) metabolism, i.e., CYP27B1 and CYP24A1. The latter enzyme has been shown to be critical for regulating calcemia, as genetic variants lead to hypersensitivity to vitamin D\(_3\) supplementation and hypercalcemia. We reproduced here the phenotype induced by decreasing CYP24A1 activity and found that vitamin D\(_3\) levels increased 8-fold, plasma calcium by 44%, and calciuria by a factor of 2.6. Overall, the model fits well with observations in infantile hypercalcemia [79].

The model takes into account a pool of calcium that is rapidly available from the bone. This pool was described several decades ago following radiolabeled calcium experiments in vivo [92]. Our model suggests that calcium exchanges between plasma and the rapidly exchangeable pool are 2- to 20-fold greater (depending upon [Ca\(^{2+}\)]\(_{p}\)) than those between plasma and the slowly exchangeable pool. As currently integrated in the model, the rapid exchange of calcium between plasma and bone plays an important role in both the dynamic evolution and the overall stability of plasma calcium levels.
In summary, this model of calcium homeostasis, which includes two important calcium regulators, i.e., the renal CaSR and the rapidly exchangeable bone calcium pool for which renewed interest has been evinced, shows robustness when challenged. In particular, it predicts that hypercalciuria in primary hyperparathyroidism is dependent upon the ratio of PTH vs. CaSR activity and upon vitamin D₃ levels. In primary hypoparathyroidism, urinary calcium excretion is partly sustained, given the absence of PTH-induced stimulation of calcium reabsorption.
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Figure Captions

Figure 1: Schematic diagram of calcium exchanges between plasma, bone, intestine, and kidney. Calcium fluxes between compartments are depicted with black arrows. Regulation by parathyroid hormone (shown in dashed arrows) acts to enhance bone resorption and renal calcium reabsorption, and to stimulate the conversion of the inactive form of vitamin D$_3$ into its active form. Regulation by vitamin D$_3$ (dotted arrows) acts to increase bone resorption, intestinal calcium absorption, and renal calcium reabsorption; vitamin D$_3$ also represses its own synthesis. In addition, plasma calcium levels regulate PTH secretion via the calcium-sensing receptor (CaSR) and inhibit the synthesis of vitamin D$_3$. CaSR-mediated effects on renal calcium reabsorption are not shown in this figure.

Figure 2: Comparison of model results (solid curves) with experimental data (circles). Panel A depicts the $[\text{Ca}^{2+}]_p$ profile obtained by Lewin et al. following a 20 minute infusion of EGTA [60]. Panel B represents the plasma concentration of PTH measured by Fox during a 2 hour administration of EGTA [39]. Panel C shows the $[\text{Ca}^{2+}]_p$ profile obtained by Bronner and Stein following the rapid intravenous injection of calcium-gluconate [17].
Figure 3: Predicted effects of primary hyperparathyroidism on concentrations and fluxes at steady-state. Panel A: normalized plasma concentration (relative to base-case value) of Ca\(^{2+}\) (solid curve), PTH (dashed curve), and vitamin D\(_3\) (dotted curve), as a function of PTH synthesis (normalized by its base-case value). The rate of PTH synthesis is increased by a factor ranging from 1 to 100. Panel B: urinary Ca\(^{2+}\) excretion (dashed curve), intestinal calcium absorption (dotted curve), bone accretion (solid curve), and bone resorption (dot-dashed curve), as a function of PTH synthesis (normalized by its base-case value); fluxes are normalized by their base-case value.

Figure 4: Predicted urinary Ca\(^{2+}\) excretion as a function of the normalized PTH synthesis rate, depending on the relative contribution of PTH to Ca\(^{2+}\) reabsorption in the proximal tubule. The rate of PTH synthesis is increased by a factor ranging from 1 to 300. The parameters \(\lambda^0_{PT}\) and \(\delta^{max}_{PT}\) are respectively taken as 0.65 and 0 (dashed curve; without PTH effects on proximal tubule reabsorption), 0.60 and 0.05 (solid curve, base-case values), and 0.55 and 0.10 (dot-dashed curve).

Figure 5: Predicted urinary Ca\(^{2+}\) excretion as a function of the normalized PTH synthesis rate, depending on the relative contribution of PTH and the renal CaSR to Ca\(^{2+}\) reabsorption in the thick ascending limb. The rate of PTH synthesis is increased by a factor ranging from 1 to 300. The parameters \(\delta^{max}_{CaSR}\) and \(\delta^{max}_{PTH}\) in Eq. 15 and Eq. 16 are respectively taken as 0.0175 and 0.0075 (solid curve; base-case values), 0.020 and 0.005 (dot-dashed curve), and 0.005 and 0.020 (dotted curve).
Figure 6: Predicted effects of primary hypoparathyroidism on concentrations and fluxes at steady-state. Panel A: fractional change in the plasma concentration of Ca\(^{2+}\), PTH, and vitamin D\(_3\), as a function of the percentage of PTH synthesis inhibition. Panel B: fractional change in urinary Ca\(^{2+}\) excretion, intestinal calcium absorption, bone accretion, and bone resorption, as a function of the percentage of PTH synthesis inhibition.

Figure 7: Predicted effects of vitamin D\(_3\) deficiency on steady-state concentrations and fluxes. Panel A: normalized plasma concentration of Ca\(^{2+}\), PTH, and vitamin D\(_3\), as a function of \([D_{3}^{\text{inact}}]_p\) (normalized by its base-case value). Panel B: normalized urinary Ca\(^{2+}\) excretion, intestinal calcium absorption, bone accretion, and bone resorption, as a function of \([D_{3}^{\text{inact}}]_p\) (normalized by its base-case value).

Figure 8: Predicted effects of inhibiting bone resorption on the evolution of concentrations and fluxes with time. In this simulation, the maximal bone resorption rate is divided by 2 starting at \(t = 2\) hours. Panels A, B, and C respectively depict the plasma concentration of Ca\(^{2+}\), PTH, and vitamin D\(_3\), as a function of time; panel D: urinary Ca\(^{2+}\) excretion; panel E: intestinal calcium absorption, accretion, and resorption.

Figure 9: Predicted effects of inhibiting bone resorption on steady-state concentrations and fluxes. Panel A: fractional change in the plasma concentration of Ca\(^{2+}\), PTH, and vitamin D\(_3\), as a function of the percentage of inhibition. Panel B: fractional change in urinary Ca\(^{2+}\) excretion, intestinal calcium absorption, bone accretion, and bone resorption, as a function of the percentage of inhibition.
Appendix

In a subset of simulations, we considered the reaction between calcium and the chelator ethylene glycol tetraacetic acid (EGTA):

\[
\begin{align*}
  k_{\text{EGTA}}^+ & \quad [Ca^{2+}] + [EGTA] \quad \Rightarrow \quad [Ca.EGTA] \\
  k_{\text{EGTA}}^- & 
\end{align*}
\]

The rate of reaction \( R_{\text{EGTA}} \) is given by:

\[
R_{\text{EGTA}} = -k_{\text{EGTA}}^+[Ca^{2+}][EGTA] + k_{\text{EGTA}}^-[Ca.EGTA] \tag{22}
\]

In the presence of EGTA in plasma, the conservation equation of \( Ca^{2+} \) in plasma (Eq. 21) is thus written as:

\[
\frac{d[Ca^{2+}]_p}{dt} \bigg|_{\text{EGTA}} = \frac{(1 - \kappa_b)}{V_p} \left\{ \Gamma_{abs}(D_3) + \Gamma_{res}(PTH, D_3) + k_{\text{Ca}}^p[Ca^{2+}]_f V_f \\
- k_{\text{Ca}}^{p-f}[Ca^{2+}]_p V_p - \lambda_v GFR[Ca^{2+}]_p \right\} - R_{\text{EGTA}} \tag{23}
\]

The kinetic rates \( k_{\text{EGTA}}^+ \) and \( k_{\text{EGTA}}^- \) are respectively taken as \( 9 \times 10^4 \text{ mM}^{-1} \cdot \text{min}^{-1} \) and \( 18 \text{ min}^{-1} \) [2, 84, 85]. To mimic the protocol used by Lewin et al. [60], we simulated a continuous intravenous injection of EGTA during 20 min using the following differential equations:

\[
\frac{d[EGTA]_p}{dt} = \frac{1}{V_p} k_{\text{inject}}^{\text{EGTA}} + R_{\text{EGTA}} \tag{24}
\]

\[
\frac{d[Ca.EGTA]_p}{dt} = k_{\text{EGTA}}^+[Ca^{2+}]_p[EGTA]_p - k_{\text{EGTA}}^-[Ca.EGTA]_p \tag{25}
\]
where $k_{\text{inject}}^{\text{EGTA}}$ is the rate of injection. During the injection, this rate is set to $3 \times 10^{-4}$ mmol.min$^{-1}$, as estimated from the concentration of the EGTA solution, the rate of injection, and the total volume injected. Before and after the 20 minute injection, $k_{\text{inject}}^{\text{EGTA}}$ is set to 0. In addition, immediately prior to injection, the plasma concentrations of EGTA and Ca.EGTA are zero, whereas $[\text{Ca}^{2+}]_p$ is equal to its equilibrium value (1.21 mM).

To reproduce the $[\text{Ca}^{2+}]_p$ profile obtained by Fox and coworkers [39], we used the same equations, but modified the injection rate. During the first 2 minutes, $k_{\text{inject}}^{\text{EGTA}}$ was set to $1.7 \times 10^{-3}$ mmol.min$^{-1}$, yielding the observed 0.3 mM decrease in $[\text{Ca}^{2+}]_p$. For the next 118 minutes, during which $[\text{Ca}^{2+}]_p$ remained stable, the rate of injection was set to:

$$k_{\text{inject}}^{\text{EGTA}} = 1.6 \times 10^{-4} \left[ e^{0.001(t-10)} + e^{0.0025(t-30)} \right].$$

(26)

where time (t) is expressed in minutes.
Table 1: PTH parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Symbol</th>
<th>Value</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>PTH(_g) synthesis rate</td>
<td>(k_{prod}^{PTH_g})</td>
<td>1.8 (\mu)mol.min(^{-1})</td>
<td>estimated</td>
</tr>
<tr>
<td>Inhibition of PTH(_g) synthesis by vitamin D(_3)</td>
<td>(\gamma_{prod}^{D_3})</td>
<td>(5 \times 10^{-3}) pM(^{-1})</td>
<td>estimated</td>
</tr>
<tr>
<td>PTH(_g) degradation rate</td>
<td>(k_{deg}^{PTH_g})</td>
<td>0.035 min(^{-1})</td>
<td>[1]</td>
</tr>
<tr>
<td>Maximal secretion rate of PTH(_g)</td>
<td>(\beta_{exo}^{PTH_g})</td>
<td>0.059 min(^{-1})</td>
<td>fitted from [39]</td>
</tr>
<tr>
<td>Maximal inhibition of secretion by Ca(^2+)</td>
<td>(\gamma_{exo}^{PTH_g})</td>
<td>0.057 min(^{-1})</td>
<td>fitted from [39]</td>
</tr>
<tr>
<td>Binding of Ca(^2+) to CaSR</td>
<td>(K_{Ca_p^{2+}})</td>
<td>1.16 mM</td>
<td>[39]</td>
</tr>
<tr>
<td></td>
<td>(n_1^{exo})</td>
<td>100</td>
<td>[80]</td>
</tr>
<tr>
<td></td>
<td>(n_2^{exo})</td>
<td>15</td>
<td>[80]</td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>1.1 mM</td>
<td>[80]</td>
</tr>
<tr>
<td></td>
<td>(\rho_{exo})</td>
<td>(10^6) mM(^{-1})</td>
<td>[80]</td>
</tr>
<tr>
<td>PTH(_p) degradation rate</td>
<td>(k_{deg}^{PTH_p})</td>
<td>2.2 min(^{-1})</td>
<td>[1]</td>
</tr>
<tr>
<td>Volume of parathyroid glands</td>
<td>(V_c)</td>
<td>0.1 (\mu)L</td>
<td>[45]</td>
</tr>
</tbody>
</table>

PTH\(_g\) and PTH\(_p\) respectively denote parathyroid hormone in the parathyroid gland and in plasma.
Table 2: Vitamin D$_3$ parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Symbol</th>
<th>Value</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minimum production rate of vitamin D$_3$</td>
<td>$k_{conv}^{min}$</td>
<td>$4.4 \times 10^{-6}$ min$^{-1}$</td>
<td>[89]</td>
</tr>
<tr>
<td>Maximal increase in vitamin D$_3$ production rate</td>
<td>$\delta_{conv}^{max}$</td>
<td>$6.02 \times 10^{-5}$ min$^{-1}$</td>
<td>[89]</td>
</tr>
<tr>
<td>Plasma concentration of D$_3^{inact}$</td>
<td>$[D_3^{inact}]_p$</td>
<td>25 nM</td>
<td>[39]</td>
</tr>
<tr>
<td>Activation of vitamin D$_3$ production by PTH</td>
<td>$K_{conv}$</td>
<td>3 pM</td>
<td>estimated</td>
</tr>
<tr>
<td>PTH sensitivity coefficient</td>
<td>$n_{conv}$</td>
<td>6</td>
<td>estimated</td>
</tr>
<tr>
<td>Time delay for PTH effects on D$_3^{inact}$ conversion</td>
<td>$\tau$</td>
<td>240 min</td>
<td>[55]</td>
</tr>
<tr>
<td>Inhibition of D$_3$ production by Ca$^{2+}$</td>
<td>$\gamma_{conv}^{Ca}$</td>
<td>0.3 mM$^{-1}$</td>
<td>estimated</td>
</tr>
<tr>
<td>Inhibition of vitamin D$_3$ production by itself</td>
<td>$\gamma_{conv}^{D_3}$</td>
<td>$1.8 \times 10^{-2}$ pM$^{-1}$</td>
<td>estimated</td>
</tr>
<tr>
<td>Degradation rate of vitamin D$_3$</td>
<td>$k_{deg}^{D_3}$</td>
<td>0.0029 min$^{-1}$</td>
<td>[6, 29, 51]</td>
</tr>
<tr>
<td>Inhibition of Cyp24a1 by PTH</td>
<td>$\gamma_{deg}^{PTH}$</td>
<td>0.52 pM$^{-1}$</td>
<td>estimated</td>
</tr>
</tbody>
</table>
Table 3: Ca\(^{2+}\) parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Symbol</th>
<th>Value</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium intake</td>
<td>I(_{Ca})</td>
<td>2.3 (\mu)mol.min(^{-1})</td>
<td>[25, 61]</td>
</tr>
<tr>
<td>Stimulation of absorption by D(_3)</td>
<td>(K_{D3}^{obs})</td>
<td>100 pM</td>
<td>estimated</td>
</tr>
<tr>
<td>Rate of Ca(^{2+}) transfer from plasma to fast bone pool</td>
<td>(k_{plf}^{Ca})</td>
<td>0.1253 min(^{-1})</td>
<td>estimated</td>
</tr>
<tr>
<td>Rate of Ca(^{2+}) transfer from fast bone pool to plasma</td>
<td>(k_{fp}^{Ca})</td>
<td>(10^{-5}) min(^{-1})</td>
<td>estimated</td>
</tr>
<tr>
<td>Initial calcium content in bone</td>
<td>(N_{Ca,b})</td>
<td>105 mmol</td>
<td>measured</td>
</tr>
<tr>
<td>Initial calcium content in rapidly exchangeable pool</td>
<td>(N_{Ca,r})</td>
<td>2.34 mmol</td>
<td>measured</td>
</tr>
<tr>
<td>Accretion rate</td>
<td>(\Gamma_{ac})</td>
<td>0.958 (\mu)mol.min(^{-1})</td>
<td>[25]</td>
</tr>
<tr>
<td>Minimal resorption rate</td>
<td>(\Gamma_{res}^{min})</td>
<td>0.142 (\mu)mol.min(^{-1})</td>
<td>[25]</td>
</tr>
<tr>
<td>Maximal resorption rate</td>
<td>(\delta_{res}^{max})</td>
<td>0.700 (\mu)mol.min(^{-1})</td>
<td>[25]</td>
</tr>
<tr>
<td>Stimulation of resorption by D(_3)</td>
<td>(K_{D3}^{D3})</td>
<td>100 pM</td>
<td>estimated</td>
</tr>
<tr>
<td>Stimulation of resorption by PTH</td>
<td>(K_{PTH}^{PT})</td>
<td>1.75 pM</td>
<td>estimated</td>
</tr>
<tr>
<td>Glomerular filtration rate</td>
<td>GFR</td>
<td>2 mL.min(^{-1})</td>
<td>[97]</td>
</tr>
<tr>
<td>Minimal fractional reabsorption of Ca(^{2+}) in the PT</td>
<td>(\lambda_{PT}^{0})</td>
<td>0.60</td>
<td>[9, 68]</td>
</tr>
<tr>
<td>Stimulation of Ca(^{2+}) reabsorption in PT by PTH</td>
<td>(\delta_{PT}^{max})</td>
<td>0.05</td>
<td>estimated</td>
</tr>
<tr>
<td>Sensitivity of Ca(^{2+}) reabsorption in PT to PTH</td>
<td>(\Gamma_{PTH}^{ref})</td>
<td>15 pM</td>
<td>estimated</td>
</tr>
<tr>
<td>Minimal fractional reabsorption of Ca(^{2+}) in the TAL</td>
<td>(\lambda_{TAL}^{0})</td>
<td>0.225</td>
<td>[9, 68]</td>
</tr>
<tr>
<td>Stimulation of Ca(^{2+}) reabsorption in TAL by PTH</td>
<td>(\delta_{TAL}^{max})</td>
<td>0.0075</td>
<td>estimated</td>
</tr>
<tr>
<td>Sensitivity of Ca(^{2+}) reabsorption in TAL to PTH</td>
<td>(K_{TAL}^{PTH})</td>
<td>1.2 pM</td>
<td>estimated</td>
</tr>
<tr>
<td>Stimulation of Ca(^{2+}) reabsorption in TAL by CaSR</td>
<td>(\delta_{CaSR}^{max})</td>
<td>0.0175</td>
<td>estimated</td>
</tr>
<tr>
<td>Sensitivity of Ca(^{2+}) reabsorption in TAL to Ca(^{2+})</td>
<td>(C_{ref})</td>
<td>1.33 mM</td>
<td>estimated</td>
</tr>
<tr>
<td>Minimal fractional reabsorption of Ca(^{2+}) in the DCT-CNT</td>
<td>(\lambda_{DCT}^{0})</td>
<td>0.080</td>
<td>[9, 68]</td>
</tr>
<tr>
<td>Stimulation of Ca(^{2+}) reabsorption in the DCT-CNT by PTH and vitamin D(_3)</td>
<td>(\delta_{DCT}^{max})</td>
<td>0.020</td>
<td>estimated</td>
</tr>
<tr>
<td>Sensitivity of Ca(^{2+}) reabsorption in the DCT-CNT to PTH</td>
<td>(K_{DCT}^{PTH})</td>
<td>1.8 pM</td>
<td>estimated</td>
</tr>
<tr>
<td>Sensitivity of Ca(^{2+}) reabsorption in the DCT-CNT to vitamin D(_3)</td>
<td>(K_{D3}^{DCT})</td>
<td>80 pM</td>
<td>estimated</td>
</tr>
<tr>
<td>Plasma volume</td>
<td>(V_{p})</td>
<td>10 mL</td>
<td>[56]</td>
</tr>
<tr>
<td>Fraction of bound calcium</td>
<td>(\kappa_{b})</td>
<td>0.4</td>
<td>[88]</td>
</tr>
</tbody>
</table>

PT: proximal tubule; TAL: thick ascending limb; DCT: distal convoluted tubule; CNT: connecting tubule. Parameters apply to a 300g (2 month-old) male rat.
Table 4: Steady-state values under normal conditions

<table>
<thead>
<tr>
<th></th>
<th>Equilibrium</th>
<th>range</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>$[\text{Ca}^{2+}]_p$ (mM)</td>
<td>1.21</td>
<td>1.1-1.3</td>
<td>[10, 15, 74, 91]</td>
</tr>
<tr>
<td>$[\text{PTH}]_p$ (pM)</td>
<td>1.93</td>
<td>1.5-13</td>
<td>[10, 22, 39, 91]</td>
</tr>
<tr>
<td>$[\text{D}_3]$ (pM)</td>
<td>94.5</td>
<td>80-250</td>
<td>[20, 23, 43]</td>
</tr>
<tr>
<td>Intestinal $\text{Ca}^{2+}$ absorption (µmol/min)</td>
<td>1.06</td>
<td>0.55-1.22</td>
<td>[38, 61]</td>
</tr>
<tr>
<td>Fractional intestinal absorption, $F_{\text{intest}}$ (%)</td>
<td>46.2</td>
<td>40-60</td>
<td>[21]</td>
</tr>
<tr>
<td>$\text{Ca}^{2+}$ accretion (µmol/min)</td>
<td>1.50</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\text{Ca}^{2+}$ resorption (µmol/min)</td>
<td>0.48</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Net $\text{Ca}^{2+}$ flux into bone (µmol/min)</td>
<td>1.02</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urinary $\text{Ca}^{2+}$ excretion (µmol/min)</td>
<td>0.047</td>
<td>0.015-0.054</td>
<td>[52, 61, 78, 97]</td>
</tr>
<tr>
<td>Fractional excretion, $\lambda_u$ (%)</td>
<td>1.95</td>
<td>0-2</td>
<td>[52, 61, 78, 97]</td>
</tr>
</tbody>
</table>
Table 5: Effects of Cinacalcet administration to rats with primary hyperparathyroidism

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>PHPT</th>
<th>PHPT with Cinacalcet</th>
</tr>
</thead>
<tbody>
<tr>
<td>$[\text{Ca}^{2+}]_p$ (mM)</td>
<td>1.21</td>
<td>1.79</td>
<td>1.24</td>
</tr>
<tr>
<td>$[\text{PTH}]_p$ (pM)</td>
<td>1.93</td>
<td>19.5</td>
<td>2.01</td>
</tr>
<tr>
<td>$[\text{D}_3]_p$ (pM)</td>
<td>94.6</td>
<td>665.5</td>
<td>100.4</td>
</tr>
<tr>
<td>Intestinal $\text{Ca}^{2+}$ absorption ($\mu$mol/min)</td>
<td>1.06</td>
<td>1.59</td>
<td>1.09</td>
</tr>
<tr>
<td>$\text{Ca}^{2+}$ accretion ($\mu$mol/min)</td>
<td>1.50</td>
<td>2.21</td>
<td>1.57</td>
</tr>
<tr>
<td>$\text{Ca}^{2+}$ resorption ($\mu$mol/min)</td>
<td>0.48</td>
<td>0.83</td>
<td>0.51</td>
</tr>
<tr>
<td>Net $\text{Ca}^{2+}$ flux into bone ($\mu$mol/min)</td>
<td>1.02</td>
<td>1.38</td>
<td>1.06</td>
</tr>
<tr>
<td>Urinary $\text{Ca}^{2+}$ excretion ($\mu$mol/min)</td>
<td>0.047</td>
<td>0.200</td>
<td>0.030</td>
</tr>
</tbody>
</table>

Primary hyperparathyroidism (PHPT) is simulated by increasing the rate of PTH synthesis ($k_{prod}^{PTH}$) by a factor of 185 (so that $[\text{PTH}]_p$ is multiplied by 10). In the presence of Cinacalcet, the rate of PTH exocytosis is set to its minimum value (0.00007 min$^{-1}$ vs. 0.0199 in the base case), and the inhibitory effects of CaSR on $\text{Ca}^{2+}$ reabsorption in the TAL are maximized ($\delta_{TAL}(Ca)$ from Eq. 15 is set to 0.0175, vs 0.010 in the base case).
Table 6: Predicted impact of age and sex on calcium homeostasis

<table>
<thead>
<tr>
<th>Source</th>
<th>2 months old</th>
<th>8 months old</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>male</td>
<td>female</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>300</td>
<td>150</td>
</tr>
<tr>
<td>Plasma volume (mL)</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td>GFR (mL.min⁻¹)</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>β_{exco}^{PTH} (min⁻¹)</td>
<td>0.059</td>
<td>0.018</td>
</tr>
<tr>
<td>γ_{exco}^{PTH} (min⁻¹)</td>
<td>0.057</td>
<td>0.017</td>
</tr>
<tr>
<td>k_{min_{conv}} (min⁻¹)</td>
<td>4.4×10⁻⁶</td>
<td>2.2×10⁻⁶</td>
</tr>
<tr>
<td>δ_{max_{conv}} (min⁻¹)</td>
<td>6.02×10⁻⁵</td>
<td>3.01×10⁻⁵</td>
</tr>
<tr>
<td>IC_{a} (µmol.min⁻¹)</td>
<td>2.3</td>
<td>1.5</td>
</tr>
<tr>
<td>R_{abs}^{D_{3}} (pM)</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Γ_{res}^{min} (µmol.min⁻¹)</td>
<td>0.142</td>
<td>0.142</td>
</tr>
<tr>
<td>δ_{res}^{max} (µmol.min⁻¹)</td>
<td>0.70</td>
<td>0.70</td>
</tr>
<tr>
<td>k_{Ca}^{p_{f}} (min⁻¹)</td>
<td>0.125</td>
<td>0.140</td>
</tr>
<tr>
<td>k_{Ca}^{f_{p}} (min⁻¹)</td>
<td>10⁻⁵</td>
<td>10⁻⁵</td>
</tr>
<tr>
<td>[Ca²⁺]_{p} (mM)</td>
<td>1.21</td>
<td>1.20</td>
</tr>
<tr>
<td>PTH (pM)</td>
<td>1.93</td>
<td>1.93</td>
</tr>
<tr>
<td>D_{3} (pM)</td>
<td>94.6</td>
<td>51.5</td>
</tr>
<tr>
<td>Intestinal Ca²⁺ absorption (µmol/min)</td>
<td>1.06</td>
<td>0.52</td>
</tr>
<tr>
<td>Fractional intestinal absorption, F_{intest} (%)</td>
<td>46.0</td>
<td>34.6</td>
</tr>
<tr>
<td>Ca²⁺ accretion (µmol/min)</td>
<td>1.5</td>
<td>0.83</td>
</tr>
<tr>
<td>Ca²⁺ resorption (µmol/min)</td>
<td>0.48</td>
<td>0.34</td>
</tr>
<tr>
<td>Net Ca²⁺ flux into bone (µmol/min)</td>
<td>1.02</td>
<td>0.49</td>
</tr>
<tr>
<td>Urinary Ca²⁺ excretion (µmol/min)</td>
<td>0.047</td>
<td>0.024</td>
</tr>
<tr>
<td>Fractional excretion, λ_u (%)</td>
<td>1.95</td>
<td>2.00</td>
</tr>
</tbody>
</table>

Shown in the upper part of the table are the parameters that we varied as a function of sex and age. Model predictions are given in the lower part of the table.
Calcium intake → intestine 

intestine 

intestinal absorption 

Fecal excretion 

Calcium intake 

Parathyroid gland 

Bone: rapidly exchangeable pool 

Resorption → bone 

Reabsorption → plasma 

Filtration → Kidney 

D3 

Urinary excretion