

Mathematical modeling of whole-body electrolyte homeostasis

by

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A thesis
presented to the University of Waterloo
in fulfillment of the
thesis requirement for the degree of
Doctor of Philosophy
in
Applied Mathematics

Waterloo, Ontario, Canada, 2025

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Author's Declaration

This thesis consists of material all of which I authored or co-authored: see Statement of Contributions included in the thesis. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners.

I understand that my thesis may be made electronically available to the public.

Statement of Contributions

Melissa M. Stadt (MS) was the sole author of this thesis, although substantial portions of the content were adapted from collaborative publications and preprints as noted below.

- Chapter 2: The work in this chapter was performed by MS, Jessica Leete (JL), and Sophia Devinyak (SD, a Physics and Astronomy co-op student at the time of the study), under the supervision of Prof. Anita Layton (AL), resulting in a co-authored paper in PLOS Computational Biology (Ref. [214]). MS, JL, and AL conceived of the study. MS, JL, and SD developed the model and associated code, and performed analysis. MS and JL wrote the first draft. AL supervised the work. MS and AL revised the final manuscript.
- Chapter 3: The work in this chapter was performed by MS, under the supervision of AL, resulting in a co-authored paper in SIAM Journal on Applied Dynamical Systems (Ref. [212]). MS conceived of the study, developed the associated code, performed analysis, and wrote the first draft of the manuscript. AL supervised the work. All authors revised the manuscript.
- Chapter 4: The work in this chapter was performed by MS, under the supervision of AL, resulting in a co-authored paper published in the American Journal of Physiology - Regulatory, Integrative, and Comparative Physiology (Ref. [213]). MS conceived of the study, developed the model and associated code, performed analysis, and wrote the first draft of the manuscript. AL supervised the work. All authors revised the manuscript.
- Chapter 5: The work in this chapter was performed by MS, under the supervision of AL resulting in a co-authored paper published in the Journal of Theoretical Biology (Ref. [211]). MS conceived of the study, developed the model and associated code, performed analysis, and wrote the first draft of the manuscript. AL supervised the work. All authors revised the manuscript.
- Chapter 6: The work in this chapter is based on a manuscript that has been submitted for publication. MS conceived of the study, developed the model and associated code, performed analysis, and wrote the first draft of the manuscript. AL supervised the work. All authors revised the manuscript.

Abstract

Electrolyte balance is crucial for many physiological processes, including cellular signaling, muscle contractions, membrane potentials, hormonal secretion, and bone structure. Disruptions to electrolyte balance, arising from disease, diet, or drugs can have severe health consequences, such as muscle weakness, bone fragility, and life-threatening cardiac arrhythmias. Therefore, a comprehensive understanding of these regulatory systems and how they may be disrupted is important for developing effective preventative and therapeutic strategies. Mathematical modeling provides a powerful tool for investigating these systems through simulations and analysis. In this thesis, we present the development and analysis of mathematical models focused on the regulation of key electrolytes, potassium and calcium.

For potassium homeostasis, we first developed a detailed, whole-body model incorporating known regulatory mechanisms. We conducted model simulations to quantify the individual contributions of these regulatory mechanisms on long-term potassium balance and responses to a meal. Additionally, we conducted sensitivity analyses to understand how parameter variations impact potassium levels in the extracellular and intracellular fluid. Furthermore, we integrated recent experimental data on renal adaptations to high potassium intake to analyze these findings from a whole-body perspective.

For calcium homeostasis, we developed mathematical models representing a male, female, late pregnant, and lactating rat to quantify sex-specific differences and maternal adaptations in calcium regulation. These models synthesized literature data to identify key mechanisms that enable females to meet the high calcium demands of pregnancy and lactation. Finally, we developed an integrated model that represents the renin-angiotensin system, calcium regulation, and bone remodeling to investigate the impact of estrogen deficiency in post-menopausal women and common antihypertensive treatments on bone density and calcium regulation.

The research provided in this thesis contributes frameworks for understanding electrolyte homeostasis and predicting the impacts of physiological changes and pharmacological interventions on electrolyte and bone homeostasis.

Acknowledgements

To everyone who has supported me along the way, thank you. The people I have met have been one of the best parts of my graduate school experience. This thesis would not be possible without the generous support, encouragement, and contributions of those who have walked this journey with me.

First and foremost, I extend my deepest gratitude to my advisor, Prof. Anita Layton, for her exceptional guidance, support, and encouragement throughout both my MMath and PhD studies. Coming to the University of Waterloo to work with you was the best decision that I could have made for my graduate studies. Thank you for your consistent availability, for challenging me to grow, and for supporting my career path.

I also would like to thank my committee members: Prof. Sue Ann Campbell, Prof. Brian Ingalls, Prof. Andrea Edginton, and Prof. Aurelie Edwards. Thank you for dedicating time to read this thesis, for your questions, and your important role in my PhD. I especially appreciate Prof. Ingalls and Prof. Campbell for introducing me to the field of mathematical biology early in my graduate studies and for their guidance through my comprehensive exam. The foundational knowledge I gained from your teaching will continue to be invaluable throughout my career.

Graduate school has offered the opportunity to meet many brilliant people. I would like to thank the past and present members of the Layton group from their contributions in challenging my work and for the many memorable moments of laughter at group meetings and other events. While there are too many people to list, I especially want to acknowledge Delaney Smith, Pritha Dutta, Stéphanie Abo, Chris West, Mehrshad Sadria, Shervin Hakimi, Zaheer Mohideen, Bing Hu, Kaixin Zheng, Gordon McNicol, and Belal Abuelsar. You have made being in this group a delightful experience.

I am also deeply grateful to the University of Waterloo Department of Applied Mathematics. It has been a privilege to do my graduate studies here surrounded by brilliant faculty and graduate students, who are also really great people. I especially would like to thank the professors who taught my courses. I learned a lot from each of you. I will remember my time at the University of Waterloo fondly because it has been a wonderful experience.

Finally, I want to thank my family who have always supported everything I do. To my parents, thank you for always encouraging my passions and for providing a constant and loving home. And to my partner, Andrew, your support through this PhD has meant the world to me. While I came to Waterloo to study mathematical biology, meeting you has been one of my favourite parts, and I am so thankful I get to take you along with me into this next chapter too.

Dedication

This thesis is dedicated to my partner, Andrew McGivery. Thank you for being my greatest supporter.

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List of Abbreviations

1,25(OH)₂D₃ calcitriol

ACE angiotensin-converting enzyme

ACEi angiotensin-converting enzyme inhibitors

AGT angiotensinogen

ALD aldosterone

Ang I angiotensin I

Ang II angiotensin II

ARB angiotensin receptor blockers

AT1R angiotensin type 1 receptor

AT2R angiotensin type 2 receptor

CaSR calcium sensing receptor

CKD chronic kidney disease

Ctrial calcitriol

DT distal tubule

GFR glomerular filtration rate

ODE ordinary differential equations

OPG osteoprotegerin

PRA plasma renin activity

PT proximal tubule

PTH parathyroid hormone

QSP quantitative systems pharmacology

RANK receptor activator of nuclear factor kappa-B

RANKL receptor activator of nuclear factor kappa-B ligand

RAS renin-angiotensin system

TAL thick ascending limb

TGF tubuloglomerular feedback

Chapter 1

Introduction

Electrolyte homeostasis is critical for various physiological functions, including nerve signaling, pH balance, muscle contractions, and bone structure. In mammals, key electrolytes such as sodium, potassium, chloride, bicarbonate, calcium, magnesium, and phosphate are maintained within normal concentration ranges by many regulatory systems. In this thesis, we use mathematical modeling to understand the regulation of electrolyte homeostasis. We specifically focus on the electrolytes calcium and potassium, although similar approaches may be used to study other electrolytes. The physiological functions and regulation of potassium and calcium are described in Sections 1.1 and 1.2, respectively.

Mathematical models are quantitative representations of systems and have been used for many years to provide insights into biological and physiological processes. Models simplify complex interactions, allowing for the prediction of system responses to various factors, such as disease states, pharmacological interventions, or environmental stressors. They also serve to explain experimental observations and to generate hypotheses about underlying mechanisms. For example, quantitative systems pharmacology (QSP) modeling, a popular approach used in the pharmaceutical industry, integrates physiological and drug-related models to predict drug effects on the body. QSP models are used to optimize clinical trials, test hypotheses, optimize dosing regimens, and unravel key underlying mechanisms.

In this thesis, we present mathematical models of potassium and calcium homeostasis to study their regulatory mechanisms, the impact of dietary intake, quantify the impact of unique physiological states such as pregnancy and lactation, and predict the impact of drugs. A description of existing mathematical models that represent potassium and calcium homeostasis is given in Sections 1.1.1 and 1.2.1, respectively.

1.1 Background: Potassium homeostasis

In humans, normal extracellular potassium concentration ranges from 3.5 to 5.0 mmol/L, while intracellular potassium concentration is approximately 120 to 140 mmol/L [95]. This transmembrane potassium concentration gradient plays a major part in maintaining the transmembrane potential gradient, which is essential for the function of electrically excitable cells [142, 234]. For example, muscle cells depend on the transmembrane potential to generate action potentials for muscle contractions. Additionally, the transmembrane potential directly impacts the activity of transporters and ion channels [142, 234].

When potassium levels are outside of normal range, there can be many dangerous health consequences, including muscle weakness, fatigue, cardiac arrhythmia, and in severe cases, fatal cardiovascular events. Too low potassium levels (i.e., hypokalemia) are often the result of insufficient dietary potassium or excessive potassium excretion due to a renal disorder or medication side effect [41, 108]. The major causes of too high potassium levels (i.e., hyperkalemia) include chronic kidney disease (CKD), uncontrolled diabetes, adrenal disease, consuming excessive potassium, and some medications like spironolactone [41, 234].

The average distribution of potassium within a human body is illustrated in Fig. 1.1. About 98% of total body potassium is stored in the intracellular fluid, primarily in the skeletal muscle cells [95]. Potassium homeostasis is maintained by balancing both internal and external potassium balance. Internal potassium balance is the balance of the distribution of potassium between the extra- and intracellular fluids (Fig. 1.1). This is primarily regulated by the activity of the $\text{Na}^+\text{-K}^+\text{-ATPase}$ pump, which actively brings potassium into the cells, against the transmembrane potassium gradient. $\text{Na}^+\text{-K}^+\text{-ATPase}$ activity is regulated by a variety of hormones, including insulin and aldosterone (ALD) [142, 163]. External potassium balance is the balance of daily potassium intake with excretion (Fig. 1.1). Since 90% of the potassium from a meal enters the bloodstream, balance with intake is primarily regulated by the kidneys [95]. Every day the entire blood volume is filtered through the glomeruli into the nephrons of the kidneys many times [53]. Since potassium is freely filtered in the glomerulus, the filtrate that enters the nephrons has the same potassium concentration as the blood plasma. As the luminal fluid passes through the nephrons, the specialized transporters along the segments of the nephrons control how much potassium is reabsorbed out of or secreted into the lumen [53, 81]. The content of the luminal fluid remaining at the end of the nephrons is excreted through urine. A schematic of basic renal potassium transport along the nephrons is illustrated in Fig. 1.2.

An interconnected system of various signals that target intracellular potassium uptake or renal function regulate potassium homeostasis. A schematic of the regulatory mechanisms of potassium is in Fig. 1.3. ALD secretion from the adrenal glands is stimulated

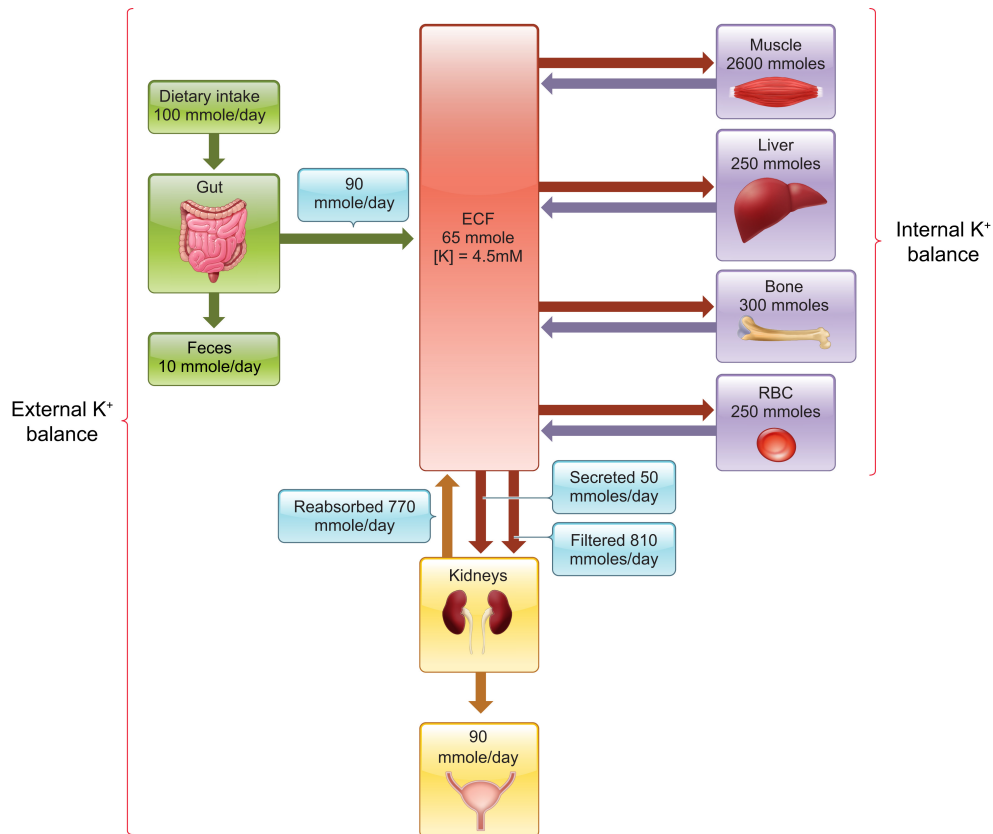


Figure 1.1: Schematic representation of potassium distribution within an average human body. External potassium balance is achieved via the balance between dietary potassium intake with excretion. Internal potassium balance is achieved with the balanced distribution of potassium between the extracellular and intracellular fluid. Figure used from Hoorn et al. [95]. ECF: extracellular fluid, RBC: red blood cell

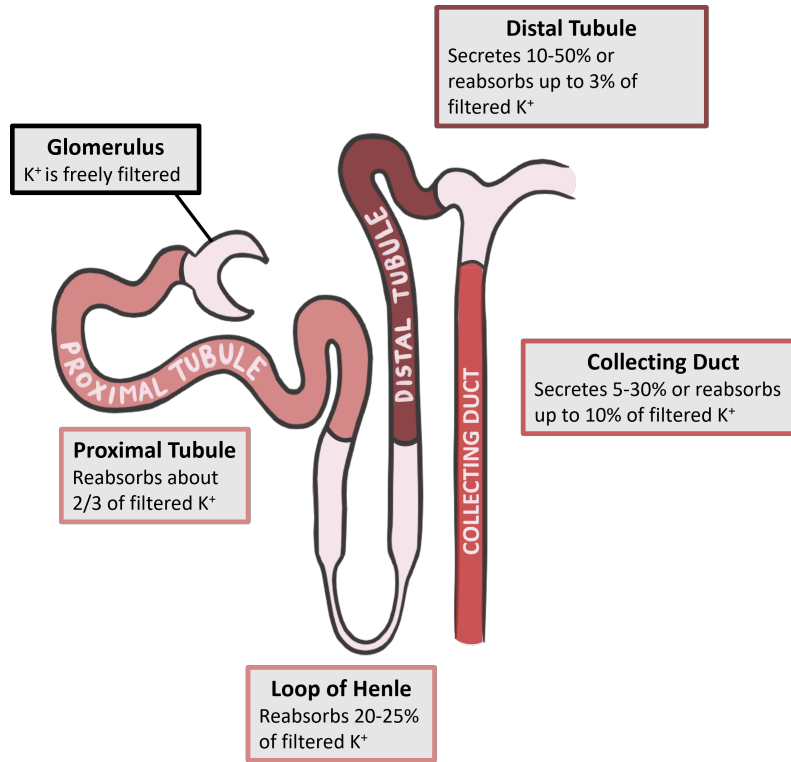


Figure 1.2: Schematic diagram of typical potassium reabsorption and secretion along key nephron segments. Figure modified from Stadt et al. [214].

by increases in extracellular potassium levels. ALD increases potassium secretion in the distal segments of the nephrons and stimulates the $Na^+-K^+-ATPase$ pump for increased cellular potassium uptake. A typical meal consists of both glucose and potassium. Glucose intake stimulates insulin secretion, which stimulates $Na^+-K^+-ATPase$ to increase cellular potassium uptake as the potassium from the meal reaches the bloodstream [142, 188]. Researchers have found that when a potassium load is given as a meal, there is more urinary potassium excretion than when an identical potassium load is given as an intravenous injection [145, 157]. Their results suggests that there are sensors in the gastrointestinal (or hepatoportal) system that signal to the kidneys to increase urinary potassium excretion before an increase in extracellular potassium levels. In the studies presented in this thesis, we refer to this effect as the “gastrointestinal feedforward effect”. Evidence for the gastrointestinal feedforward effect has been reported in rodents [145, 157], sheep [178], and humans [172].

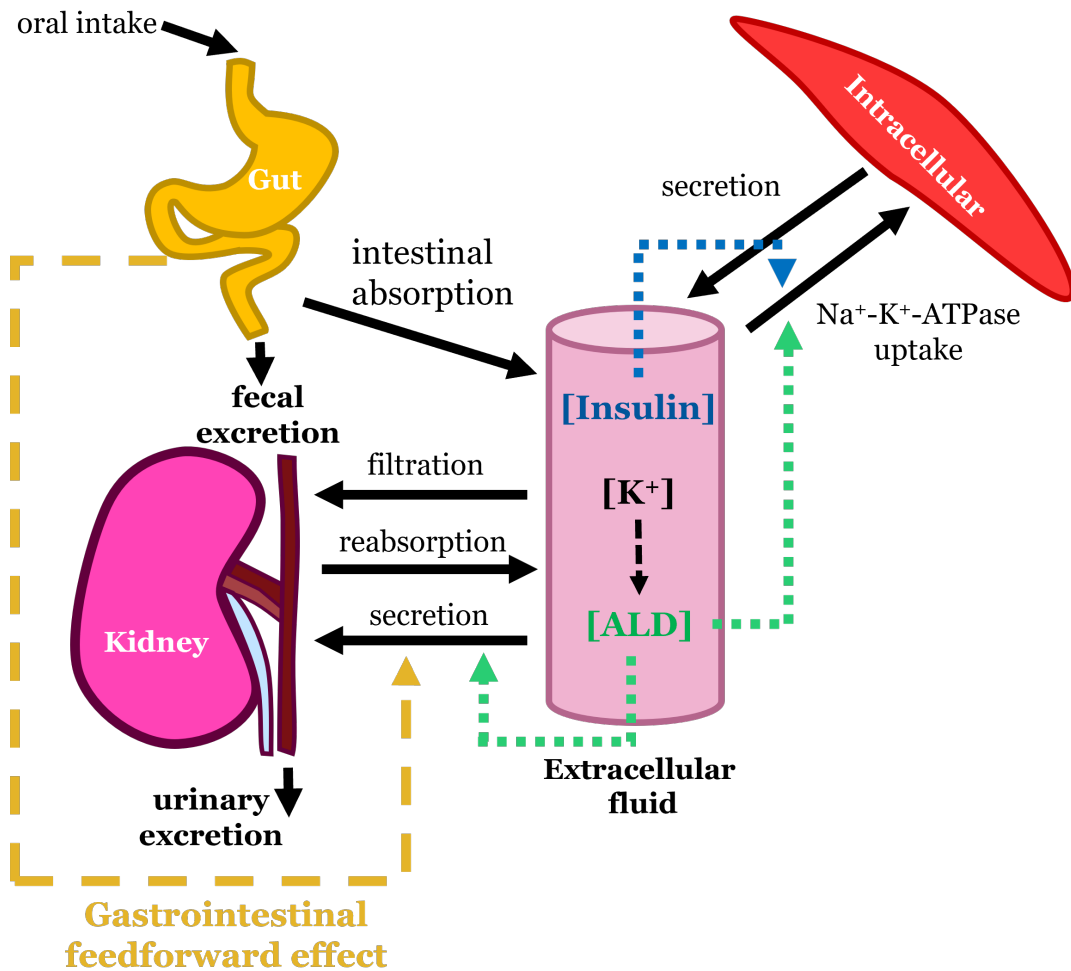


Figure 1.3: Schematic representation of the primary organs and hormones involved in potassium regulation. Solid black arrows represent transport of potassium between the compartments. Green, light blue, and yellow arrows represent the stimulating effects of aldosterone, insulin, and the gastrointestinal feedforward effect, respectively. ALD: aldosterone

Together these regulatory mechanisms ensure that potassium levels are maintained within the normal range. Potassium homeostasis and approaches to manage or prevent potassium dysregulation remains an open and active area of research. Due to the complexity of the physiological processes involved, mathematical modeling serves as a powerful tool

to explore these systems, test hypotheses, and predict the impacts of drugs and disease on potassium balance in patients.

1.1.1 Mathematical modeling of potassium homeostasis

Mathematical models have been developed to study potassium regulation for various applications. Pettit and Vick [168] developed a two-compartment model representing the exchange of potassium between the extracellular and intracellular fluid with the effect of insulin on intracellular potassium uptake to understand the contribution of insulin in extrarenal potassium homeostasis in dogs. Rabinowitz [178] used a similar approach to evaluate the role of the gastrointestinal feedforward effect in sheep. More recently, Youn et al. [245] used two-compartment and three-compartment models to analyze fluxes of potassium between the extracellular and intracellular fluid for understanding potassium uptake by red blood cells in rats.

In recent years, several mathematical models of potassium regulation in humans have been developed. We developed a detailed physiology-based mathematical model of potassium homeostasis that represents the plasma, interstitial, and intracellular fluids as well as a detailed representation of the kidneys that includes reabsorption and secretion of potassium along key nephron segments. Additionally, we included known feedforward and feedback mechanisms: the gastrointestinal feedforward effect, insulin and ALD stimulation of $\text{Na}^+\text{-K}^+\text{-ATPase}$ cellular uptake, and ALD stimulation of renal potassium secretion. This model was published in Ref. [214] and is described in Chap. 2 of this thesis. We used this model to quantify the impacts of the individual feedforward and feedback mechanisms on a typical (Western) diet as well as investigate a hypothesized control mechanism called “muscle-kidney cross talk” under periods of high and low potassium intake [214]. A sensitivity analysis study of this model is in Chap. 3 (published in Ref. [212]). After our original model was published (Ref. [214]), new experimental results about the impact of a high potassium diet on kidney function was published by Wang et al. [230]. We applied these findings to our published model (Ref. [214], Chap. 2) to study the whole-body impacts of these changes in the kidney. This study was published in Ref. [213] and is in Chap. 4 of this thesis.

The same year our model in Ref. [214] (Chap. 2) was published, Maddah and Hallow [140] published a QSP model of potassium regulation to study the impact of dietary potassium, spironolactone, hyperaldosteronism, and renal impairment on plasma potassium levels. Spironolactone is a mineralocorticoid antagonist [109]. Given that ALD is a major mineralocorticoid, spironolactone is an ALD antagonist. It is commonly used as a

potassium-sparing diuretic and for the treatment of hypertension, hypokalemia, hyperaldosteronism, and acne [109]. It is also used as an androgen blocker in transgender women undergoing feminizing hormone replacement therapy [8]. A common side effect of spironolactone is hyperkalemia, especially in patients with renal impairment. Model simulations in Ref. [140] showed that for small declines in renal impairment, there was a small impact on plasma potassium levels, but when increasing potassium intake with major renal impairment there was a high risk of hyperkalemia. Additionally, model simulations showed that plasma potassium remained stable with spironolactone treatment, but increased as renal impairment became more severe. Similar to our model developed in Ref. [214] (Chap. 2), the model by Maddah and Hallow [140] included potassium exchange between the intracellular and extracellular fluid and a detailed representation of renal potassium handling by the nephron segments. However, this model did not include regulatory processes of insulin, the gastrointestinal feedforward effect, or ALD impact on potassium uptake into the intracellular fluid.

Another QSP model was developed by Clegg et al. [42] to investigate the effects of sodium zirconium cyclosilicate for emergency lowering of serum potassium levels in patients with severe hyperkalemia and prevention of hyperkalemia in patients with end-stage CKD on dialysis treatment. Sodium zirconium cyclosilicate binds with potassium in the gastrointestinal tract, but is not absorbed into systemic circulation [96]. This reduces absorption of potassium from the gastrointestinal tract after a meal, thereby reducing the amount of potassium entering the bloodstream. Similar to our model in Ref. [214] (Chap. 2), the model developed by Clegg et al. [42] has compartments for potassium in the gastrointestinal tract, plasma, interstitial fluid, and intracellular fluid. This model has a more simple representation of renal potassium handling by not including potassium transport along the different segments of the nephrons as in our model (Ref. [214], Chap. 2) and the model by Maddah and Hallow [140]. Instead, Clegg et al. [42] represented renal clearance by filtration (i.e., plasma potassium concentration times the filtration rate) with a function that represented the impact of circadian rhythms on glomerular filtration rate (GFR). Clegg et al. [42] fit model parameters to data from individual patients to simulate virtual clinical trials with patient-specific parameter sets and inform clinical trial design. This is different from the approach of using population data to fit model parameters as in Refs. [140, 214].

1.2 Background: Calcium homeostasis

Calcium is the most abundant mineral in the human body. About 99% of the total body calcium is stored within the bones [175, 184]. By binding with phosphate to produce the compound hydroxyapatite, calcium forms the major structural components of the bone. The remaining 1% of total body calcium is in the cells and blood to be used for critical physiological functions such as vascular contraction, muscle function, blood clotting, intracellular signaling, and hormone secretion [29, 32, 175]. Regulation of extracellular calcium concentration is critical because both excess calcium (i.e., hypercalcemia) and insufficient calcium (i.e., hypocalcemia) levels can lead to dangerous and potentially fatal health outcomes.

Maintaining calcium homeostasis involves balanced calcium fluxes between key organs: the intestine, the bones, and the kidneys. Given that phosphate is tightly connected with calcium, due to hydroxyapatite formation, it is similarly regulated [184]. Fluxes between the key organs are primarily regulated by the calciotropic hormones: parathyroid hormone (PTH) and calcitriol. PTH is secreted by the parathyroid glands and affects several calcium regulatory pathways including renal reabsorption and bone remodeling. Calcitriol is synthesized in the proximal tubules of the kidneys from vitamin D by the enzyme 1α -hydroxylase. Calcitriol primarily regulates intestinal calcium absorption by binding to the vitamin D receptor [64].

The calcium sensing receptor (CaSR) is expressed on the parathyroid glands and in the kidneys to regulate PTH secretion, calcitriol synthesis, and renal reabsorption based on calcium levels in the blood [91]. CaSR is also expressed in the mammary glands of lactating women to regulate calcium levels for sufficient milk production [91, 118]. Dysregulation of the CaSR can lead to many dangerous health consequences [91]. A schematic diagram of the key processes involved in calcium regulation is in Fig. 1.4.

Blood is continuously filtered through the glomeruli of the kidneys. Calcium and phosphate in the blood, that is not bound to proteins, is filtered through the glomeruli into the nephrons where specific transporters control how much is reabsorbed back into systemic circulation, while what is remaining at the end of the nephrons is excreted in urine [4, 50]. The transporters along the nephrons are regulated by PTH, calcitriol, and the CaSR.

The bones are dynamic organs that continuously exchange calcium and phosphate with the extracellular fluid. Most of this exchanged calcium and phosphate undergoes a process known as accretion, resulting in the formation of hydroxyapatite. Bone formation is regulated by cells called osteoblasts. Bone resorption, the process of breaking down bone tissue, is done by osteoclasts. PTH plays a key role in regulating activity of osteoblasts

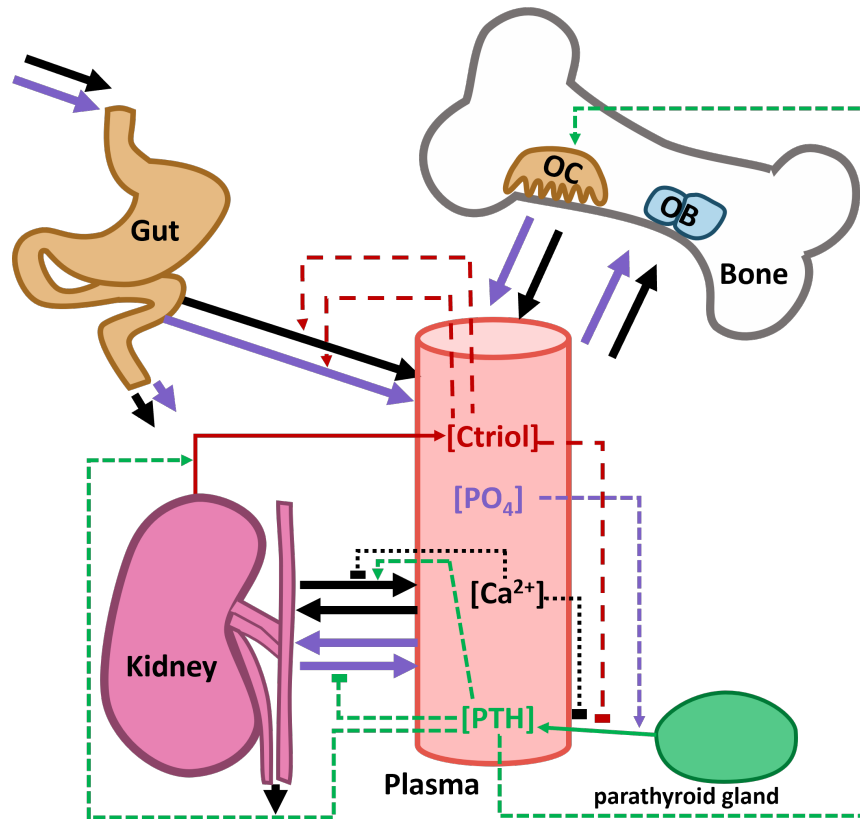


Figure 1.4: Schematic representation of the primary organs and hormones involved in calcium regulation. Solid lines indicate transport of calcium (black), phosphate (purple), secretion of parathyroid hormone (green), or calcitriol synthesis (red). Dashed lines indicate the effects of the respective electrolyte or hormone levels with blunted arrows indicating inhibition and pointed arrows indicating stimulation. Ca^{2+} : calcium, Ctriol: calcitriol, PTH: parathyroid hormone, PO_4 : phosphate, OC: osteoclast, OB: osteoblast

and osteoclasts to ensure balanced bone remodeling.

1.2.1 Mathematical modeling of calcium homeostasis

Many mathematical models have been developed to study calcium homeostasis. One of the first mathematical models was developed by Aubert and Milhaud [10], which included a central and bone compartment with calcium entering the central compartment through calcium absorption by the intestines and leaving the central compartment through urinary excretion. A more complex model, which included hormonal regulation by PTH was later developed by Aubert and Bronner [9]. Powell also developed a calcium homeostasis model with PTH regulation [170]. These models were all developed based on rodent studies. A mathematical model of calcium regulation in humans was first published by Knop et al. [115]. This model was used to study the impact of hyperparathyroidism and hypoparathyroidism on calcium homeostasis [115]. Raposo et al. [180] developed a mathematical model of calcium-phosphate homeostasis that included the regulation of bone resorption by PTH and calcitriol, but did not include the effect of calcitriol on intestinal absorption.

Granjon et al. [78, 79] developed calcium and calcium-phosphate homeostasis models for rats. The models included a detailed representation of calcium reabsorption along the nephron segments. Additionally, Granjon et al. [79] included the impact of fibroblast growth factor 23, a hormone that regulates reabsorption of phosphate in the kidneys and calcitriol synthesis. The authors also included a brief analysis on the effects of sex and age on model parameters and predictions [78]. Dutta and Layton [51] integrated a magnesium homeostasis model with this calcium model (Ref. [78]) to study the interconnected physiological processes regulating calcium and magnesium homeostasis. The authors used this model to predict the impact of magnesium and calcium deficient diets as well as vitamin D deficiency [51]. We extended the work by Granjon et al. [78] to analyze the impacts of sex differences and the unique physiological states of pregnancy and lactation in females on calcium regulation [211]. This study is included in Chap. 5.

Peterson and Riggs [165] developed a QSP model based on previous models of calcium-phosphate homeostasis coupled to a detailed bone model that included bone formation and resorption by osteoblasts and osteoclasts, respectively. This model was used to study the impact of hypoparathyroidism and hyperparathyroidism, renal insufficiency, and treatment with teriparatide, a synthetic PTH [165]. This model has been extensively used for clinical applications such as predicting the risk of bone loss for patients on gonadotropin-releasing hormone treatment of endometriosis [186], mineral bone disorder in patients with

CKD [187], predicting bone density for treatment of osteoporosis [166], and included the impact of osteocytes to study pharmaceutical interventions of sclerostin inhibition [59]. In 2015, the Peterson and Riggs [165] model was one of the first QSP models to be used to evaluate a dosing regimen of a new drug by the US Food and Drug Administration [167]. This was a milestone moment for the use of QSP models in the pharmaceutical industry.

Gaweda et al. [72] extended the Peterson and Riggs [165] model to include fibroblast growth factor 23 and a smooth muscle compartment to predict vascular calcification in patients with CKD mineral bone disorder. The authors have continued work in this area by using this model to inform artificial intelligence models (specifically reinforcement learning) for precision treatment for patients with CKD mineral bone disorder [71].

Outside of medical sciences, calcium regulation is also of a large concern in the dairy industry, where milk fever (i.e., hypocalcemia in dairy cows) is an issue due to the excessive demands of lactation for dairy milk [47]. In the studies presented in this thesis, we focus on rat and human applications, although a similar approach may be used to study dairy cows.

1.3 Outline of thesis

In this thesis, we present mathematical models and analysis of potassium and calcium regulation. The remainder of the manuscript is structured as follows:

Chapter 2 presents a mathematical model of potassium regulation in an average healthy man. The model compartments represent the potassium distribution between the plasma, interstitial, and intracellular fluids and includes known regulatory mechanisms. Potassium enters the plasma through a gut compartment for oral potassium intake and excretion is determined by a detailed representation of the kidneys. We use this model to quantify the impacts of the individual regulatory mechanisms. Also, we conduct simulations to investigate a hypothesized regulatory mechanism during periods of high and low potassium intake. The work in this chapter was conducted with Dr. Jessica Leete, Sophia Devinyak, and Prof. Anita Layton and has been published as the paper titled “A mathematical model of potassium homeostasis: Effect of feedforward and feedback controls” in *PLOS Computational Biology* (Ref. [214]).

Chapter 3 presents a global sensitivity analysis of our potassium regulation model described in Chap. 2. This analysis investigates the impact of individual and combined changes in model parameters on both the steady-state and the dynamic response to a single meal. By investigating the sensitivity of different model outputs

to changes in parameter values, we can determine which parameters and their corresponding biological components contribute the most to variations in long-term and short-term potassium regulation. Our analyses support that our model captures known physiological function of potassium regulation within the parameter space investigated. The work in this chapter was supervised by Prof. Anita Layton and has been published in the paper titled “A mathematical model of whole-body potassium regulation: Global parameter sensitivity analysis” in the *SIAM Journal of Applied Dynamical Systems* (Ref. [212]).

Chapter 4 presents an extension of our potassium regulation model (Chap. 2) to investigate experimental data published by Wang et al. [230] that reported changes in renal function during high potassium diets. In particular, the authors reported that rats fed high potassium diets had much lower proximal tubule fractional reabsorption and GFR than control diet rats. In Chap. 4, we conduct model simulations and sensitivity analysis to investigate how these renal adaptations impact whole-body potassium regulation. The work in this chapter was supervised by Prof. Anita Layton and has been published in the paper titled “A modeling analysis of whole body potassium regulation on a high-potassium diet: Proximal tubule and tubuloglomerular feedback effects” in the *American Journal of Physiology - Regulatory, Integrative, and Comparative Physiology* (Ref. [213]).

Chapter 5 presents mathematical models of calcium homeostasis that represent (i) sex differences between male and female rats and (ii) the physiological adaptations in females during pregnancy and lactation to meet increased calcium demands of a growing fetus and neonate, respectively. We conduct model simulations to quantify the impact of these sex differences on long-term calcium homeostasis and analyze the individual contributions of pregnancy and lactation. In addition, we conduct theoretical simulations to explore the hypothesis that sex differences in calcium regulation may provide a better physiological basis for female bodies to support the substantial calcium demands of fetal and neonatal development than males. The work in this chapter was supervised by Prof. Anita Layton and has been published in the paper titled “Mathematical modeling of calcium homeostasis in female rats: An analysis of sex differences and maternal adaptations” in the *Journal of Theoretical Biology* (Ref. [211]).

Chapter 6 presents a mathematical model that represents the interconnected physiological systems of estrogen, the renin-angiotensin system, bone remodeling, and calcium regulation. This model is used to investigate the impact of rapidly declining estrogen levels in post-menopausal women on bone health. Additionally, we modeled

how renin-angiotensin system inhibitors, specifically angiotensin-converting enzyme inhibitors and angiotensin receptor blockers may improve or decrease bone loss in aging women with declining estrogen levels.

Chapter 7 summarizes the main findings of the studies presented in this thesis and proposes future research directions using the models and their analyses presented in this thesis.

Chapter 2

A mathematical model of potassium homeostasis: Effect of feedforward and feedback controls

Abstract

Maintaining normal potassium (K^+) concentrations in the extracellular and intracellular fluid is critical for cell function. K^+ homeostasis is achieved by ensuring proper distribution between extracellular and intracellular fluid compartments and by matching K^+ excretion with intake. The $Na^+-K^+-ATPase$ pump facilitates K^+ uptake into the skeletal muscle, where most K^+ is stored. $Na^+-K^+-ATPase$ activity is stimulated by insulin and ALD. The kidneys regulate long term K^+ homeostasis by controlling the amount of K^+ excreted through urine. Renal handling of K^+ is mediated by a number of regulatory mechanisms, including by ALD, in which high extracellular K^+ concentration stimulates ALD secretion, which enhances urine K^+ excretion, and a gastrointestinal feedforward effect, in which dietary K^+ intake increases K^+ excretion. Additionally, we conduct theoretical simulations to investigate a muscle-kidney cross talk signal, where the K^+ concentration in skeletal muscle cells directly affects urine K^+ excretion without changes in extracellular K^+ concentration. To understand how these mechanisms coordinate under different K^+ challenges, we have developed a compartmental model of whole-body K^+ regulation. The model represents the intra- and extracellular fluid compartments in a human

(male) as well as a detailed kidney compartment. We included (i) the gastrointestinal feedforward effect, (ii) the effect of insulin and (iii) ALD on Na^+ - K^+ -ATPase K^+ uptake, and (iv) ALD stimulation of renal K^+ secretion. We used this model to investigate the impact of regulatory mechanisms on K^+ homeostasis. Model predictions showed how the regulatory mechanisms synthesize to ensure that the extracellular and intracellular fluid K^+ concentrations remain in normal range in times of K^+ loading and fasting. Additionally, we predict that without the hypothesized muscle-kidney cross talk signal, the model was unable to predict a return to normal extracellular K^+ concentration after a period of high K^+ loading or depletion.

2.1 Introduction

Potassium (K^+) plays an essential role in maintaining normal cellular function. In particular, the transmembrane K^+ gradient maintains the transmembrane potential gradient, which is critical for cellular function [142]. In electrically excitable cells, such as neurons and muscle cells, the transmembrane potential gradient is used for transmitting signals between different parts of a cell [234]. Muscle cells, for instance, rely on the gradient to drive action potentials to facilitate muscle contractility [234]. The transmembrane potential gradient also impacts transporter and ion channel activity [142, 234]. In humans, normal extracellular K^+ concentration is about 3.5-5.0 mEq/L; intracellular K^+ concentration is about 120-140 mEq/L [95, 142]. To achieve K^+ homeostasis, K^+ excretion must first match intake in the long run to avoid net accumulation or loss; then the body's K^+ must be properly distributed between extracellular and intracellular fluids [41].

When extracellular K^+ concentrations are outside the tight range of 3.5–5.0 mEq/L, cellular balances of Na^+ , K^+ , and Ca^{2+} are altered, which can have dangerous consequences [142, 234]. The distribution of K^+ between the extracellular and intracellular fluid, as well as renal K^+ handling (which impacts total body K^+) can be altered by hormones, drugs, various pathological states, or even just a simple meal. Uncompensated K^+ imbalance or dyskalemia (either hyperkalemia or hypokalemia) can lead to constipation, muscle weakness, fatigue, and cardiac arrhythmias, and in severe cases could be fatal. Hypokalemia can be a result of insufficient dietary K^+ or excessive K^+ excretion [41, 108]. The major causes of hyperkalemia include CKD, uncontrolled diabetes, adrenal disease, consuming excessive dietary K^+ , and some medications [41, 234].

K^+ homeostasis has received much attention, from the research community as well as the general public, because not only can large fluctuations in the extracellular K^+ concen-

trations cause potentially life-threatening consequences, maintaining the extracellular K^+ concentration within a small range is also a daily homeostatic challenge. First, unless an opposing transmembrane potential is sustained, the large transmembrane K^+ concentration gradient (noted above) would drive a massive K^+ flux out of the cells. That could produce a substantial perturbation in extracellular K^+ concentration, given that the extracellular pool is much smaller than the intracellular pool (70 compared to 3,500 mEq [95, 142]). A second challenge is that K^+ has the highest ratio of daily intake to extracellular pool size of all the major electrolytes: recommended daily intake of K^+ is 120 mEq [142], about 70% more than the extracellular K^+ pool. (In comparison, the recommended daily intake of Na^+ is 2,300 mEq, which is only slightly higher than its extracellular pool of about 2,100 mEq [216].) As one can imagine, a simple meal could have nearly as much K^+ as in the extracellular fluid! As such, without rapid cellular K^+ uptake, a meal could cause drastic changes in extracellular fluid K^+ concentration. Finally, since about 90% of the K^+ excretion occurs via urine (remaining via feces and excretion through sweat), urinary excretion of K^+ must be nearly equal to K^+ intake. Because K^+ intake varies widely each day, the kidneys must be capable of varying urinary K^+ excretion to match varied K^+ intake to avoid excessive K^+ retention or loss.

Mammals have evolved to develop a number of regulatory mechanisms that target renal function and cellular K^+ uptake. *How do these mechanisms interact? What role does each of these mechanisms play under different types of K^+ perturbations (e.g., a K^+ -rich meal, repeated K^+ loading, or K^+ depletion)? Given the potentially and immediate dire consequences of hypokalemia and hyperkalemia, how can the kidneys sense that extracellular K^+ may be in danger of going out of range before it happens, and promptly adjust K^+ excretion appropriately?* Indeed, understanding the big picture of K^+ homeostasis is an open area of study with significant pieces of the puzzle that have yet to be put together. Knowledge gaps primarily lie in understanding how signals and sensors on both cellular uptake and renal K^+ handling synthesize to maintain an extracellular fluid K^+ concentration within normal range [142]. Below we give a brief overview of the individual mechanisms involved in K^+ regulation. For more details we refer the readers to Refs. [81, 95, 142].

Kidney function

In the long term, the kidney is the principal defense against chronic K^+ imbalance. Potassium is freely filtered through the glomerulus and into the nephrons. As the luminal fluid passes through the nephrons, various regulatory processes control how much K^+ is secreted or reabsorbed [81, 151, 241]. A schematic of general renal K^+ handling along the nephrons is shown in Fig 2.1. In a male rat, the proximal tubules consistently reabsorb about two-

thirds of filtered K^+ and then the loops of Henle reabsorb about 20-25% more so that together these segments reabsorb around 90% of the filtered K^+ [81]. This leaves the fine tuning to the downstream segments, namely the distal tubules and collecting ducts.

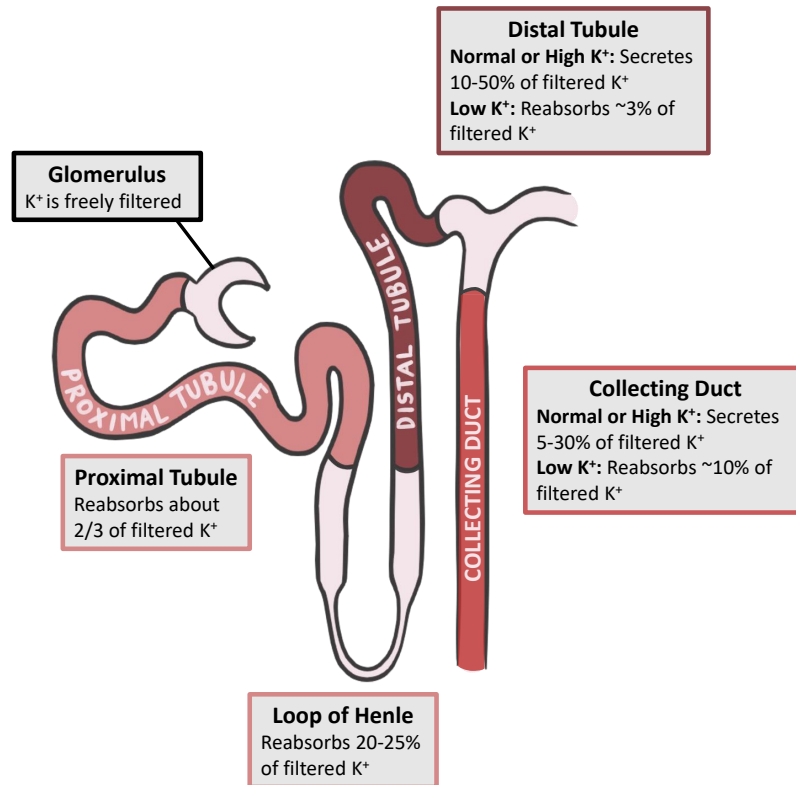


Figure 2.1: Transport of K^+ in key nephron segments during normal, low, and high K^+ intake.

The distal tubular segments (i.e., the connecting tubule and distal convoluted tubule) have the ability to secrete or reabsorb K^+ , depending on the state of the body. During normal or high K^+ intake, the distal tubular segments secrete 10% to 50% of filtered K^+ [81]. Secretion rate is largely controlled by ALD, which increases the luminal permeability of K^+ along the distal tubular segments. However, the distal tubule's capacity to reabsorb K^+ is limited (up to 3% of filtered K^+ in male rats), meaning that in times of drastically low K^+ intake, it is up to the downstream collecting ducts to conserve K^+ via reabsorption [81, 241].

The cortical collecting duct and outer medullary collecting duct consist of the principal cells and intercalated cells, which are responsible for K^+ secretion and reabsorption, respectively. Principal cells comprise approximately 70–75% of collecting duct cells in rats [53]. These cells primarily secrete K^+ . The rate of K^+ secretion is regulated by dietary K^+ intake, intracellular K^+ concentration, Na^+ uptake into the cells, urine flow rate, and hormones such as ALD and angiotensin II [81, 241]. Through upregulation of $H^+-K^+-ATPase$ in the intercalated cells, the collecting duct can reabsorb up to 10% of filtered K^+ [81, 151].

Internal K^+ balance

While the kidney determines overall K^+ conservation and loss, it takes time to have an effect [142]. After a K^+ load, in the first 3-6 hours, only about 50% of the K^+ load is excreted via urine; the remaining K^+ load is excreted over the next 10 hours [16, 142]. This time scale gives rise to the question: *Following a large K^+ intake (e.g., a K^+ -rich meal), how can the low extracellular K^+ concentration be maintained?* This is an important question because even transient rises in extracellular K^+ concentration can be dangerous. If the remaining half of the K^+ load (not excreted in urine) stayed in the extracellular fluid, plasma K^+ concentration would rise significantly, potentially to dangerous levels. To avoid that scenario, the body rapidly increases uptake of K^+ into the cells, primarily, the skeletal muscle cells. This quick storage of K^+ allows the body to maintain an appropriately low extracellular K^+ concentration, and to later slowly release K^+ back into the bloodstream without large perturbations in concentration. Cellular uptake of K^+ can be enhanced by the activation of $Na^+-K^+-ATPase$ pumps by insulin and ALD [146, 163].

Feedback and feedforward controls

A number of feedback and feedforward controls are involved in K^+ homeostasis by targeting either cellular K^+ uptake or renal function. Feedback controls respond to changes in extracellular K^+ concentration. High extracellular K^+ concentration increases ALD secretion. As mentioned previously, ALD impacts K^+ regulation by increasing K^+ secretion into the distal segments of the nephrons, as well as increasing cellular K^+ uptake via $Na^+-K^+-ATPase$ pump stimulation [142, 163].

Feedforward control refers to a pathway that reacts to an environmental input in a predetermined way, without reference to the value being controlled. A typical meal consists of both glucose and K^+ . Glucose intake stimulates insulin secretion, which then stimulates cellular uptake of K^+ . K^+ intake has been shown to activate a feedforward control in

which urinary K^+ excretion is stimulated before changes in plasma K^+ concentration or ALD concentration can be observed [142, 172, 179, 244]. This signal will be referred to as the *gastrointestinal feedforward effect*.

Identifying all the K^+ regulatory mechanisms remains an open area of research [142]. For example, following a significant elevation in total body K^+ , how does the body safely get rid of the excess K^+ without significantly, and potentially dangerously, raising extracellular K^+ levels? One hypothesized mechanism is *muscle-kidney cross talk*, whereby intracellular K^+ concentration of skeletal muscle cells can directly affect urine K^+ excretion without first causing changes in extracellular K^+ concentration [142, 145, 146].

With the multitude of physiological processes involved in K^+ balance, mathematical modeling is well suited for studying their interactions. Pettit and Vick [168] presented a two-compartment model to analyze data from dogs to understand the contribution of insulin to extrarenal K^+ homeostasis. Their model represents K^+ storage in the extracellular and intracellular compartments, together with their K^+ exchange. A similar model was developed by Rabinowitz [178] to analyze data in sheep and understand the role of a gastrointestinal feedforward effect. Youn et al. [245] used two-compartment and three-compartment models to analyze fluxes of K^+ between the extracellular and intracellular fluid as well as K^+ uptake by red blood cells. Maddah and Hallow [140] developed a QSP model that captures the effect of ALD on K^+ homeostasis to simulate the effects of spironolactone treatment in patients with hyperaldosteronism.

To unravel the roles of the multitudes of organ systems and regulatory mechanisms involved in maintaining K^+ balance, we built upon the above studies and developed a more comprehensive computational model of K^+ homeostasis. We apply the model to investigate how each of the feedback and feedforward controls contributes to the regulation of plasma K^+ concentration, to the rapid excretion of K^+ following a K^+ -rich meal, and to the preservation of K^+ during periods of K^+ depletion. We also simulate muscle-kidney cross talk and predict how this hypothesized mechanism may impact recovery from repeated K^+ loading or K^+ depletion.

2.2 Materials and methods

We developed a compartmental mathematical model of K^+ regulation in a man. (We refer to this as a model of a man, instead of more generally as a human model, due to the known sex differences in renal K^+ handling [98, 100, 225], ALD concentration [148], and plasma K^+

concentration [162], all of which affect K^+ homeostasis. Parameters for the present models were fitted for a man, not a woman.) The model represents the gut and hepatoportal circulation, intracellular fluid, extracellular fluid, and the kidney. The extracellular fluid compartment is divided into two sub-compartments: blood plasma and interstitial fluid. The physiological processes that are represented include dietary K^+ intake, delivery of K^+ through the hepatoportal circulation to the blood plasma, intracellular and extracellular K^+ exchange, renal K^+ transport, the gastrointestinal feedforward effect, the effect of insulin on $Na^+-K^+-ATPase$ K^+ uptake, and feedback control of ALD on both $Na^+-K^+-ATPase$ and distal segment K^+ secretion. A flow chart depicting the system is shown in Fig 2.2. Model parameter values are listed in Table 2.1.

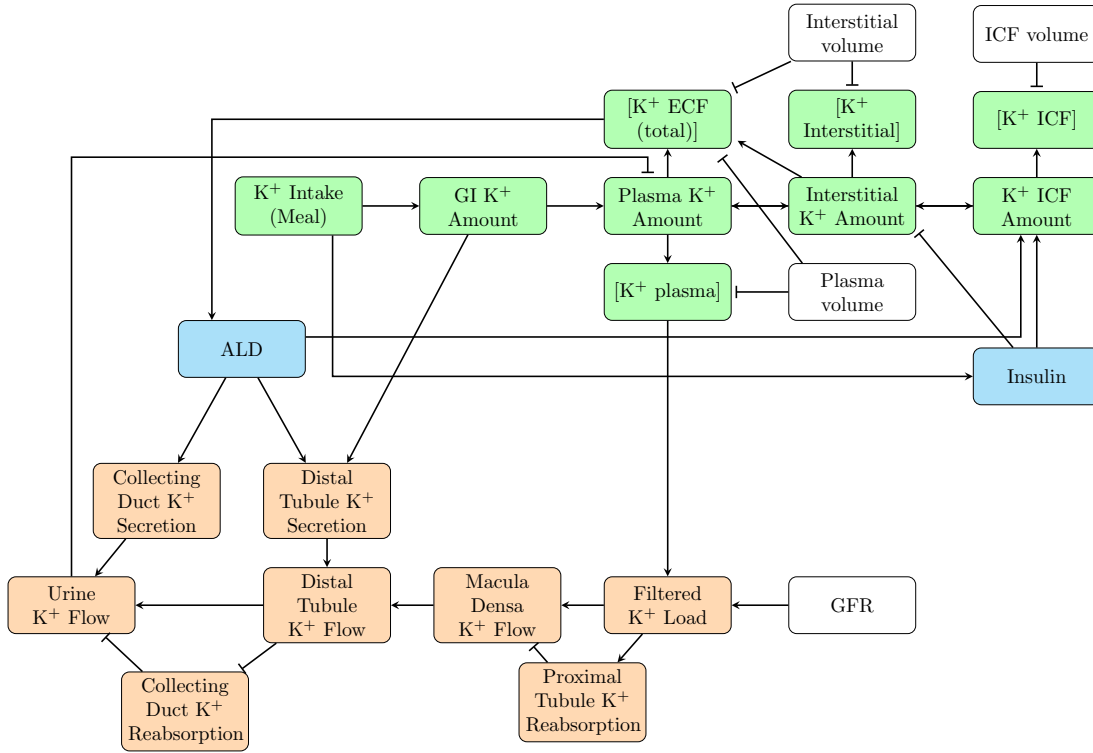


Figure 2.2: Schematic representation of our mathematical model of K^+ regulation. Green nodes represent internal K^+ balance; orange, kidney function; blue, hormones (i.e., aldosterone and insulin); and white, constant model parameters. Pointed arrows indicate stimulation; blunted arrows indicate inhibition. ALD: aldosterone, GFR: glomerular filtration rate, GI: gastrointestinal, Interstitial: interstitial fluid, ICF: intracellular fluid, ECF: extracellular fluid

Parameter	Description	Value	Unit	Ref
k_{gut}	K^+ delivery rate to plasma	0.01	min^{-1}	
P_{ECF}	effective K^+ permeability in extracellular fluid	0.3	L/min	
V_{plasma}	plasma fluid volume	4.5	L	[107]
$V_{\text{interstitial}}$	interstitial fluid volume	10	L	[107]
V_{max}	maximum K^+ flow into cells	130	mEq/min	[40]
K_{m}	half maximal activation	1.4	mEq/L	[40]
P_{trans}	effective transmembrane K^+ permeability	0.697	L/min	
$V_{\text{intracellular}}$	intracellular fluid volume	24	L	[107]
Φ_{GFR}	glomerular filtration rate	0.125	L/min	[107]
$\Phi_{\text{dt-Ksec}}^{\text{eq}}$	baseline distal tubule K^+ secretion	0.0410	mEq/min	[81]
$\Phi_{\text{cd-Ksec}}^{\text{eq}}$	baseline collecting duct K^+ secretion	0.0022	mEq/min	[81]
$A_{\text{cd-Ksec}}$	parameter that determines λ_{al} (Eq. 2.24)	0.161		
$B_{\text{cd-Ksec}}$	parameter that determines λ_{al} (Eq. 2.24)	0.411		
$A_{\text{cd-Kreab}}$	parameter that determines $\Phi_{\text{cd-Kreab}}$ (Eq. 2.17)	0.499		
T_{al}	aldosterone regulatory time constant	60	mins	[107]
C_{sod}	plasma Na^+ concentration	144	mEq/L	[107]
$A_{\text{dt-Ksec}}$	parameter that determines γ_{al} (Eq. 2.23)	0.348		
$B_{\text{dt-Ksec}}$	parameter that determines γ_{al} (Eq. 2.23)	0.238		
A_{insulin}	parameter that determines ρ_{insulin} (Eq. 2.26)	1.11		
α_{al}	parameter that determines ρ_{al} (Eq. 2.22)	0.74		
β_{al}	parameter that determines ρ_{al} (Eq. 2.22)	0.003		
A_{Kin}	parameter that determines γ_{Kin} (Eq. 2.27)	0.250		

Table 2.1: Model parameter values used in the baseline K^+ homeostasis mathematical models.

2.2.1 Internal K^+ balance

Oral K^+ intake (Φ_{Kin}) is treated as a model input. By varying Φ_{Kin} , one can study the model's response to changes in K^+ intake. For instance, if Φ_{Kin} is set to a constant ($\Phi_{\text{Kin}}^{\text{ss}}$), one may obtain a steady-state solution to the model equations. Realistically, K^+ intake varies throughout the day, with periods of high intake (meals) and periods of no intake (between meals). Thus, K^+ intake is best approximated by a time-dependent function, as done in the simulations in Section 2.3.

Potassium intake

When K^+ is consumed orally (i.e., a meal), about 90% of the K^+ is absorbed by the intestine, then goes through the hepatoportal circulation into the blood plasma of the general circulation. The remaining 10% is excreted through feces. The amount of K^+ in the gut and hepatoportal circulation is denoted by $M_{K_{\text{gut}}}$ and is determined by

$$\frac{dM_{K_{\text{gut}}}}{dt} = 0.9 \times \Phi_{K_{\text{in}}} - k_{\text{gut}} \times M_{K_{\text{gut}}} \quad (2.1)$$

where k_{gut} is a parameter that determines how fast the K^+ is delivered to the plasma.

Extracellular fluid

The extracellular fluid is made up of the blood plasma and the interstitial fluid. As noted above, after K^+ is ingested and processed, it is absorbed into the blood. The amount of K^+ in the blood plasma ($M_{K_{\text{plasma}}}$) is given by

$$\frac{dM_{K_{\text{plasma}}}}{dt} = k_{\text{gut}} \times M_{K_{\text{gut}}} - \Phi_{\text{ECF}} - \Phi_{\text{uK}} \quad (2.2)$$

where Φ_{uK} denotes urine K^+ excretion; Φ_{ECF} denotes the diffusion of K^+ from the blood plasma to the interstitial fluid and is given by

$$\Phi_{\text{ECF}} = P_{\text{ECF}} \times (K_{\text{plasma}} - K_{\text{interstitial}}) \quad (2.3)$$

where P_{ECF} is a permeability parameter (see Table 2.1), K_{plasma} denotes the plasma K^+ concentration, and $K_{\text{interstitial}}$ denotes K^+ concentration in the interstitial fluid.

Note that the total extracellular fluid is about 14.5 L in the average male, where about 4.5 L is plasma fluid [107]. Letting the volume of plasma be given by $V_{\text{plasma}} = 4.5$ L and interstitial volume $V_{\text{inter}} = 10$ L, we have

$$K_{\text{plasma}} = \frac{M_{K_{\text{plasma}}}}{V_{\text{plasma}}} \quad (2.4)$$

$$K_{\text{interstitial}} = \frac{M_{K_{\text{inter}}}}{V_{\text{inter}}} \quad (2.5)$$

where $M_{K_{\text{inter}}}$ is the amount of K^+ in the interstitial fluid and is determined by

$$\frac{dM_{K_{\text{inter}}}}{dt} = \Phi_{\text{ECF}} - \Phi_{\text{ECtoIC}} + \Phi_{\text{ICtoEC}} \quad (2.6)$$

where Φ_{ECtoIC} and Φ_{ICtoEC} are the flux of K^+ from the extracellular to the intracellular fluid and flux of K^+ from the intracellular to the extracellular fluid, respectively (described below).

Intracellular fluid

Most K^+ (about 75%) within the intracellular fluid is stored in skeletal muscle cells [95]. Hence, the amount of K^+ in the skeletal muscle is the major driving factor in intracellular K^+ concentration. The amount of K^+ in the intracellular compartment is denoted by M_{KIC} and is determined by

$$\frac{dM_{KIC}}{dt} = \Phi_{ECtoIC} - \Phi_{ICtoEC}. \quad (2.7)$$

Flow of K^+ into the intracellular fluid (Φ_{ECtoIC}) is driven by Na^+ - K^+ -ATPase. This is modeled using Michaelis-Menten kinetics so that

$$\Phi_{ECtoIC} = \rho_{al} \times \rho_{insulin} \times \frac{V_{max} \times K_{interstitial}}{K_m + K_{interstitial}} \quad (2.8)$$

where V_{max} is the maximum rate, K_m denotes the half maximal activation level, ρ_{al} is the effect of ALD on Na^+ - K^+ -ATPase, and $\rho_{insulin}$ is the effect of insulin (see Eqs. 2.22 and 2.26).

Flow from the intracellular to extracellular compartments (i.e., Φ_{ICtoEC}) is modeled by diffusion through a permeable membrane

$$\Phi_{ICtoEC} = P_{trans} \times (K_{IC} - K_{interstitial}) \quad (2.9)$$

where P_{trans} denotes the transmembrane permeability, K_{IC} is the concentration of K^+ in the intracellular fluid. Note that

$$K_{IC} = \frac{M_{KIC}}{V_{intracellular}} \quad (2.10)$$

where $V_{intracellular}$ is the volume of the intracellular fluid (see Table 2.1).

2.2.2 Renal handling of K^+

Potassium is freely filtered at the glomerulus. As such, the filtered K^+ load (Φ_{filK}) is the GFR (denoted by Φ_{GFR}) multiplied by the plasma K^+ concentration, i.e.,

$$\Phi_{filK} = \Phi_{GFR} \times K_{plasma}. \quad (2.11)$$

Note that, in this model, Φ_{GFR} is assumed to be constant based on average GFR in a man (Table 2.1).

As the filtrate passes through the nephrons, K^+ is reabsorbed and returned to the general circulation along some tubular segments, and secreted into the lumen along the distal segments (Fig. 2.1). Indeed, K^+ transport is coupled to other solutes, either directly via a cotransporter or indirectly via membrane potential. But for simplicity, the transport of other solutes and fluid is not represented in this model. Eventually, what is remaining at the end of the collecting ducts is excreted in urine. Despite the heterogeneity among nephron populations, for simplicity the model represents a single nephron, divided into three model segments: the “proximal segment (ps)” which includes the proximal tubule and the loop of Henle, the “distal tubule (dt)” that includes the distal convoluted tubule and the connecting tubule, and the collecting duct (cd).

The proximal segment is assumed to reabsorb a fixed fraction (92%) of the filtered K^+ based on Ref. [81]:

$$\Phi_{ps-Kreab} = 0.92 \times \Phi_{filK}. \quad (2.12)$$

The proximal segment (which includes the loop of Henle) ends at the macula densa. Macula densa K^+ flow (Φ_{mdK}) is the difference between filtered K^+ load and proximal segment reabsorption:

$$\Phi_{mdK} = \Phi_{filK} - \Phi_{ps-Kreab}. \quad (2.13)$$

The macula densa marks the beginning of the distal tubule, where most of the renal K^+ handling is finely regulated.

Along the distal tubule, K^+ secretion occurs. Distal tubule K^+ secretion ($\Phi_{dt-Ksec}$) has a baseline value of $\Phi_{dt-Ksec}^{eq}$ (see Table 2.1), chosen to yield a urine K^+ flow of $0.9 \times \Phi_{Kin}^{ss}$ at steady-state. Distal tubule K^+ secretion is regulated by ALD and the gastrointestinal feedforward effect so that

$$\Phi_{dt-Ksec} = \Phi_{dt-Ksec}^{eq} \times \gamma_{al} \times \gamma_{Kin} \quad (2.14)$$

where γ_{al} denotes the regulatory effect of ALD and γ_{Kin} represents the gastrointestinal feedforward effect (see Eqs. 2.23 and 2.27).

Distal tubule K^+ outflow (Φ_{dtK} ; mEq/min) is given by the macula densa K^+ flow plus net distal tubule K^+ secretion:

$$\Phi_{dtK} = \Phi_{mdK} + \Phi_{dt-Ksec} \quad (2.15)$$

which then enters the collecting duct. The collecting duct both secretes and reabsorbs K^+ through the function of principal and intercalated cells, respectively [81]. Collecting duct K^+ secretion ($\Phi_{cd-Ksec}$) has a baseline value of $\Phi_{cd-Ksec}^{eq}$ (see Table 2.1), and is regulated by ALD. As such we let

$$\Phi_{cd-Ksec} = \Phi_{cd-Ksec}^{eq} \times \lambda_{al} \quad (2.16)$$

where λ_{al} denotes the regulatory effect of ALD on collecting duct K^+ secretion (see Eq. 2.24).

Collecting duct K^+ reabsorption ($\Phi_{\text{cd-Kreab}}$) is modeled as a fraction of distal tubule K^+ outflow:

$$\Phi_{\text{cd-Kreab}} = \Phi_{\text{dtK}} \times A_{\text{cd-Kreab}} \quad (2.17)$$

where $A_{\text{cd-Kreab}}$ is a fitting parameter (see Table 2.1).

Finally, urine K^+ excretion (Φ_{uK}) is given by the difference between distal tubule K^+ outflow and net collecting duct K^+ transport ($\Phi_{\text{cd-Ksec}} - \Phi_{\text{cd-Kreab}}$) so that

$$\Phi_{\text{uK}} = \Phi_{\text{dtK}} + \Phi_{\text{cd-Ksec}} - \Phi_{\text{cd-Kreab}}. \quad (2.18)$$

2.2.3 Aldosterone effects on cellular K^+ uptake and kidney function

Aldosterone plays a major role in K^+ homeostasis via its effect on K^+ secretion along the distal tubule (see Eq. 2.14) and Na^+ - K^+ -ATPase abundance (see Eq. 2.8). To model ALD concentration (C_{al}), we let N_{al} be the normalized ALD concentration so that

$$C_{\text{al}} = N_{\text{al}} \times 85 \text{ ng/L} \quad (2.19)$$

where baseline C_{al} is 85 ng/L. Then following the approach in Ref. [107] and letting N_{als} denote normalized ALD secretion, we have that

$$\frac{dN_{\text{al}}}{dt} = \frac{1}{T_{\text{al}}} (N_{\text{als}} - N_{\text{al}}) \quad (2.20)$$

where T_{al} is the time constant governing regulation of C_{al} .

In this model, ALD secretion is affected by letting

$$N_{\text{als}} = \max \left(0, \frac{K_{\text{ECFtotal}}/C_{\text{sod}}}{\alpha_{\text{al-sec}}} - \xi_{\text{al-sec}} \right) \quad (2.21)$$

where $\alpha_{\text{al-sec}}$ and $\xi_{\text{al-sec}}$ are fitting parameters,

$$K_{\text{ECFtotal}} = (M_{\text{Kplasma}} + M_{\text{Kinterstitial}}) / (V_{\text{plasma}} + V_{\text{interstitial}})$$

is the extracellular fluid concentration, C_{sod} is the extracellular Na^+ concentration ($C_{\text{sod}} = 144 \text{ mEq/L}$), and is parameterized so that $N_{\text{als}} = 1$ when K_{ECFtotal} is at its steady-state value (see Table 2.2).

An increase in ALD concentration leads to a modest increase in $\text{Na}^+\text{-K}^+\text{-ATPase}$ abundance, which increases K^+ flux into cells [40, 169]. We model this effect by the scaling factor ρ_{al} (from Eq. 2.8)

$$\rho_{\text{al}} = \alpha_{\text{al}} + \beta_{\text{al}} \times C_{\text{al}} \quad (2.22)$$

where α_{al} and β_{al} are parameters fit to the data in Phakdeekitcharoen et al. [169].

Distal tubule and collecting duct K^+ secretion both increase with increased ALD concentration. Field et al. [61] found that in adult male Sprague-Dawley rats, an increase in plasma ALD concentration from 4.4 ng/dl to 55.8 ng/dl resulted in an 83% increase in distal tubule secretion. Humans have a much higher baseline ALD concentration. Given that interspecies difference, we parameterize the equation so that the same percentage increase from the baseline ALD concentration induces the same percentage increase in secretion

$$\gamma_{\text{al}} = A_{\text{dt-Ksec}} \times (C_{\text{al}})^{B_{\text{dt-Ksec}}} \quad (2.23)$$

where $A_{\text{dt-Ksec}}$ and $B_{\text{dt-Ksec}}$ are fitting parameters. Similarly, Schwartz et al. [195] measured K^+ flux in the cortical collecting ducts of rabbits at various ALD levels. Using this data, we fit the curve connecting ALD levels to collecting duct K^+ secretion so that

$$\lambda_{\text{al}} = A_{\text{cd-Ksec}} \times (C_{\text{al}})^{B_{\text{cd-Ksec}}} \quad (2.24)$$

where $A_{\text{cd-Ksec}}$ and $B_{\text{cd-Ksec}}$ are fitting parameters. See Table 2.1 for parameter values of $A_{\text{dt-Ksec}}$, $B_{\text{dt-Ksec}}$, $A_{\text{cd-Ksec}}$, and $B_{\text{cd-Ksec}}$.

2.2.4 Insulin effect on cellular K^+ uptake

We approximate the relationship between serum insulin levels and time after a meal based on Benedict et al. [14]. Letting t_0 denote the time at the beginning of the meal (in minutes) we have

$$C_{\text{insulin}} = \begin{cases} \frac{(325-22.6) \text{ pmol/L}}{90 \text{ min}} \times (t - t_0 \text{ min}) + 22.6 \text{ pmol/L} & \text{if } t_0 \leq t < t_0 + 90 \\ \frac{-(325-22.6) \text{ pmol/L}}{270 \text{ min}} \times (t - (t_0 + 360 \text{ min})) + 22.6 \text{ pmol/L} & \text{if } t_0 + 90 \leq t \leq t_0 + 360 \\ 22.6 \text{ pmol/L} & \text{otherwise.} \end{cases} \quad (2.25)$$

where C_{insulin} denotes the concentration of serum insulin (pmol/L).

Serum insulin concentration stimulates $\text{Na}^+\text{-K}^+\text{-ATPase}$ uptake [142, 188]. Thus, we let ρ_{insulin} (from Eq. 2.8) be given by

$$\rho_{\text{insulin}} = \max(1, A_{\text{insulin}} \times (C_{\text{insulin}} - C_{\text{insulin}}^{\text{ss}}) + 1) \quad (2.26)$$

where $C_{\text{insulin}}^{ss} = 123.4$ pmol/L denotes steady-state C_{insulin} and A_{insulin} is a fitting parameter (see Table 2.1). This function is bounded below by 1 so that insulin only impacts Na^+ - K^+ -ATPase activity when C_{insulin} is larger than baseline insulin levels (i.e., C_{insulin}^{ss}).

2.2.5 Gastrointestinal feedforward control mechanism

In experimental animals, when plasma K^+ concentration is artificially maintained constant, the kaliuretic response to a K^+ load is greater when given as a meal than with an intravenous infusion [145, 157]. This evidence suggests the existence of K^+ sensors in the gut capable of responding to dietary K^+ to signal to the kidney to rapidly alter K^+ excretion before plasma K^+ levels increase. The model represents this feedforward effect via the term γ_{Kin} , which alters distal tubule K^+ secretion with M_{Kgut} (see Eq. 2.14) where

$$\gamma_{\text{Kin}} = \max(1, A_{\text{Kin}} \times (M_{\text{Kgut}} - M_{\text{Kgut}}^{ss}) + 1) \quad (2.27)$$

where A_{Kin} is a fitting parameter (see Table 2.1) and M_{Kgut}^{ss} is the steady-state value of M_{Kgut} . Note that we have

$$M_{\text{Kgut}}^{ss} = \frac{0.9 \times \Phi_{\text{Kin}}^{ss}}{k_{\text{gut}}}$$

by solving Eq. 2.1 at steady-state.

2.2.6 Muscle-kidney cross talk mechanism

As previously noted, following a K^+ load or depletion, K^+ moves into or out of muscle cells as needed to stabilize plasma K^+ concentration. However, sustained high or low intracellular K^+ concentrations can be harmful. A muscle-kidney cross talk signal may allow intracellular K^+ concentration, K_{IC} , to more rapidly return to its baseline level, without significant variations in plasma K^+ concentration so that a sufficiently high (muscle) K_{IC} elevates urine K^+ excretion and vice versa.

Because there has not been definitive evidence for this muscle-kidney cross talk, it is not included in the baseline model. However, we conducted simulations for these “*what-if*” scenarios to study its potential impact. Muscle-kidney cross talk is represented in these scenarios via a coupling function that links intracellular K^+ to either (i) distal tubule K^+ secretion, (ii) collecting duct K^+ secretion, or (iii) collecting duct K^+ reabsorption. The coupling function (ω_{Kic}) is taken to be a linearly increasing function of K_{IC} , bounded above 0:

$$\omega_{\text{Kic}} = \max(0, m_{\text{Kic}} (K_{\text{IC}} - K_{\text{IC}}^{\text{baseline}}) + 1) \quad (2.28)$$

where the value for m_{Kic} depends on the case being simulated (see Eqs. 2.29, 2.30, and 2.31).

At baseline, $K_{\text{IC}} = K_{\text{IC}}^{\text{baseline}} = 130 \text{ mEq/L}$, thus $\omega_{\text{Kic}} = 1$. To model a muscle-kidney cross talk signal that targets distal tubule K^+ secretion, ω_{Kic} can be included in Eq. 2.14 so that

$$\Phi_{\text{dt-Ksec}} = \Phi_{\text{dt-Ksec}}^{eq} \times \gamma_{\text{al}} \times \gamma_{\text{Kin}} \times \omega_{\text{Kic}}, \quad m_{\text{Kic}} = 0.1 \quad (2.29)$$

We will refer to this scenario as Case MKX-DT-sec. If collecting duct secretion is the target, then we modify Eq. 2.16 by

$$\Phi_{\text{cd-Ksec}} = \Phi_{\text{cd-Ksec}}^{eq} \times \lambda_{\text{al}} \times \omega_{\text{Kic}}, \quad m_{\text{Kic}} = 0.1. \quad (2.30)$$

Similarly, if collecting duct reabsorption is the target, we modify Eq. 2.17 by

$$\Phi_{\text{cd-Kreab}} = \Phi_{\text{dtK}} \times A_{\text{cd-Kreab}} \times \omega_{\text{Kic}}, \quad m_{\text{Kic}} = -0.1. \quad (2.31)$$

We refer to these two cases as Case MKX-CD-sec and Case MKX-CD-reab, respectively.

2.2.7 Model parameters

Model parameter values are listed in Table 2.1. References are given for parameter values that were found in the literature; other parameters were fitted during model construction.

To fit P_{trans} , we applied the constraint that at steady-state, the flows into and out of cells are equal. That is we set the right sides of Eqs. 2.8 and 2.9 equal with the feedback effects set to 1 (due to steady-state) and used the baseline values for K_{IC} and $K_{\text{interstitial}}$ (130 mEq/L and 4.3 mEq/L, respectively), and solved for P_{trans} .

Phakdeekitcharoen et al. [169] found a linear relationship between ALD levels and skeletal muscle Na^+ - K^+ -ATPase activity. We fit Eq. 2.22 to the results presented in their study normalized to baseline Na^+ - K^+ -ATPase activity to represent the impact of ALD concentration on K^+ uptake into the intracellular fluid. Field et al. [61] found that in adult male Sprague-Dawley rats, an increase in plasma ALD concentration from 4.4 ng/dl to 55.8 ng/dl causes distal tubule K^+ secretion to increase by 83%. Our (human male) model has a much higher baseline ALD concentration ($C_{\text{al}}^{\text{baseline}} = 85 \text{ ng/l}$). Given that discrepancy, we chose values for parameters $A_{\text{dt-Ksec}}$ and $B_{\text{dt-Ksec}}$ in Eq. 2.23 so that the same percent increase from ALD baseline concentration causes the same percent increase in K^+ secretion. Scharz et al. [195] measured K^+ flux in the cortical collecting ducts of rabbits at various plasma ALD concentration. Using this data, we fit the curve connecting ALD levels to collecting duct K^+ secretion to get values for the parameters $A_{\text{cd-Ksec}}$ and $B_{\text{cd-Ksec}}$ in Eq. 2.24.

Some model parameters had to be estimated by fitting to K^+ regulation data for a single meal intake. Specifically, the magnitude of the gastrointestinal feedforward effect (A_{Kin}), collecting duct K^+ reabsorption parameter ($A_{cd-Kreab}$), and insulin sensitivity parameter ($A_{insulin}$) were fitted to match model behavior to the plasma K^+ concentration and urine K^+ excretion data from Preston et al. [172].

2.2.8 Numerical methods

Model simulations were conducted using a variable-step, variable-order implicit differential algebraic equation solver (`ode15i`, MATLAB R2021a). Parameter fitting was conducted using the interior-point algorithm (`fmincon`, MATLAB R2021a) to minimize model predicted values from given data. The code used for this study is available at

<https://github.com/Layton-Lab/Kregulation>.

2.3 Results

2.3.1 Baseline steady-state results

We computed the baseline steady-state solution by assuming a daily K^+ intake of 70 mEq a day distributed evenly over 24 hours, corresponding to steady-state K^+ intake of $\Phi_{Kin}^{ss} = 0.049$ mEq/min. We chose this value for Φ_{Kin}^{ss} due to the experimental procedures used in Preston et al. [172] that was used to fit the baseline model parameters. We also note that, while the daily recommended intake of K^+ is 120 mEq/day, the average person on a Western diet consumes much less [145]. Using the model parameter values listed in Table 2.1, we integrated baseline model equations (Eqs. 2.1–2.27) to steady-state. Model steady-state solution values are summarized in Table 2.2. We report normal ranges for K^+ amount and concentrations in Table 2.3.

At steady-state, 97.9% of the total body K^+ is stored intracellularly. This is consistent with estimates in the literature [95, 162]. Model steady-state plasma and intracellular K^+ concentrations are 4.2 and 129.3 mEq/L, respectively. These concentrations are within normal range [95]. Urine K^+ excretion is 0.044 mEq/min, which is 90% of Φ_{Kin}^{ss} reflecting that 90% of K^+ intake is excreted through urine, while the remaining 10% is excreted via feces [95]. The filtered K^+ load ($\Phi_{filK}^{ss} = 0.53$ mEq/min) is determined by the fixed GFR parameter (Φ_{GFR}) and the steady-state plasma K^+ concentration (K_{plasma}^{ss}). About 92% of the filtered K^+ is reabsorbed along the proximal segments, followed by substantial

Variable	Description	Value	Unit
M_{Kgut}	amount of K^+ in gut and heptatoportal circulation	4.4	mEq
M_{Kplasma}	amount of K^+ in plasma	18.9	mEq
$M_{\text{Kinterstitial}}$	amount of K^+ in interstitial fluid	42.1	mEq
M_{KIC}	amount of K^+ in intracellular fluid	3104.2	mEq
K_{plasma}	plasma K^+ concentration	4.2	mEq/L
$K_{\text{interstitial}}$	interstitial fluid K^+ concentration	4.2	mEq/L
K_{IC}	intracellular fluid K^+ concentration	129.3	mEq/L
Φ_{ECtoIC}	K^+ flow into cells	97.6	mEq/min
Φ_{ICtoEC}	K^+ flow out of cells	97.6	mEq/min
Φ_{filK}	filtered K^+ load	0.53	mEq/min
$\Phi_{\text{ps-Kreab}}$	proximal segment K^+ reabsorption	0.48	mEq/min
Φ_{mdK}	tubular K^+ flow at macula densa	0.042	mEq/min
$\Phi_{\text{dt-Ksec}}$	distal tubule K^+ secretion	0.041	mEq/min
Φ_{dtK}	distal tubule K^+ outflow	0.083	mEq/min
$\Phi_{\text{cd-Ksec}}$	collecting duct K^+ secretion	0.0022	mEq/min
$\Phi_{\text{cd-Kreab}}$	collecting duct K^+ reabsorption	0.042	mEq/min
Φ_{uK}	urine K^+ flow	0.044	mEq/min

Table 2.2: Baseline model steady-state solution. These values are used as the initial conditions in the model simulations.

K^+ secretion along the distal tubule ($\Phi_{\text{dt-Ksec}}^{\text{ss}} = 0.041$ mEq/min). Both reabsorption and secretion happens along the collecting duct, resulting in net reabsorption at steady-state ($\Phi_{\text{cd-Kreab}}^{\text{ss}} - \Phi_{\text{cd-Ksec}}^{\text{ss}} = 0.039$ mEq/min). Taken together, urine K^+ excretion corresponds to about 8.3% of the filtered K^+ load.

2.3.2 Response to a single meal of varying content

What are the physiological responses to meals with different contents? We followed experimental protocols in Preston et al. [172] and simulated a K^+ deficient meal (referred to as “Meal only” experiment), 35 mEq of orally ingested K^+ only (referred to as “KCl only” experiment), and a K^+ deficient meal with 35 mEq of K^+ (referred to as the “Meal + KCl” experiment). This was accomplished by varying K^+ intake (Φ_{Kin}) as an independent variable. Initial conditions were taken to be the baseline steady-state solution (Table 2.2). To simulate a fasting state, K^+ intake was initialized at the steady-state value ($\Phi_{\text{Kin}}^{\text{ss}} = 0.049$ mEq/min), then decreased to $\Phi_{\text{Kin}} = 0$ mEq/min and kept at no K^+ intake for six

Compartment	Range	Unit	Ref
Amount of K^+ in extracellular fluid	60-70	mEq	[95, 142]
Amount of K^+ in intracellular fluid	3400 - 3900	mEq	[95]
Extracellular K^+ concentration	3.5-5.0	mEq/L	[95, 142]
Intracellular K^+ concentration	120-140	mEq/L	[95, 142]

Table 2.3: Normal ranges for K^+ concentration and amount.

hours (see Fig 2.3a). After this fasting period, a meal was simulated depending on the experiment type (i.e., “Meal only”, “KCl only”, or “Meal + KCl”). For the experiments that include K^+ ingestion (i.e., “KCl only” and “Meal + KCl”), a dose of 35 mEq of K^+ is given over 30 minutes of meal ingestion (see Fig 2.3a). Fig 2.3 shows simulation results for the impact of K^+ intake on K^+ amounts and concentrations in the gut, plasma, interstitial, and intracellular fluid for each of the three experiments. Fig 2.4 shows the effects of K^+ intake on the kidney and Fig 2.5 shows the predicted effects of insulin and ALD as well as the gastrointestinal feedforward effect.

When a meal containing glucose is ingested, insulin is secreted. Besides its role in glucose metabolism, insulin also activates cellular uptake of K^+ by increasing Na^+ - K^+ -ATPase activity. To isolate insulin’s effect on K^+ regulation, we simulate a K^+ deficient meal (“Meal only” experiment) by running the baseline model with $\Phi_{Kin} = 0$, with the stimulation of insulin as given by Eqs. 2.25 and 2.26. The model predicts that the insulin in a K^+ -deficient meal elevates Na^+ - K^+ -ATPase uptake, by up to 10% (Fig 2.5a), resulting in an immediate increase of about 5 mEq in the intracellular K^+ fluid. However, that increase is small relative to the baseline intracellular K^+ amount (3104.2 mEq; Table 2.2); thus, there is essentially no change in the intracellular K^+ concentration (Fig 2.3e). In contrast, the baseline amount of K^+ in plasma and interstitial fluids are significantly less than in the intracellular fluid (18.9 and 42.1 mEq, respectively, vs. 3104.2 mEq; Table 2.2); thus, the higher cellular K^+ uptake is enough to reduce both the interstitial and plasma K^+ concentration from about 4.0 mEq/L at the end of the fasting period to about 3.5 mEq/L (Fig 2.3c). The predicted trend in plasma K^+ concentration is consistent with data reported by Preston et al. [172] (Fig 2.3c). This simulation illustrates the mechanism by which insulin lowers the extracellular K^+ concentration without significantly elevating intracellular K^+ concentration following a meal. We also note that the predicted urine K^+ excretion (Fig 2.4d) captures the observed trend of a decrease in urine K^+ excretion [172].

Next we considered a K^+ load not accompanied by a meal containing glucose (“KCl only”). We did this by increasing the independent variable Φ_{Kin} so that there is a 35 mEq dose of K^+ (Fig 2.3a), but did not include the effect of insulin (i.e., $\rho_{insulin} = 1$ for the full

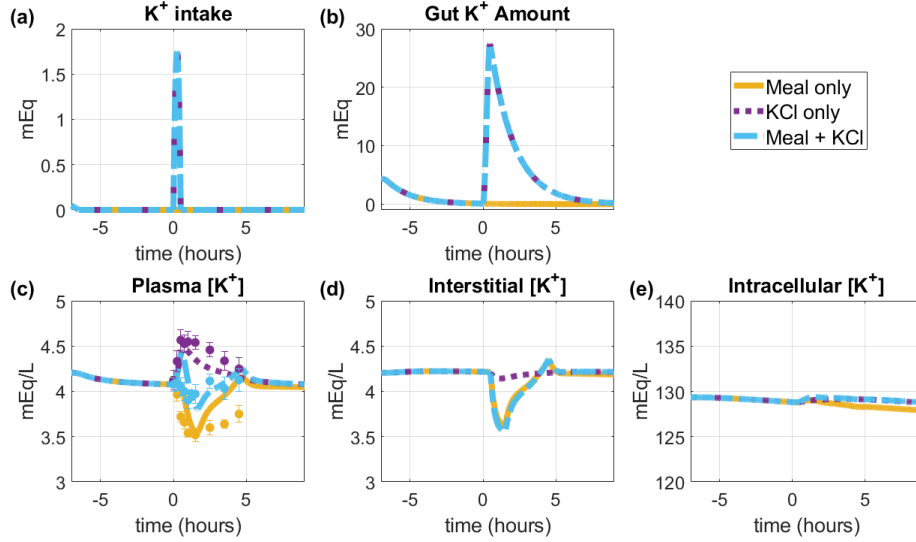


Figure 2.3: (a) Prescribed K^+ intake, (b) predicted gut K^+ amount, and (c)-(e) predicted K^+ concentrations for a single meal load. The meal experiment occurs at time = 0 hours. Initial conditions are the baseline steady-state values (see Table 2.2) followed by a fasting state set before the meal experiment. The yellow lines denote the K^+ deficient meal (i.e., “Meal only” experiment); purple, only K^+ ingestion (i.e., “KCl only” experiment); blue, meal with K^+ (i.e., “Meal + KCl” experiment). Points and error bars for plasma K^+ concentration (c) show experimental data from Preston et al. [172].

simulation time; Fig 2.5a). When K^+ is added into the model system, the gut K^+ amount increases from 0 mEq to a peak of 27 mEq (Fig 2.3b). The K^+ is eventually absorbed into the plasma, causing plasma K^+ concentration to increase by 12% (Fig 2.3c). The gastrointestinal feedforward effect from the increased gut K^+ amount (Fig 2.5c) signals to the distal tubule to rapidly increase K^+ secretion (Fig 2.4b). This in turn increases urine K^+ excretion so that plasma K^+ levels can return to baseline after about 6 hours (Fig 2.3c). The predicted plasma K^+ concentration and urine K^+ excretion (Figs 2.3c and 2.4d) match the observed trends [172].

The baseline model represents a typical meal that contains both K^+ and glucose, therefore includes both the effect of insulin on cellular uptake and gastrointestinal feedforward effect of K^+ . The model predicts an initial rise in plasma K^+ concentration, but then the insulin effect on cellular uptake (ρ_{insulin}) leads to about 14 mEq K^+ being moved into the intracellular compartment, quickly returning the plasma K^+ concentration back to baseline

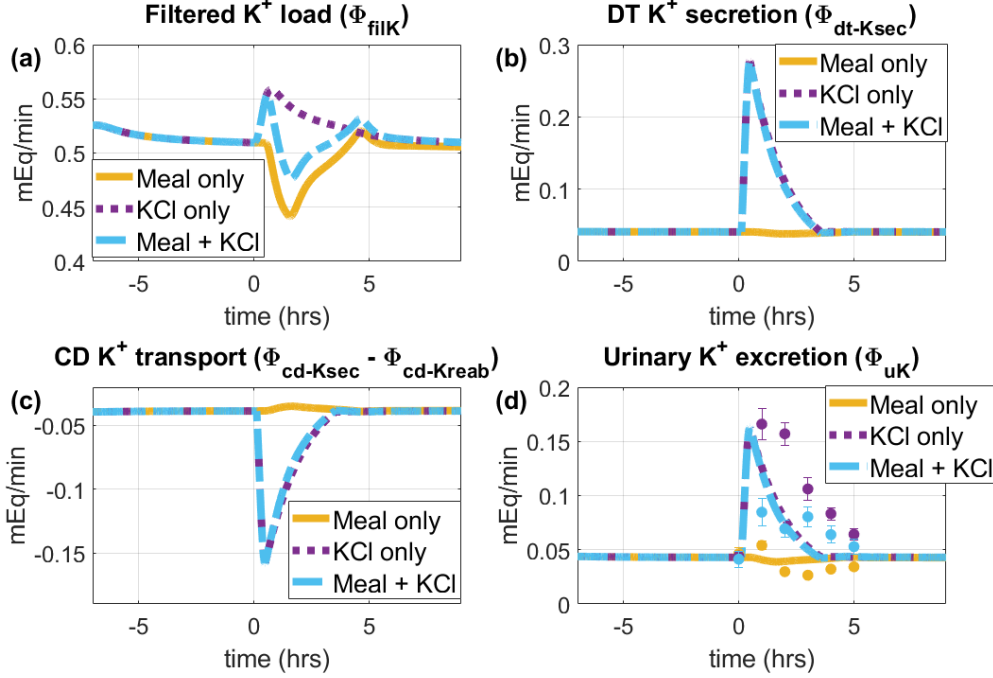


Figure 2.4: Model simulation predictions for kidney K^+ handling during a single meal load. The meal experiment occurs at time = 0 hours. Initial conditions are the baseline steady-state values (see Table 2.2) with a fasting state before the meal experiment. The yellow lines denote the K^+ deficient meal (i.e., “Meal only” experiment); purple, only K^+ ingestion (i.e., “KCl only” experiment); blue, meal with K^+ (i.e., “Meal + KCl” experiment). Points and error bars for urine K^+ excretion show experimental data from Preston et al. [172]. CD: collecting duct, DT: distal tubule

level (Fig 2.3c). As in the “Meal only” case, there is not a notable change in intracellular K^+ concentration (Fig 2.3e). The gastrointestinal feedforward effect elevates distal tubule K^+ secretion, which in turn increases urine K^+ excretion (Fig 2.4; Fig 2.5). Together these effects stabilize plasma K^+ concentration around its baseline value.

2.3.3 Effect of feedforward and feedback controls

We assessed the effect of the feedforward and feedback controls on K^+ regulation by conducting *in silico* experiments of the baseline model without individual control mechanisms.

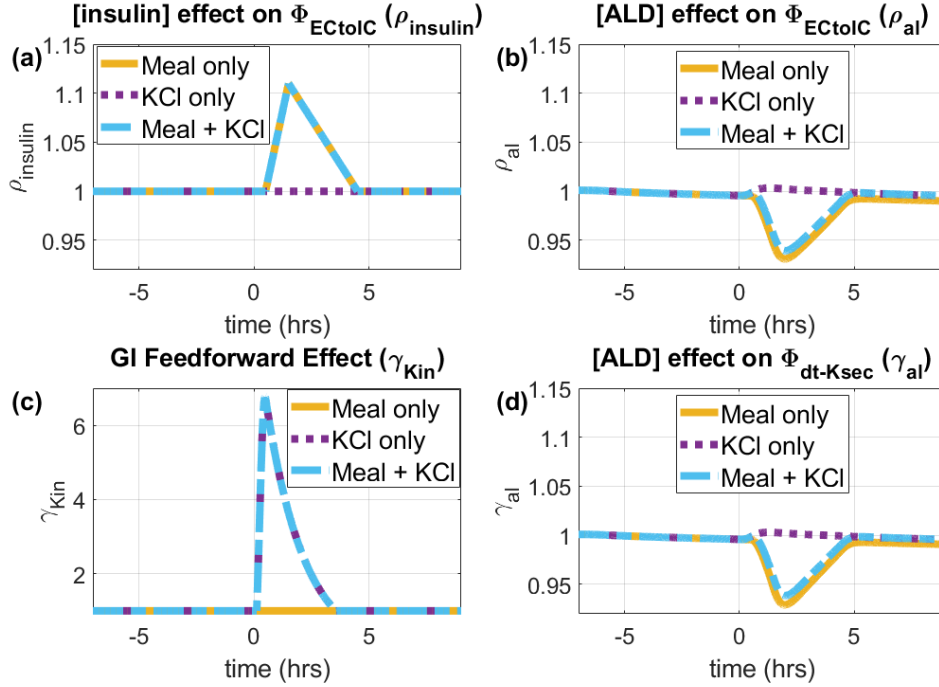


Figure 2.5: Model simulation predictions for (a) effect of insulin concentration ($[\text{insulin}]$) on $\text{Na}^+\text{-K}^+\text{-ATPase K}^+$ uptake, (b) aldosterone concentration effect on $\text{Na}^+\text{-K}^+\text{-ATPase K}^+$ uptake, (c) gastrointestinal (GI) feedforward effect on distal tubule K^+ secretion, and (d) aldosterone effect on distal tubule K^+ secretion. The meal experiment occurs at 0 hours. Initial conditions are steady-state with a fasting state set before the meal experiment. The yellow lines denote the K^+ deficient meal (i.e., “Meal only” experiment); purple, only K^+ ingestion (i.e., “KCl only” experiment); blue, meal with K^+ (i.e., “Meal + KCl” experiment). ALD: aldosterone, GI: gastrointestinal

In these simulations, three equal typical meals were given (i.e., including glucose and K^+) each day, totaling 100 mEq of K^+ intake per day for 10 days (see Fig 2.6a).

With all control mechanisms intact, the baseline model maintains a relatively steady plasma K^+ concentration through the day with fluctuations around meal times (Fig 2.6b). Each day the predicted plasma K^+ concentration varied between 3.8 and 4.4 mEq/L with an average daily plasma K^+ concentration of 4.2 mEq/L (Fig 2.6b); intracellular K^+ concentration remained stable at about 130 mEq/L throughout the 10 day simulation (Fig 2.6c). Both plasma K^+ and intracellular K^+ concentration were within normal ranges for the

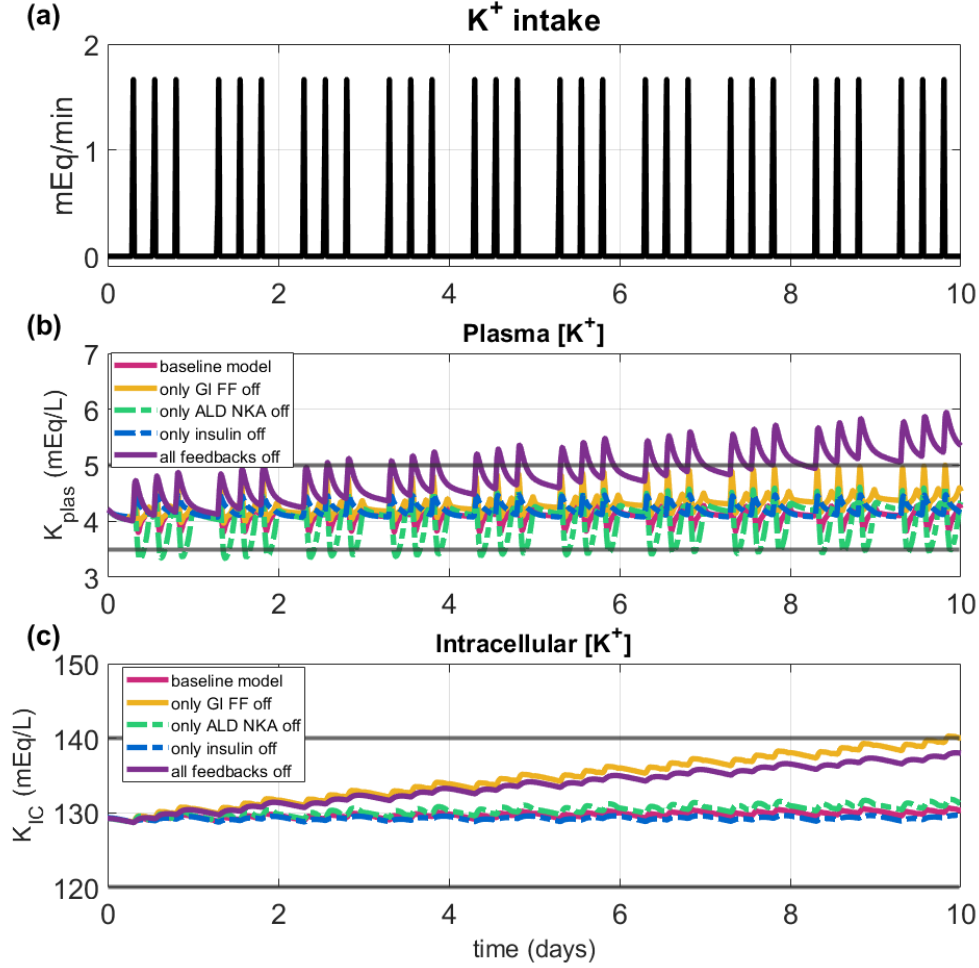


Figure 2.6: Simulation results for plasma K⁺ concentration (b) and intracellular K⁺ concentration (c) for 10 days of normal K⁺ intake ((a); 100 mEq/day, 3 typical meals) for the baseline model, baseline without gastrointestinal feedforward (GI FF) effect ($\gamma_{\text{Kin}} = 1$), baseline without the aldosterone effect on Na⁺-K⁺-ATPase uptake ($\rho_{\text{al}} = 1$), baseline model without insulin effect on Na⁺-K⁺-ATPase uptake ($\rho_{\text{insulin}} = 1$), and baseline model with all feedback effects off. Grey lines indicate normal range for K_{plasma} and K_{IC} . ALD: aldosterone, NKA: Na⁺-K⁺-ATPase, GI: gastrointestinal, FF: feedforward

entire 10 day intake simulation.

Both insulin and ALD affect K^+ balance by stimulating uptake of K^+ into the intracellular fluid via the $Na^+-K^+-ATPase$ pump. When the effect of insulin on $Na^+-K^+-ATPase$ uptake is turned off (i.e., $\rho_{insulin} = 1$), less K^+ enters the intracellular fluid, with plasma K^+ concentration reaching an average low of about 4.1 mEq/L, compared to the baseline value of 3.8 mEq/L. When the effect of ALD is turned off (i.e., $\rho_{al} = 1$), at meal times, predicted plasma K^+ concentration increases similarly to the baseline model. But as plasma K^+ begins to decrease after the meal, without the inhibitory effect of the resulting low ALD on $Na^+-K^+-ATPase$ activity, plasma K^+ concentration dips below normal range to 3.4 mEq/L (Fig 2.6b). Over time, it seems that through the day plasma K^+ concentration is able to stabilize and intracellular K^+ concentration is slightly higher at the end of the 10 days at 132 mEq/L. We also simulated the effect of turning off γ_{al} and λ_{al} , which affect distal tubule and collecting duct K^+ secretion, respectively, but they did not have a notable predicted impact on plasma or intracellular K^+ concentration (not shown in Fig 2.6).

The gastrointestinal feedforward effect is predicted to have the largest impact on K^+ regulation. Specifically, when this effect is turned off (i.e., $\gamma_{Kin} = 1$), on day 1 of normal K^+ intake the peak plasma K^+ concentration is 7% higher than the baseline model (Fig 2.6b). Each day the average plasma K^+ concentration and intracellular K^+ concentration increase so that at the end of the 10 days, average intracellular and plasma K^+ concentration are 9% higher than day 1 (Fig 2.6b; Fig 2.6c). This shows that without the gastrointestinal feedforward effect, total body K^+ increases by the same percentage in each of the compartments, hence increasing both plasma and intracellular K^+ concentrations. With continued normal K^+ intake, intracellular K^+ concentration is predicted to rise higher than normal range.

When all the feedforward and feedback effects are turned off, there is a large impact on both plasma and intracellular K^+ concentration (Fig 2.6b; Fig 2.6c). Both plasma K^+ and intracellular K^+ concentrations increase so that by day 6, average plasma K^+ concentration is above normal range (Fig 2.6b). At the end of the 10 day simulation, peak plasma K^+ concentration is a severe hyperkalemic level of 5.9 mEq/L. Notably, while intracellular K^+ concentration does steadily increase through the 10 day simulation, the increase is not as high as when only the gastrointestinal feedforward effect is turned off. This is likely because insulin and ALD (eliminated in this case) both stimulate K^+ uptake into the intracellular fluid. Without this stimulation, the muscle cells fail to sufficiently buffer the increase in plasma K^+ concentration, which reaches a dangerously high level. Notably, average daily plasma K^+ concentration increases by 33% while intracellular K^+ concentration increases by 7%, showing that it is not only whole body K^+ that is increasing, but the distribution of K^+ between the extracellular and intracellular fluid that is affected by not having all of these signals activated.

2.3.4 Response to repeated K^+ loading

How does the model respond to repeated K^+ loading, and what might the effect of muscle-kidney cross talk be? To answer this question, we initialized the model at steady-state, and then for 2 days we gave 3 meals of equal amounts so that total K^+ consumption was 100 mEq per day. Then for the next 4 days we increased the meal K^+ amounts so that the total K^+ consumption was 400 mEq per day. After the 4 days of K^+ loading, we returned to the baseline of 100 mEq per day. The 14-day K^+ intake values are shown in Fig 2.7a. This protocol is similar to the experiments conducted in Rabelink et al. [177]. Simulations were conducted for the baseline model, and separately with each of the 3 muscle-kidney cross talk mechanisms (Case MKX-DT-sec, Case MKX-CD-sec, and Case MKX-CD-reab).

When muscle-kidney cross talk is implemented to target collecting duct K^+ secretion (Case MKX-CD-sec), the model predicts negligible effects (results not included in Fig 2.7). This is likely because baseline collecting duct K^+ secretion is much smaller than either collecting duct K^+ reabsorption or distal tubule K^+ secretion (see Table 2.2). Muscle-kidney cross talk mechanisms that target distal tubule K^+ secretion or collecting duct K^+ reabsorption yield significant and similar effects on plasma K^+ concentration and intracellular K^+ concentration (Fig 2.7).

The baseline model, which does not have any muscle-kidney cross talk signal, predicts a strong response to a high K^+ diet (during days 3-6). During high K^+ intake, the average total body K^+ increases by 16.2%, with most of this excess K^+ stored in the intracellular fluid compartment. With the inclusion of the distal tubule muscle-kidney cross talk mechanism (Case MKX-DT-sec), average distal tubule K^+ secretion increases to 57.5% above baseline (Fig. 2.7b). For Case MKX-CD-reab, collecting duct reabsorption is inhibited so that on day 6, average collecting duct K^+ reabsorption is 61.6% below baseline (Fig. 2.7d). The two muscle-kidney cross talk mechanisms have a similar impact on urine K^+ excretion; on day 6, average urine K^+ excretion is $\sim 47\%$ above baseline (Fig 2.7f). As a result, at the end of day 6, the peak intracellular K^+ concentration is about 137 mEq/L with muscle-kidney cross talk compared to 150 mEq/L for the baseline model. At the end of the 14 days, intracellular K^+ concentration remains out of normal range for the baseline model at 148.5 mEq/L, whereas intracellular K^+ concentration is near baseline values at 131 mEq/L with muscle-kidney cross talk (Fig 2.7e). Similarly, mean plasma K^+ concentration at day 6 is about 4.3 mEq/L and 4.9 mEq/L, respectively, with and without muscle-kidney cross talk (Fig 2.7c). While the latter is within normal range *on average*, it is on the high end and its peak value easily went out of range to a maximum of 5.8 mEq/L, which would be diagnosed as hyperkalemia. Indeed, repeated K^+ loading results in an increasing trend in the plasma K^+ concentration in the baseline model, but with muscle-kidney cross talk

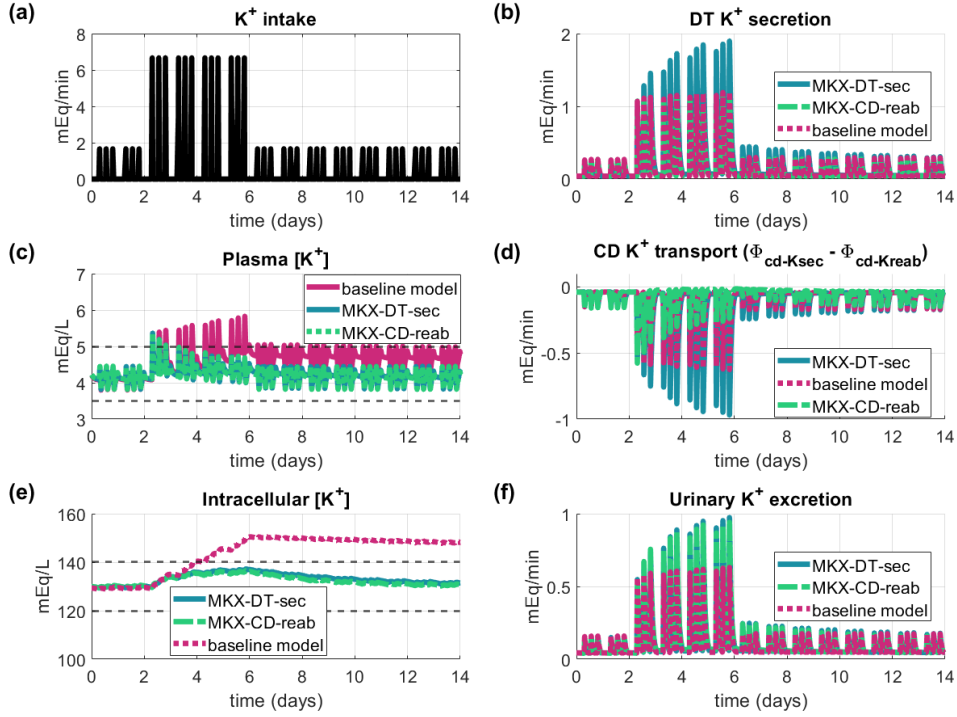


Figure 2.7: Simulation results for K^+ loading experiments for the baseline model and muscle-kidney cross talk simulations (Case MKX-DT-sec and Case MKX-CD-reab). Potassium intake (a) is the same for all three simulation types. Note that Case MKX-CD-sec is not plotted since it had little impact from the baseline model results. Horizontal grey line shows normal range for plasma K^+ concentration (c) and intracellular K^+ concentration (e). CD: collecting duct, DT: distal tubule

(Case MKX-DT-sec and Case MKX-CD-reab) plasma K^+ concentrations stays within a tight range. In other words, by signalling to the kidneys to excrete more K^+ when there are high intracellular K^+ concentrations, the body is better able to handle significant K^+ loads, thus preventing hyperkalemia. We conducted these simulations with varying values of the parameter m_{Kic} to show that the decrease in intracellular K^+ concentration is consistent for varied parameter values (see Appendix A.3; Fig. A.3). Model predictions suggest that the signal to collecting duct K^+ reabsorption or to distal tubule K^+ secretion would have a similar effect.

2.3.5 Response to K^+ depletion

We next analyzed the model's response to K^+ depletion. To that end, we started by simulating a normal daily K^+ intake of 100 mEq per day. Then we restricted the model's daily K^+ intake to 25 mEq a day for 30 days to see when or if the model would predict hypokalemia. After the 30 day depletion period we returned K^+ intake to 100 mEq per day (Fig 2.8A). Simulations were conducted with and without each of the 3 muscle-kidney cross talk mechanisms (Case MKX-DT-sec, Case MKX-CD-sec, and Case MKX-CD-reab). Similar to the K^+ loading experiment done previously (Fig 2.7), Case MKX-CD-sec had little effect on the model simulation results from baseline (not shown).

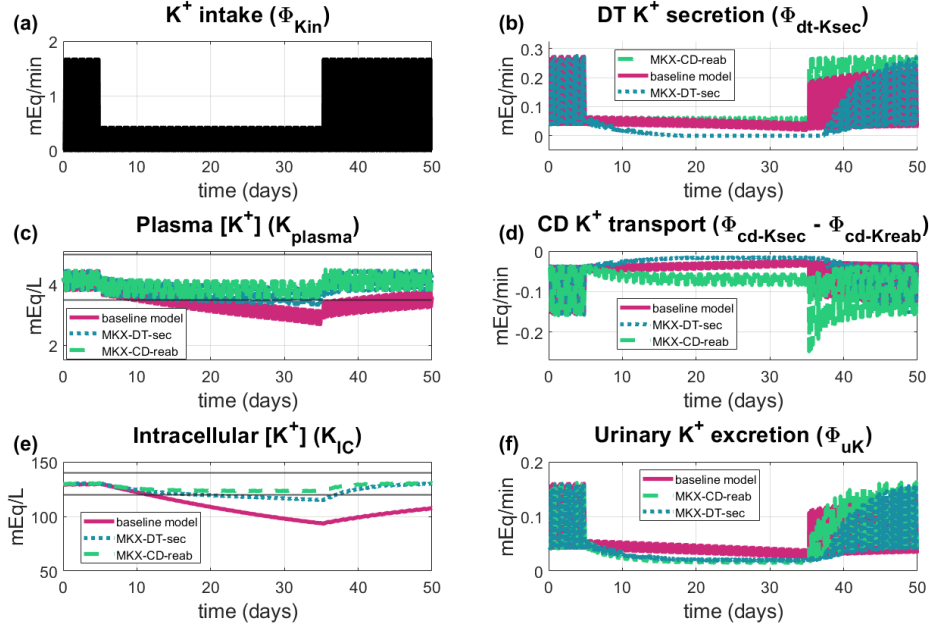


Figure 2.8: Simulation results for K^+ depletion experiments for the baseline model and muscle-kidney cross talk simulations (Case MKX-DT-sec and Case MKX-CD-reab). Potassium intake (Φ_{Kin}) is the same for all three simulation types (a). Note that Case MKX-CD-sec is not plotted since it had little impact from the baseline model results. Horizontal grey line shows normal range for plasma K^+ concentration (c) and intracellular K^+ concentration (e). CD: collecting duct, DT: distal tubule

During K^+ depletion, total body K^+ rapidly declines by 27.5% in the absence of muscle-

kidney cross talk, with intracellular K^+ concentration reduced from ~ 130 mEq/L to 93.8 mEq/L, significantly below normal range of 120-140 mEq/L (Fig 2.8e). Plasma K^+ concentration decreases from an average of 4.1 mEq/L to a severely hypokalemic daily average plasma K^+ concentration of 2.9 mEq/L by day 30 of K^+ depletion period (Fig 2.8c).

Model simulations predict that muscle-kidney cross talk can be highly protective in retaining K^+ by massively decreasing urine K^+ as intracellular K^+ decreases, thereby preserving total body K^+ and plasma K^+ . Specifically, Case MKX-DT-sec reduces distal tubule K^+ secretion to almost 0, resulting in a 30.8% decrease in average urine K^+ from the baseline model on day 30 of K^+ depletion (Fig 2.8b; Fig 2.8f). Case MKX-CD-reab more than doubles collecting duct K^+ reabsorption so that average urine K^+ is 46.0% lower than baseline on day 30 of K^+ depletion (Fig 2.8d; Fig 2.8f). These drastically reduced urine K^+ excretion rates limit K^+ loss, resulting in only 11.5% and 5.3% reduction in total K^+ for Case MKX-DT-sec and Case MKX-CD-reab, respectively, compared to 27.5% for the baseline model. Consequently, intracellular and plasma K^+ concentrations remain within normal ranges. Notably, in Case MKX-CD-reab neither plasma nor intracellular K^+ concentration falls below normal range (Fig 2.8c; Fig 2.8e). While plasma K^+ concentration for Case MKX-DT-sec eventually falls below 3.5 mEq/L, the average plasma K^+ concentration remains within normal range for the full K^+ depletion experiment (Fig 2.8c). Intracellular plasma K^+ concentration decreases substantially, but much slower than the baseline model simulations, reaching an average of 115 mEq/L at day 30 (Fig 2.8e). Both muscle-kidney cross talk simulations return to baseline plasma K^+ and intracellular K^+ concentrations within a few days of normal K^+ intake, whereas the baseline model takes more than 15 days to recover normal levels. In summary, muscle-kidney cross talk makes the body more robust to periods of severe K^+ depletion by substantially decreasing urine K^+ excretion. This is consistent for varied values of the parameter m_{Kic} (Appendix A.3; Fig. A.4).

2.4 Discussion

Maintaining extracellular and intracellular K^+ concentrations in the body within physiological ranges is crucial for proper cell function. The transmembrane K^+ gradient is a major contributor in maintaining a proper membrane potential, which helps determine contractility of skeletal and cardiac muscle [234]. Compared to other major electrolytes, the ratio of daily K^+ intake (about 70-120 mEq in a Western diet) to extracellular fluid amount (about 70 mEq) is the highest [142]. This high ratio means that maintaining the extracellular K^+ concentration within a tight normal range is a daily homeostatic chal-

lenge because of frequent periods of K^+ loading (e.g., at meal times) and fasting. Mammals have evolved feedforward and feedback mechanisms to maintain a normal extracellular K^+ concentration by (i) ensuring a proper distribution of K^+ between the extracellular and intracellular fluids and (ii) regulating urinary excretion of K^+ . These mechanisms are complex and are not fully understood.

The mathematical model presented in this study includes a comprehensive description of the regulatory mechanisms involved in K^+ handling. In particular, represented here is the gastrointestinal feedforward effect, in which higher dietary K^+ intake stimulates urinary K^+ excretion. Additionally, the model represents a feedback control, in which elevated plasma K^+ concentration stimulates ALD secretion to elevate renal tubular K^+ secretion and excretion, and vice versa. The model also includes the impact of both ALD and insulin secretion on K^+ uptake into the skeletal muscle.

Model simulations were conducted to predict the impact of the individual feedback and feedforward mechanisms on K^+ balance. We predict that the gastrointestinal feedforward effect has the largest role in regulating total body K^+ in the long term by stimulating distal tubule K^+ secretion during times of K^+ loading (Fig 2.6). Additionally, since typical meals contain glucose and thus stimulate insulin secretion, insulin's impact on $Na^+-K^+-ATPase$ uptake likely evolved to store extra K^+ in the skeletal muscles to later be secreted back into the extracellular fluid (Fig 2.6c). While the model predicts that the insulin signal only has a small impact on plasma K^+ concentration, it is likely that during periods of K^+ loading, the slight increase in $Na^+-K^+-ATPase$ uptake plays an important role in ensuring extra K^+ is stored in the intracellular fluid. ALD affects both the uptake of K^+ by $Na^+-K^+-ATPase$ as well as targets K^+ secretion in the distal segments of the nephron. In this way, during low extracellular K^+ concentrations, secretion is decreased in the nephrons and uptake by $Na^+-K^+-ATPase$ is decreased so that K^+ remains in the extracellular fluid. Model simulations predict that the ALD feedback stabilizes plasma K^+ concentration (Fig 2.6b).

It has been shown that after a period of high K^+ loading, plasma K^+ concentrations return to normal almost immediately after the high K^+ intake is discontinued [177]. In contrast, the baseline model yields a markedly slower excretion of the excess K^+ , with plasma and intracellular K^+ concentration remaining significantly above baseline after four days (Fig 2.7c; Fig 2.7e). Recovery is accelerated with the addition of a hypothesized regulatory process: muscle-kidney cross talk, by which variations in intracellular K^+ concentration induce adjustments in renal K^+ transport. That cross talk enhances urine K^+ excretion to stabilize plasma and intracellular K^+ concentration in face of a massive K^+ load. In a period of K^+ depletion, without this cross talk, plasma and intracellular K^+ concentration quickly decreased below normal ranges (Fig 2.8c; Fig 2.8e) and remained low even after normal K^+ intake resumed. With the addition of a muscle-kidney cross talk signal,

urine K^+ excretion was significantly suppressed (Fig 2.8c; Fig 2.8e), resulting in much more stable plasma and intracellular K^+ concentration. These results suggest that muscle-kidney cross talk may significantly improve recovery times and prevent hyperkalemia and hypokalemia. That said, it is prudent to note that conclusive data on a muscle-kidney cross talk signal has not yet emerged. Furthermore, even if muscle-kidney cross talk exists, it remains unclear which tubular segment(s) the signal acts on. Simulation results suggest that an effect on distal tubule K^+ secretion or collecting duct K^+ reabsorption is likely to impact urine K^+ excretion enough to sufficiently handle a major K^+ load or depletion.

Model limitations and future work

Regulation of K^+ transport is coupled to other solutes. In particular, the secretion of K^+ along the distal tubules depends in part on Na^+ reabsorption. Na^+ regulation is not represented explicitly in this study, because our goal is to explore the impact of feedforward and feedback effects on K^+ homeostasis in isolation. The present model can be extended to incorporate Na^+ regulation. The resulting integrative model can be used to study the synergistic roles of Na^+ and K^+ in blood pressure regulation [2, 89, 131]. (Update: Since the publication of the work presented in this chapter, we have coupled the K^+ model in this study to the blood pressure model from Ref. [131]. This is published in Ref. [207].)

Another limitation is the uncertainty of the muscle-kidney cross talk hypothesis. Our baseline model does not include such mechanism, but without it the model was unable to capture normal K^+ regulation when significant K^+ loading or depletion was added. There are other mechanisms that impact K^+ homeostasis that have not been captured in the model presented in this study. One such mechanism is circadian rhythms which are known to impact secretion of hormones (such as ALD) as well as impact renal function [83, 126, 208, 232]. To incorporate circadian rhythms, some model parameters, e.g., Φ_{GFR} , would need to be made time dependent. Progesterone has also shown to play a role in K^+ regulation in both males and females [18, 56]. Future work may study the impact of other regulatory mechanisms.

Sex differences have been reported in renal K^+ handling [98, 100, 225], Na^+ balance [99, 127], ALD concentration [130, 148], and plasma K^+ concentration [162], all of which affect K^+ homeostasis. The current model parameters were determined for a male human. Sex-specific K^+ models will need to be developed to capture female-specific K^+ regulation. That would require a better characterization of the effect of sex on model components and overall behaviors, and to incorporate those effects by setting sex-specific model parameter values. Additionally, pregnancy is known to alter K^+ regulation, leading to K^+ retention, to deliver sufficient K^+ to the rapidly developing fetus [209, 229, 236]. Lactation also would require

similar changes to support the K^+ needs of breastmilk. Female-specific mathematical models may be used to study the unique physiological states of pregnancy or lactation [1]. Another unique state of K^+ retention is in neonates and early childhood [48, 84]. These altered K^+ handling states may benefit from investigation through mathematical modeling.

This K^+ homeostasis model may also be adapted to investigate K^+ -related diseases and treatments. Hypokalemia and hyperkalemia are common electrolyte disorders caused by changes in K^+ intake, altered excretion, or transcellular shifts. Diuretic use and gastrointestinal losses are common causes of hypokalemia, whereas kidney disease, hyperglycemia, and medication use are common causes of hyperkalemia. Both hypokalemia and hyperkalemia can be potentially fatal [41, 108]. Why does substantial K^+ imbalance emerge in some patients but not others? Simulations of a cohort of virtual patients by sampling selected model parameters for some distribution (e.g., Ref. [3]) may yield insights for predicting individual risks of dyskalemia.

Chapter 3

A mathematical model of whole-body potassium regulation: Global parameter sensitivity analysis

Abstract

The maintenance of stable potassium levels is essential for many physiological systems, requiring a robust system of mechanisms to maintain homeostasis. In this study, we analyze our mathematical model of whole-body K^+ regulation to investigate the sensitivity of different model outcomes to variations in parameter values. We used the Morris method, a global sensitivity analysis method, to evaluate the impact of the parameters on both steady-state results and simulations of a single-meal. Our results show that the most influential parameters and processes depend on what is being measured. Specifically, variations in steady-state results relied primarily on parameters that were involved in kidney function, while transient results relied on hormonal feedback mechanisms. This study supports that our mathematical model of whole-body potassium regulation represents known physiological function of potassium regulation despite a large number of uncertain parameters. Furthermore, this analysis will serve as a foundation for future model development and applications.

3.1 Introduction

Maintaining potassium (K^+) levels within normal range is essential for normal cellular function as it plays a major role in maintaining the resting cellular-membrane potential, which is critical for the propagation of action potentials in neuronal, muscular, and cardiac tissue [234]. K^+ also plays a vital role in many physiological systems such as hormone secretion and action, systemic blood-pressure control, acid–base homeostasis, and fluid balance [111]. Serious and potentially deadly health consequences can occur when K^+ levels are too high or too low [234]. Given its importance, mammals (likely) have evolved a robust system of mechanisms to maintain a steady plasma K^+ concentration ($[K^+]$) [111, 142].

To achieve K^+ homeostasis, the concentration gradient between the intracellular and extracellular fluids (120–140 mmol/L vs. 3.5–5.0 mmol/L in humans) as well as the total amount of K^+ in the body must be maintained. The kidneys primarily regulate the total body K^+ content by balancing total daily K^+ intake with excretion [81, 121]. The intracellular fluid (largely made up of skeletal muscle) stores about 98% of total body K^+ content, while the remaining 2% is in the extracellular fluid [95, 142]. This means that the extracellular fluid, which contains about 65 mmol of K^+ on average, could be significantly changed by dietary K^+ intake in isolation [95, 142]. Because adjustments in renal K^+ excretion happen on a time-scale of several hours, changes in extracellular $[K^+]$ are initially buffered by movement of K^+ into or out of the intracellular fluid. Since either a major shift in total body K^+ content or the distribution of K^+ between the intracellular and extracellular fluid can lead to large fluctuations in the extracellular $[K^+]$, a robust system of K^+ regulation that controls both K^+ excretion and intracellular K^+ uptake is essential.

The interactions among the multitude of physiological systems and processes involved in K^+ regulation remain to be incompletely understood. For example, which processes are more critical in maintaining K^+ at steady-state versus immediately after a meal? To answer this question, we analyze a mathematical model of whole-body K^+ regulation developed in Ref. [214] (Chap. 2). The model represents intracellular, plasma, and interstitial fluid compartments as well as a detailed kidney. The model includes the effect of insulin and ALD on Na^+ - K^+ -ATPase K^+ uptake, ALD stimulation of renal K^+ secretion, and the gastrointestinal feedforward effect. We conduct parameter sensitivity analysis to investigate the impact of different regulatory mechanisms on K^+ homeostasis in steady-state and transient states.

Models of complex systems often involve a large number of parameters, many of which have unknown or uncertain values [182, 191]. There are two types of uncertainty: epistemic uncertainty and aleatory uncertainty [182]. Epistemic uncertainty arises from incomplete

knowledge of a system. This is frequently encountered when modeling physiological systems, as direct parameter measurements are often extremely expensive, invasive, or even impossible to do ethically. For example, exactly measuring how much K^+ secretion increases in the distal segments of the kidney for elevated hormone levels (e.g., ALD) would be an extremely invasive and costly experiment. Aleatory uncertainty arises from the uncertainty that comes from the inherent heterogeneity in the system [182]. Indeed, even if every parameter could be measured directly, allowing for a model that precisely represents an individual or the population mean, it would not accurately represent every individual for all time. Rather, physiological differences exist inter-individually as well as at different states due to varying factors such as diet [230], exercise [135], and time of day [82], which influence how an individual regulates potassium. By investigating the impacts of varied parameters using analysis methods can help us gain deeper insights into the uncertainties inherent to modeling complex systems.

Sensitivity analysis can be used to evaluate the relationship between parameters and mechanisms to the model output [176, 182, 191]. It has been utilized to understand mathematical models in many biological system applications such as, epidemiology [139], blood coagulation [136], oncology [183], and more. There are many types of sensitivity analysis methods, which are generally categorized into two types: local and global methods. Local methods examine sensitivity of the model inputs at one specific point in the input space by changing a single input at a time and evaluating the change in the model output. While local sensitivity analysis is a popular technique because it is relatively easy to implement and compute, it has a number of disadvantages, such as neglecting how parameters interact with other parameters, and being heavily dependent on the initial parameter set. In contrast, global methods evaluate the importance of each parameter at many points in the given parameter space as well as the interactions between parameters.

Global sensitivity analysis methods are generally divided into two categories: one-at-a-time or many-at-a-time methods [176]. The former involves altering a single model input for each evaluation, while the latter modifies two or more inputs simultaneously for every model evaluation. These methods can be used for different purposes, including screening, ranking, and mapping. Screening is used to identify which model inputs affect model variability and to identify inputs that have negligible impact or are unidentifiable. Ranking orders model inputs based on their effect on output variability. Mapping determines the impact of the inputs on the model output. It is important to note that no individual sensitivity analysis method is suitable for all of these applications. Furthermore, the computational costs associated with different sensitivity analysis methods vary and can significantly increase with the addition of each model input. For these reasons, it is important to choose a method that is suitable for the objectives of the study. For a more comprehensive description of

sensitivity analysis methods we refer readers to Refs. [176, 182, 191].

The Morris method is a randomized one-at-a-time global sensitivity analysis method [35, 152]. This method involves evaluating many local changes at different points within a given parameter space, thereby functioning as a global sensitivity analysis method. This approach captures the impact of changes in individual parameters on the overall model output. Compared to other global methods, the Morris method is computationally efficient and has been shown to be effective for screening significant parameters [35, 152, 176]. In this study, we use the Morris method to identify which parameters contribute to the variability in both the steady-state and single-meal simulations of our K^+ homeostasis model from Ref. [214] (Chap. 2).

3.2 Methods

We conduct a sensitivity analysis by applying the Morris method (see Section 3.2.4) to the mathematical model of whole-body K^+ regulation (described in Section 3.2.1). We refer to sensitivity analysis using the Morris method as a “Morris analysis” in short. We analyze the impacts of parameter values on (i) the steady-state solution (see Section 3.2.2) and (ii) single-meal simulations (see Section 3.2.3).

3.2.1 Mathematical model of whole-body potassium regulation

We apply Morris analysis to the mathematical model of K^+ homeostasis developed in Ref. [214] (Chap 2). The model is a compartmental ordinary differential equations (ODE) model, which incorporates feedforward and feedback effects representing the regulation of K^+ levels in a man. A brief description of the model equations is given in Appendix B.1 and a model schematic is shown in Fig. 3.1. Baseline model parameters are listed in Table 3.1. For a more comprehensive description of the model development and baseline parameter fitting, see Ref. [214] (Chap. 2).

3.2.2 Steady-state simulations

In the Morris analysis, we compute the steady-state solution for the given parameter set by fixing K^+ intake (Φ_{Kin} ; Eq. B.1) to baseline K^+ intake ($\Phi_{Kin}^{ss} = 70$ mmol/day) as done in Ref. [214] (Chap. 2). We used the R package `sensitivity` [104] to conduct the sensitivity

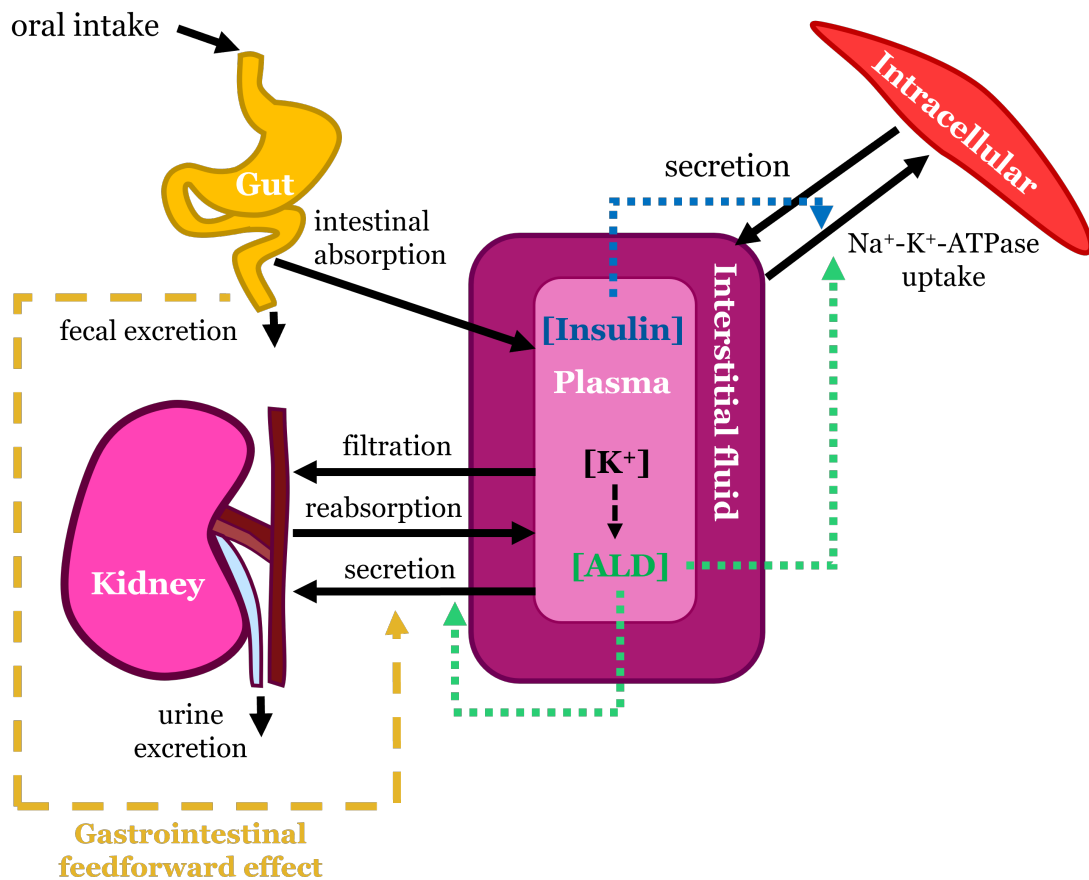


Figure 3.1: Baseline schematic of K^+ regulation. Black arrows represent transport of K^+ between the compartments. Green, light blue, and yellow arrows represent the stimulating effects of aldosterone, insulin, and the gastrointestinal feedforward effect, respectively. Internal K^+ balance is the distribution of K^+ between the intracellular and extracellular fluid (plasma and interstitial fluid) to maintain the K^+ concentration gradient, while total body K^+ content depends on the balance on oral K^+ intake with total K^+ excretion (urinary and fecal K^+ excretion). Note that K^+ is assumed to diffuse between the interstitial fluid and plasma, which together make up the extracellular fluid. ALD: aldosterone

Name	Range	Original	Description
V_{plasma}	2.25 - 6.75	4.5	plasma volume
V_{inter}	5 - 15	10	interstitial volume
$V_{\text{intracellular}}$	12 - 36	24	intracellular volume
k_{gut}	0.05 - 0.015	0.01	K^+ delivery rate to plasma
K_{m}	0.8 - 1.5	1.4	half maximal activation of Na^+ - K^+ -ATPase
V_{max}	65 - 195	130	maximum K^+ flow into cells
$m_{\text{K-ald}}$	0.25 - 0.75	0.5	parameter that determines C_{al}
P_{ECF}	0.15 - 0.45	0.3	effective K^+ permeability in extracellular fluid
A_{Kin}	0.13 - 0.38	0.25	parameter that determined γ_{Kin}
Φ_{GFR}	0.1 - 0.13	0.12	glomerular filtration rate
$\Phi_{\text{dt-Ksec}}^{\text{eq}}$	0.03 - 0.05	0.04	baseline distal tubule K^+ secretion
$A_{\text{dt-Ksec}}$	0.17 - 0.52	0.35	parameter that determines γ_{al}
$B_{\text{dt-Ksec}}$	0.12 - 0.36	0.24	parameter that determines γ_{al}
$\Phi_{\text{cd-Ksec}}^{\text{eq}}$	1.65e-3 - 2.75e-3	2.2e-3	baseline collecting duct K^+ secretion
$A_{\text{cd-Ksec}}$	0.08 - 0.24	0.16	parameter that determines λ_{al}
$B_{\text{cd-Ksec}}$	0.21 - 0.62	0.41	parameter that determines λ_{al}
$A_{\text{cd-Kreab}}$	0.37 - 0.62	0.50	parameter that determines $\Phi_{\text{cd-Kreab}}$
A_{insulin}	0.56 - 1.67	1.11	parameter that determines ρ_{insulin}
$K_{\text{IC}}^{\text{base}}$	120 - 140	130	baseline intracellular concentration
$K_{\text{ECF}}^{\text{base}}$	3.5 - 5.0	4.2	baseline extracellular concentration
$C_{\text{al}}^{\text{base}}$	45 - 300	85	baseline ALD concentration
$\eta_{\text{ps-Kreab}}$	0.85 - 0.95	0.92	fractional proximal segment reabsorption

Table 3.1: Parameter ranges and baseline values (used from Ref. [214], Chap 2) used for the Morris analysis and baseline model simulations.

analysis for the steady-state. Numerical steady-states were computed using the function `stode` from the library `rootSolve` [201], which uses the Newton-Raphson method to find zeros for the given system of equations. Parameter sets that could not produce a zero within the tolerance were eliminated from the Morris analysis. The number of simulations removed was less than 0.1% of the total model evaluations.

3.2.3 Single-meal simulations

In our previous study (Ref. [214], Chap. 2), we investigated the physiological responses to meals of different contents by conducting *in silico* experiments of whole-body K^+ regulation

during a single meal of varying contents. To do this, we simulated three experiments based on the methods of the clinical trial presented in Preston et al. [172]. The experiments consisted of a meal deficient in K^+ (“Meal only”), an oral ingestion of K^+-Cl^- only (“KCl only”), and typical meal made up of a combination of the K^+ -deficient meal with K^+ intake (“Meal + KCl”). Model simulations for the three meal types are shown in Fig. 3.2. The meal is given at time 0 after a fasting period of 6 hours from the initial condition as done in Ref. [214] (Chap. 2). In this study, we refer to these simulations as the “single-meal simulations” and conduct a Morris analysis to determine the impacts of parameters on these results at time points of every 10 minutes in the simulation. The same initial condition was used in every simulation in the Morris analysis.

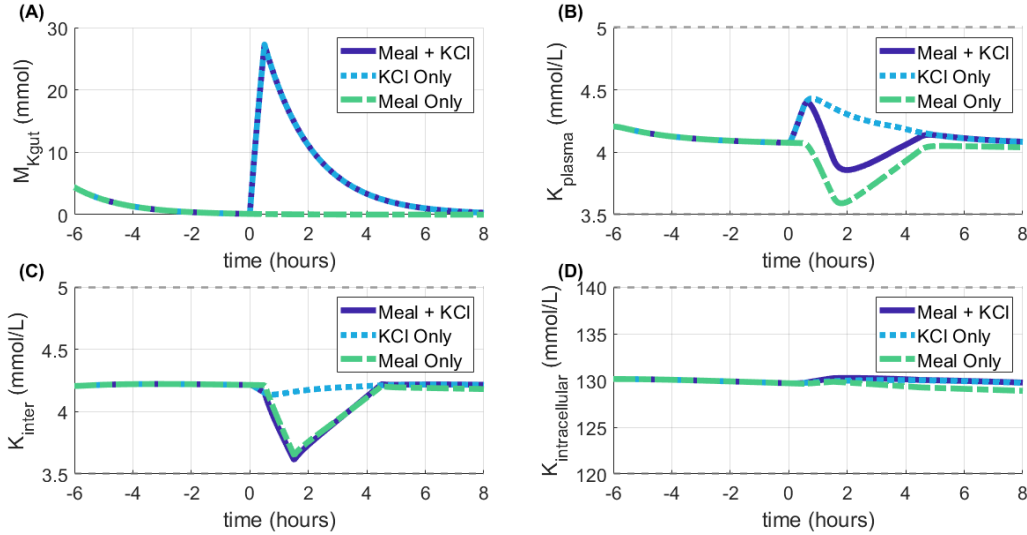


Figure 3.2: Model simulations of the (A) amount of K^+ in the gut, (B) plasma K^+ concentration, (C) interstitial K^+ concentration, and (D) intracellular K^+ concentration using baseline parameters from Ref. [214] (Chap 2; listed in Table 3.1 as “Original”) for three meal simulation types: Meal + KCl, Meal only, and KCl only (Section 3.2.3). The meal starts at time = 0 after a 6 hour fasting period. Gray lines indicate normal range for (B) plasma, (C) interstitial, and (D) intracellular K^+ concentration. The initial condition are the steady-state solution. $M_{K_{gut}}$: gastrointestinal K^+ amount; K_{plasma} : plasma K^+ concentration, K_{inter} : interstitial fluid K^+ concentration; $K_{intracellular}$: intracellular fluid K^+ concentration

We used the package `ODEsensitivity` [231] to conduct our sensitivity analysis for the single-meal simulations. `ODEsensitivity` uses the `lsoda` method for solving ODEs, which

automatically selects between non-stiff and stiff methods [202].

3.2.4 Morris method

Here we briefly describe the Morris analysis; for more details see Refs. [35, 152, 191]. We first choose the model output and inputs we are interested in analyzing. For a mathematical model, f , that is a system of ODE, the model inputs being varied are typically the model parameters. The model output measured could be a variety of model outcomes, depending on the problem being investigated. In this study, the inputs are the model parameters and we consider two types of model outputs: (i) the steady-state solution (see Section 3.2.2) and (ii) the plasma and intracellular $[K^+]$ at given time points in a single-meal simulation (see Section 3.2.3). We determine a range for each of the model parameters based on physiologically feasible ranges. We varied parameters that we could not identify a physiological range for by $\pm 50\%$ the baseline value. The ranges and baseline values for each parameter are listed in Table 3.1.

Let $f(X_1, X_2, \dots, X_n)$ denote the model output for model parameters $X_1, X_2, \dots, X_n$ where n is the number of parameters. In our Morris analysis, the output is either the steady-state solution (see Section 3.2.2) or a single-meal simulation (see Section 3.2.3) solved based on a given parameter set. To start the Morris analysis, a random value is selected for each parameter based on the given ranges (see Table 3.1). Subsequently, one random parameter, X_i , is incremented by a step size Δ (typically chosen to be $\Delta = n/(2(n-1))$). The elementary effect for the i -th parameter, denoted by EE_i , is computed by:

$$EE_i = \frac{f(X_1, X_2, \dots, X_i + \Delta, \dots, X_n) - f(X_1, X_2, \dots, X_i, \dots, X_n)}{\Delta}. \quad (3.1)$$

From this parameter set, another random parameter is incremented by Δ and the subsequent elementary effect is computed. This is repeated for all n parameters so that an elementary effect for each of the n parameters has been computed. This process is repeated r times by sampling at different initial parameter sets. As a result, there will be a total of r elementary effects per parameter at the end of the Morris analysis. We chose $r = 1000$ in our analysis for single-meal simulations and $r = 10,000$ in our steady-state analysis.

After computing the r elementary effects for each parameter, we find the average value (μ_i), average of the absolute values (μ^*), standard deviation (σ), and Morris Index (MI_i)

by:

$$\mu_i = \frac{\sum_{k=1}^r EE_i^k}{r} \quad (3.2)$$

$$\mu_i^* = \frac{\sum_{k=1}^r |EE_i^k|}{r} \quad (3.3)$$

$$\sigma_i = \sqrt{\frac{\sum_{k=1}^r (EE_i^k - \mu_i)^2}{r - 1}} \quad (3.4)$$

$$MI_i = \sqrt{(\mu_i)^2 + (\sigma_i)^2} \quad (3.5)$$

where EE_i^k denotes the elementary effect of the i -th parameter during the k -th evaluation.

The values computed for each input can be interpreted in the following ways:

- μ_i^* gives a parameter ranking: a greater μ_i^* value indicates that the i -th parameter affects the model output more
- σ_i indicates how much the i -th parameter interacts with other inputs and/or is non-linear
- MI_i : The Morris index is another way to rank inputs by giving a single metric that incorporates both the mean (μ) and standard deviation (σ) of the elementary effects

A limitation of using μ in isolation, the original metric used in Ref. [152], is that for non-monotonic models (as in our case here), the positive and negative elementary effects can cancel each other out when computing μ (Eq. 3.2). Therefore, in this study, we primarily focus on the metric μ^* as proposed by Campolongo et al. [35], which computes the mean of the absolute values of the elementary effects (Eq. 3.3) so that the elementary effects cannot cancel each other out. Note that in the Morris index (MI_i), μ_i is used.

3.2.5 Software

The simulations and Morris analyses were conducted using R. All code and Morris analysis results used for this study are openly available at:

https://github.com/Layton-Lab/Kreg_GSA.

3.3 Results

3.3.1 Steady-state analysis

The results for the Morris analysis for parameter effects on model steady-state plasma and intracellular $[K^+]$ are shown in Fig. 3.3 and Fig. 3.4. Any parameters not included in the figures had inconsequential μ^* , σ , and Morris index values for both plasma and intracellular $[K^+]$.

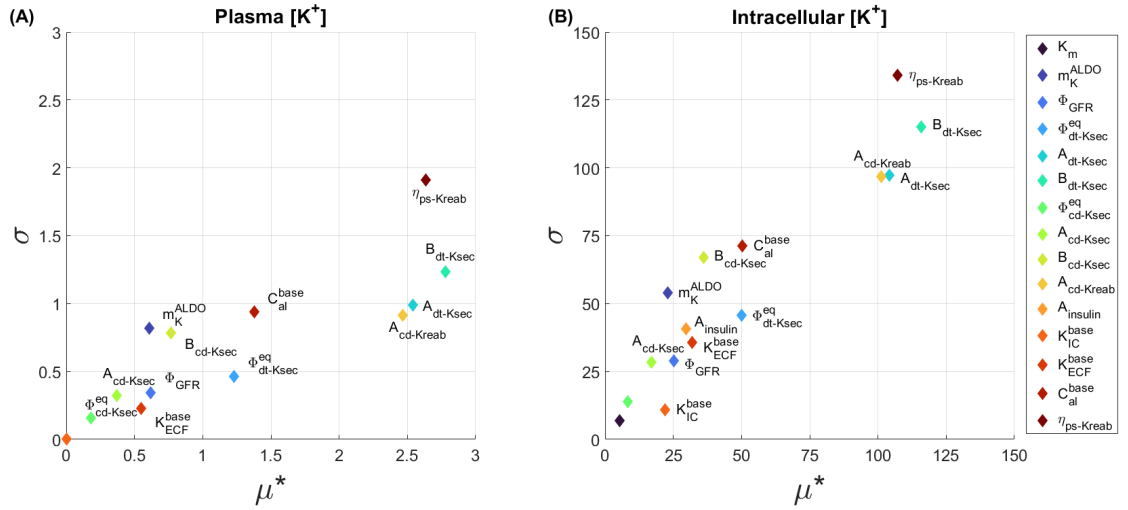


Figure 3.3: Morris plots for model steady-state (A) plasma K^+ concentration and (B) intracellular K^+ concentration. Parameters not shown had μ^* and σ values at or near 0 for both plasma and intracellular K^+ concentration.

While the μ^* , σ , and Morris index values are larger for the intracellular $[K^+]$ than plasma $[K^+]$, the seven largest μ^* values are the same parameters for both the plasma and intracellular $[K^+]$ (Fig. 3.3). Notably, the parameters with the top four Morris index values for both plasma and intracellular $[K^+]$ are $\eta_{ps-Kreab}$, $B_{dt-Ksec}$, $A_{dt-Ksec}$, and $A_{cd-Kreab}$, which are all parameters related to renal K^+ handling (Fig. 3.4). Specifically, $\eta_{ps-Kreab}$ represents fractional proximal segment K^+ reabsorption (Eq. B.9), $A_{dt-Ksec}$ and $B_{dt-Ksec}$ determine distal tubule K^+ secretion (Eq. B.16), and $A_{cd-Kreab}$ determines collecting duct K^+ reabsorption (Eq. B.12). The parameter with the fifth highest Morris index is C_{al}^{base} , which represents baseline [ALD] (Fig. 3.4). The insulin parameter $A_{insulin}$, which represents how much insulin affects intracellular K^+ intake (Eq. B.19), does not have much impact on

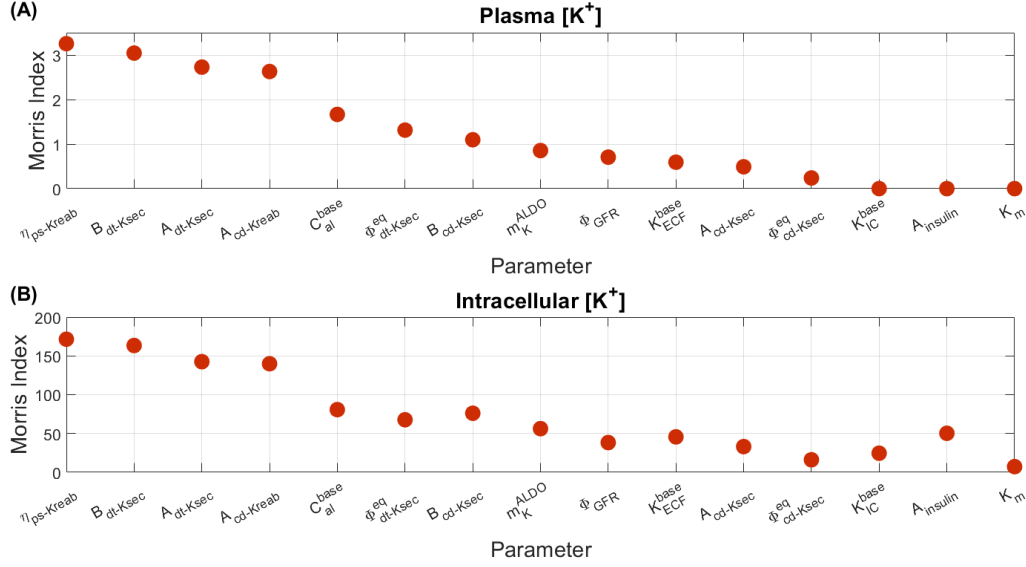


Figure 3.4: Morris indices (MI_i ; see Eq. 4.12) for model steady-state (A) plasma K^+ concentration and (B) intracellular K^+ concentration. Parameters not shown had small Morris index values.

steady-state plasma $[K^+]$ with a Morris index of about 0 (Fig. 3.4A). The insulin parameter ($A_{insulin}$) does have some impact on the intracellular $[K^+]$ (Fig. 3.4B).

In summary, results of our Morris analysis indicate that the steady-state plasma and intracellular $[K^+]$ depend primarily on the renal handling parameters and baseline [ALD].

3.3.2 Single-meal simulations

The μ^* and σ values for parameter impact on on model predictions of plasma and intracellular $[K^+]$ over time during a single “Meal + KCl” simulation are shown in Fig. 3.5. During the fasting state (before time = 0; see Section 3.2.3), the μ^* and σ values do not change much for both plasma and intracellular $[K^+]$ from their initial value. When the meal is added (at time = 0), different parameters have a larger or smaller impact on model results, as determined by an increase or decrease in μ^* (Fig. 3.5A1-B1).

The largest change in μ^* and σ is for the parameter $A_{insulin}$, which determines insulin impact on $Na^+-K^+-ATPase$ K^+ uptake (denote by $\rho_{insulin}$; Eq. B.19). Before the meal, μ^* and σ remains stable for both plasma and intracellular $[K^+]$. Then after the meal, initially

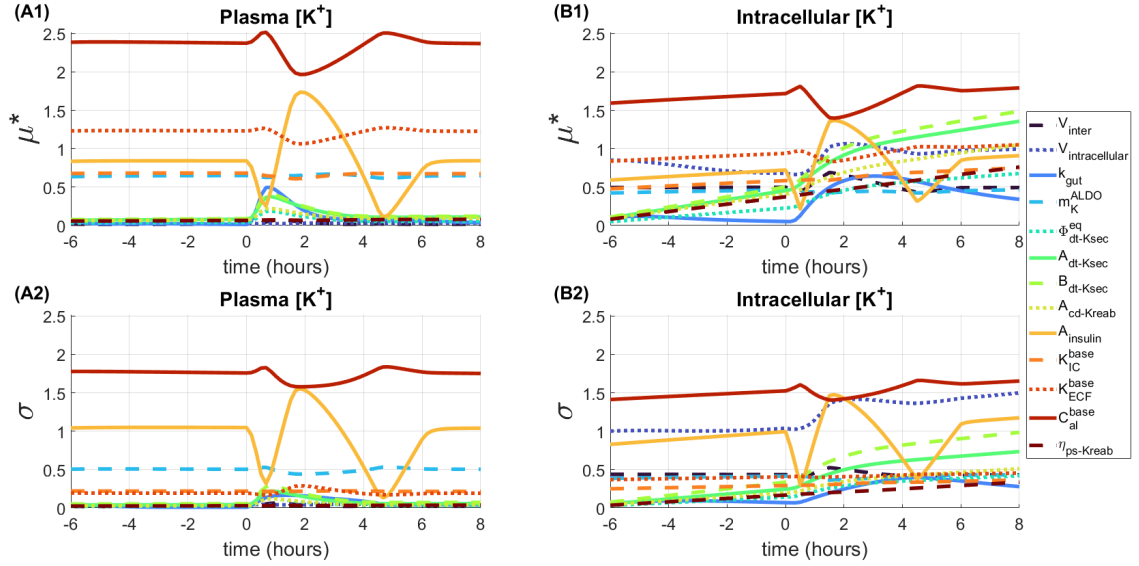


Figure 3.5: Morris (A1-B1) μ^* and (A2-B2) σ values for time points along the Meal + KCl simulation. Plasma $[K^+]$ effects are shown in panels (A1-A2) and intracellular $[K^+]$ effects are shown in (B1-B2). Elementary effects were computed for every 10 minutes of simulations. Only parameters that had significant μ^* and σ values through the simulations are shown.

μ^* and σ decrease due to Eq. B.19 being bounded below by 1, so A_{insulin} does not impact ρ_{insulin} . However, after the meal is fully delivered and C_{insulin} (Eq. B.18) increases above C_{insulin}^{ss} , the impact of variations in A_{insulin} on model output greatly increases and peaks when C_{insulin} is at its peak value (time = 1.5 hours; Eq. B.18). This shows that insulin is a key regulator of K^+ homeostasis after a meal.

The rate of delivery of K^+ from $M_{K_{\text{gut}}}$ to the plasma (Eq. B.1; Eq. B.2) has μ^* and σ values around 0 for both the plasma and intracellular $[K^+]$ during the fasting period (Fig. 3.5). However, after the meal, μ^* and σ values increase since k_{gut} regulates how fast K^+ enters the bloodstream. Variations in this rate can slow down or increase plasma $[K^+]$ changes for large changes in $M_{K_{\text{gut}}}$, like after a meal (Fig. 3.2A). This is captured in the Morris analysis results.

Since the μ and σ values change over time in the simulation (as shown in Fig. 3.5), to compare each parameter more directly we computed the mean and standard deviation of the Morris indices over the full simulation for each parameter. These values are plotted with

the minimum and maximum values in Fig. 3.6. For both plasma and intracellular $[K^+]$, C_{al}^{base} has the highest mean Morris index (Fig. 3.6). This is due to high μ and σ values for both plasma and intracellular $[K^+]$, showing that this parameter is both highly influential and interacts significantly with other parameters during any part of the simulation.

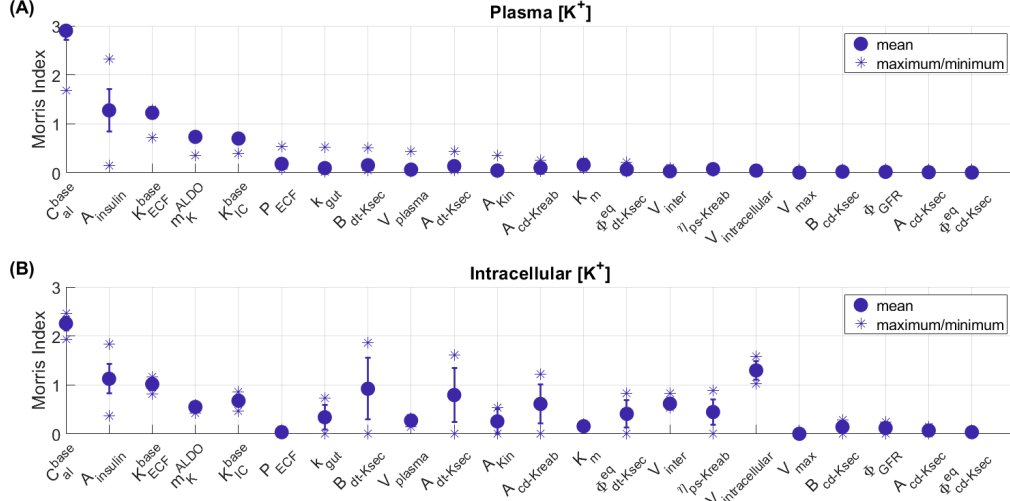


Figure 3.6: Mean, standard deviation (error bar), minimum, and maximum values of Morris indices along full Meal + KCl simulation for each parameter value for (A) plasma $[K^+]$ and (B) intracellular $[K^+]$.

Based on the maximum Morris index, the top four Morris indices for plasma $[K^+]$ are C_{al}^{base} , $A_{insulin}$, K_{ECF}^{base} , and m_K^{ALDO} . Note that these parameters are all related to hormonal (i.e, ALD and insulin) signalling (Eq. B.14; B.19). Notably, for plasma $[K^+]$, the Morris index for $A_{insulin}$ was nearly 0 in the steady-state Morris analysis (Fig. 3.4A), but $A_{insulin}$ is the second highest Morris index value on average in the Meal + KCl simulation (Fig. 3.6A).

For intracellular $[K^+]$, the top four largest maximum Morris indices are C_{al}^{base} , $B_{dt-Ksec}$, $A_{dt-Ksec}$, and $V_{intracellular}$ (Fig. 3.6B). Notably, the intracellular fluid volume ($V_{intracellular}$) and ALD impact on distal tubule K^+ secretion (shown by $A_{dt-Ksec}$ and $B_{dt-Ksec}$) were relatively insignificant for plasma $[K^+]$ (Fig. 3.6A). This shows that variability in different types of model outputs can be influenced by different parameters.

We conducted a Morris analysis for both the KCl only and Meal only single-meal simulations. The Morris indices are shown in Fig. 3.7 and the time series results are given in Appendix B.2 (Fig. B.2; Fig. B.3). For KCl only, the results are about the same as the

Meal + KCl results except that A_{insulin} has no impact since $\rho_{\text{insulin}} = 1$ (Eq. B.19) for the full KCl only simulation due to no insulin changes. Similarly, for Meal only, the results are similar to Meal + KCl except for a lower impact of A_{Kin} , k_{gut} , $B_{\text{dt-Ksec}}$, and $A_{\text{dt-Ksec}}$ on intracellular $[\text{K}^+]$ (Fig. 3.7B) due to no addition of K^+ into the system.

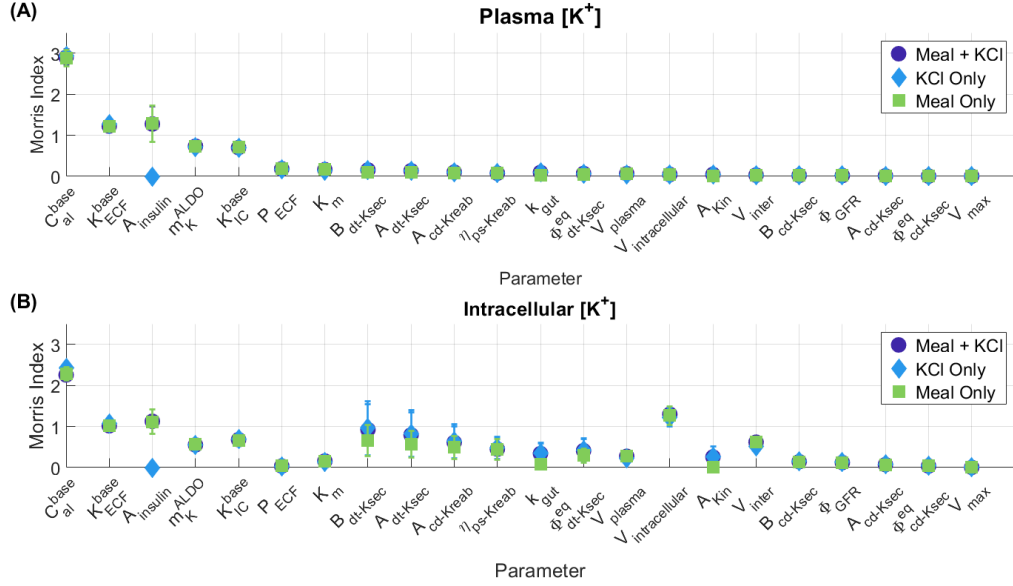


Figure 3.7: Mean and standard deviation of Morris indices along full Meal + KCl, KCl Only, and Meal Only simulation for each parameter value for (A) plasma $[\text{K}^+]$ and (B) intracellular $[\text{K}^+]$.

3.4 Discussion

In this study, we used the Morris method to conduct a global sensitivity analysis of our mathematical model of whole-body K^+ regulation (Ref. [214]; Chap. 2) to quantify how variations in model parameters contribute to variation in the model steady-state and single-meal simulation predictions. Our model analysis shows that the K^+ regulation model captures what is expected biologically: steady-state plasma K^+ regulation is driven by renal K^+ handling and internal K^+ balance after a shift in plasma $[K^+]$ (i.e., a single-meal) is driven by hormonal signalling. This analysis can also be used to guide future model building and applications by informing what parameters have a negligible impact on model outputs to determine where the model can be reduced.

3.4.1 Steady-state results depend on renal handling of K^+

The top four parameters that contribute to model variation of steady-state plasma and intracellular $[K^+]$ are involved in renal K^+ handling: $\eta_{ps-Kreab}$, $B_{dt-Ksec}$, $A_{dt-Ksec}$, $A_{cd-Kreab}$ (Fig. 3.3). Indeed, physiologically, long-term K^+ homeostasis is controlled by the kidneys, which excrete excess K^+ through urine in order to maintain a stable total body K^+ content.

The μ^* , σ , and Morris index values are larger for the intracellular than plasma $[K^+]$ in the steady-state Morris analysis (Fig. 3.3; Fig. 3.4). This makes sense biologically because baseline plasma $[K^+]$ levels are around 3.5–5.0 mmol/L and baseline intracellular $[K^+]$ is about 120–140 mmol/L. Therefore, intracellular $[K^+]$ levels have a wider possible range. Additionally, biologically, plasma $[K^+]$ levels are controlled more tightly, whereas intracellular $[K^+]$ levels are able to fluctuate more to ensure a safe plasma $[K^+]$ [146]. Our model seems to more tightly regulate plasma $[K^+]$ levels than intracellular $[K^+]$ levels.

3.4.2 Aldosterone has a large impact on K^+ regulation as well as interactions with other parameters

The parameter C_{al}^{base} , which represents baseline $[ALD]$, was a top parameter for plasma and intracellular $[K^+]$ in both the steady-state Morris analysis and the single-meal experiments (Fig. 3.4; Fig. 3.7). ALD is known to play a key role in K^+ homeostasis. Notably, normal $[ALD]$ levels have a wide range (about 45–300 ng/L; Ref. [49, 140]). We allowed C_{al}^{base} to vary by this known physiological range (Table 3.1), which is the largest range of all the parameters. This is likely a reason that this parameter had such large μ^* and σ values

(Fig. 3.5). We investigated the Morris analysis with smaller ranges for this parameter and it consistently was a top parameter (results not shown).

$C_{\text{al}}^{\text{base}}$ had a high variance in the elementary effects values, as given by large values for σ , especially in the single-meal experiments (Fig. 3.5). This indicates that this parameter interacts with other parameters. Biologically this makes sense because a patient that has typically low [ALD] level might respond differently to higher levels of [ALD] than a patient with typically higher [ALD]. Indeed, our results show that, in isolation, [ALD] levels alter K^+ regulation significantly, which is indeed biological as demonstrated by patients with hyperaldosteronism having altered K^+ levels [49, 111, 163].

3.4.3 Plasma K^+ regulation after a single-meal is driven by hormonal signalling

The parameter A_{insulin} , which represents how insulin affects intracellular K^+ uptake, does not have much impact on steady-state plasma $[\text{K}^+]$ with a Morris index of about 0 (Fig. 3.4A). However, in the single-meal experiments, A_{insulin} became a significant parameter for plasma $[\text{K}^+]$ with increasing μ^* and σ values after the meal, when insulin levels are increased (Fig. 3.5A1-A2).

Additionally, for the single-meal simulations $C_{\text{al}}^{\text{base}}$ and m_K^{ALDO} , which are both parameters related to [ALD] (Eq. B.14), are in the top 5 parameters for plasma $[\text{K}^+]$ (Fig. 3.7A). Therefore, our model captures that after a single-meal, which causes a shift in plasma $[\text{K}^+]$, is regulated primarily by hormonal signalling.

3.4.4 Parameter screening with Morris analysis

The Morris method is a computationally efficient and effective parameter screening method for complex systems [35, 152, 176]. We analyzed 22 parameters in the Morris analysis (listed in Table 3.1). Indeed, not all parameters significantly influenced the variation in each type of model output, as shown by their low μ^* and Morris index values. For example, the parameters V_{max} and K_{m} , which are the Michaelis-Menten parameters for Na^+ - K^+ -ATPase K^+ uptake (Eq. B.6), had μ^* , σ , and Morris index values that were close to 0 for all simulation types (Fig. 3.4; Fig. 3.7). This shows that these parameters have little impact on the model's steady-state and single-meal simulations when considering plasma and intracellular $[\text{K}^+]$. Other parameters, such as those involved in collecting duct K^+ secretion ($A_{\text{cd-Ksec}}$, $B_{\text{cd-Ksec}}$ and $\Phi_{\text{cd-Ksec}}^{\text{eq}}$), had a negligible impact on model variability in the single-meal

experiments with near-zero Morris indices (Fig. 3.7). However, these parameters had small but clearly non-zero Morris indices for the steady-state model output (Fig. 3.4). This indicates that while these parameters have minimal impact on short-term dynamic model simulations, such as those for a single meal, they become potentially significant as the model reaches a steady-state. This shows that the type of model output being investigated should be considered when conducting a parameter screening.

In conclusion, the Morris analysis results of our study capture the expected biological behaviour of K^+ regulation under homeostasis conditions (i.e., steady-state) and after a single meal of varying types. Results of the analysis can be used to inform future modeling development decisions, e.g., which parameters may best capture disease state models or therapeutic treatments. The analysis and model code presented in this study will also serve as a foundation for future model development and applications by providing insights into which parameters or model components can be altered or held constant without significantly impacting the model output.

3.4.5 Limitations and future work

A limitation of the Morris method arises from non-monotonicity, where elementary effects may cancel one another out in complex systems that are non-monotonic. To overcome this, we chose to focus on the metric μ^* (Eq. 3.3) instead of μ , which is a more recent advancement of this method found by Campolongo et al. [35].

Another limitation of the Morris method is its inability to quantify the proportion that each parameter contributes to the total variance in the model output. This can be computed using the first- and total-order indices from Sobol’ analysis, which is a widely used global variance-based method for sensitivity analysis [176, 183]. Indeed, the Sobol’ method may be applied to the model presented in this chapter.

The study of K^+ regulation is an active area of research. In recent years several mathematical models of this system have been published by other groups (Refs. [42, 140]). These models are QSP models, which investigate clinical applications of drugs for patients with dysregulated K^+ levels. Our group also recently published a model extension studying the impact of a high K^+ diet on K^+ regulation based on new experimental results [213] (Chap. 4). There are many more applications of K^+ regulation that can be studied using modeling. Some clinical examples are CKD [41], diabetes [44, 111], diuretics [52], and other drug impacts [111, 150]. Examples of physiological applications are the impact of circadian rhythms [52, 82, 208], sex differences [122], pregnancy [1, 215], and exercise [135] impact on

potassium regulation. The analysis presented in this study can serve as a foundation for future model extensions.

Chapter 4

A modeling analysis of whole-body potassium regulation on a high potassium diet: Proximal tubule and tubuloglomerular feedback effects

Abstract

Potassium (K^+) is an essential electrolyte that plays a key role in many physiological functions. Indeed, maintaining K^+ balance is critical for normal cell function, as too high or too low K^+ levels can have serious and potentially deadly health consequences. K^+ homeostasis is achieved by an intricate balance between the intracellular and extracellular fluid as well as balance between K^+ intake and excretion. This is achieved via the coordinated actions of regulatory mechanisms such as the gastrointestinal feedforward effect, insulin and aldosterone upregulation of $Na^+-K^+-ATPase$ uptake, and hormone and electrolyte impacts on renal K^+ handling. We recently developed a mathematical model of whole-body K^+ regulation to unravel the individual impacts of these regulatory mechanisms. In this study, we extend our mathematical model to incorporate recent experimental findings that showed decreased glomerular filtration rate and fractional proximal tubule reabsorption under a high K^+ diet. We conducted model simulations and sensitivity analyses to investigate how these renal alterations impact whole-body K^+ regulation. Model predictions quantify the sensitivity of K^+ regulation to various levels of proximal tubule

K^+ reabsorption adaptation and tubuloglomerular feedback. Our results suggest that the reduced proximal tubule K^+ reabsorption under a high K^+ diet could achieve K^+ balance in isolation, but the resulting tubuloglomerular feedback reduces the filtration rate and thus K^+ excretion.

4.1 Introduction

Potassium (K^+) homeostasis is critical for normal cell function. The function of excitable cells is controlled by membrane voltage, which is primarily determined by the ratio between the extracellular and intracellular K^+ concentrations ($[K^+]$) [234]. K^+ also plays a key role in other physiological processes such as systemic blood-pressure regulation as well as hormone secretion and action [111, 142, 144, 164].

About 98% of total body K^+ is stored in the intracellular fluid, primarily within the skeletal muscle cells [95, 142]. This results in a concentration gradient between the intracellular and extracellular fluid with a high intracellular $[K^+]$ (120-140 mmol/L) and a low extracellular $[K^+]$ (3.5-5.0 mmol/L) [95, 111, 142]. In total, this means the amount of K^+ in the extracellular fluid is approximately 65 mmol, which can easily be exceeded by dietary K^+ intake [95, 142]. This daily homeostatic challenge is overcome by regulatory mechanisms such as insulin signalling and $Na^+-K^+-ATPase$ uptake that moves excess extracellular K^+ into the intracellular fluid [95, 142]. In a healthy person, the kidneys ensure that K^+ excretion is balanced with K^+ intake [81, 95, 121, 127]. K^+ homeostasis is achieved by balancing the distribution between the extracellular and intracellular fluids as well as balancing excretion with intake, primarily via urinary excretion. When one of these mechanisms are altered, hyperkalemia or hypokalemia may occur. Due to the importance of K^+ , and the dangers of hyperkalemia and hypokalemia, humans have several regulatory mechanisms that ensure extracellular $[K^+]$ stays within a normal range.

For much of the evolution of the K^+ regulatory systems, humans consumed a diet rich in fruits, vegetables, nuts, and seeds. This diet contained ample amounts of K^+ and very little Na^+ . Specifically, it is estimated that in Paleolithic times, the average intake for K^+ was about 230-400 mmol/day, which is about four-fold the amount of K^+ in the modern Western diet (about 70-100 mmol/day) due to significant changes in food sources [95, 111]. This change from a K^+ -rich diet to a K^+ -poor diet (in Western cultures) is estimated to have occurred within the last several centuries, which is a short time frame from a mammalian evolution perspective. Therefore, it is likely that our K^+ regulatory systems are better suited for a K^+ -rich diet [57, 111, 164]. This may explain why the modern Western high Na^+ , low K^+ diet is associated with many health problems such as high blood pressure,

cardiovascular disease, and strokes [57, 111, 144, 164, 248]. Notably, it has been found that the Yanomamö natives in the Amazon, who are known to have a K^+ -rich, low Na^+ diet, closer to that of Paleolithic times, do not have the same increases in blood pressure as they age in contrast to Western cultures [159].

To unravel the complexities of K^+ regulation, in a recent study, we developed a detailed compartmental model of whole-body K^+ regulation (Ref. [214], Chap. 2). The model, which consists of a system of coupled ODE, represents the intracellular and extracellular fluid compartments as well as a detailed renal representation. The model includes a gut compartment to simulate oral K^+ intake and includes the following known regulatory mechanisms: (i) the gastrointestinal feedforward effect, (ii) the effect of insulin on Na^+ - K^+ -ATPase K^+ -mediated uptake and (iii) ALD stimulation of renal K^+ secretion, and (iv) ALD stimulation of Na^+ - K^+ -ATPase-mediated cellular K^+ uptake. We used this model to quantify the impacts of individual feedback and feedforward effects [214] (Chap. 2). In our previous study, we found that the model predicted high extracellular and intracellular $[K^+]$ under high K^+ intake despite it being known that healthy persons can sustain a high K^+ diet [177, 214]. It is likely that there are other mechanisms of K^+ handling during a high K^+ diet that have not been discovered. In our previous study, we proposed an additional hypothesized regulatory mechanism, muscle-kidney cross talk, where $[K^+]$ in the skeletal muscle cells directly affect renal K^+ transport without changes in extracellular $[K^+]$ [214] (Chap. 2). Our simulation results supported that this signal would enable mammals to sustain normal levels of plasma and intracellular $[K^+]$ during high K^+ intake [214] (Chap. 2).

A recent experimental study in rats showed evidence for inhibited proximal tubule (PT) K^+ reabsorption under a high K^+ diet when compared to controls (Ref. [230]). The authors also showed decreased GFR likely due to the tubuloglomerular feedback (TGF) signal from inhibited Na^+ , K^+ , and Cl^- reabsorption along the PT in rats on a high K^+ diet [230]. In this study, we extend our mathematical model to include the impact of a high K^+ diet on PT K^+ reabsorption as well as TGF effects reported by Wang et al. [230]. The objectives of this study are to: (i) update our mathematical model of whole-body K^+ regulation to include new experimental findings, (ii) quantify how renal adaptations of PT K^+ reabsorption and TGF effects impact whole-body potassium homeostasis, (iii) investigate whole-body impacts of varied renal adaptations under a high K^+ diet, and (iv) conduct sensitivity analysis to unravel how changes to parameters impact model predictions.

4.2 Materials and Methods

In this study, we investigate the PT and TGF effects of high K^+ intake reported by Wang et al. [230] by adapting our previously developed mathematical model of whole-body K^+ regulation (Ref. [214], Chap. 2). (Note that this is also the same model used and analyzed in Chap. 3). The model consists of a system of coupled ODE, representing intracellular and extracellular fluid compartments as well as known regulatory mechanisms. The model is parameterized to represent an average healthy male body. The modifications made to the model that represent PT and TGF effects of high K^+ intake are described below and are illustrated in Fig. 4.1. A more detailed model description is available in Appendix B.1.

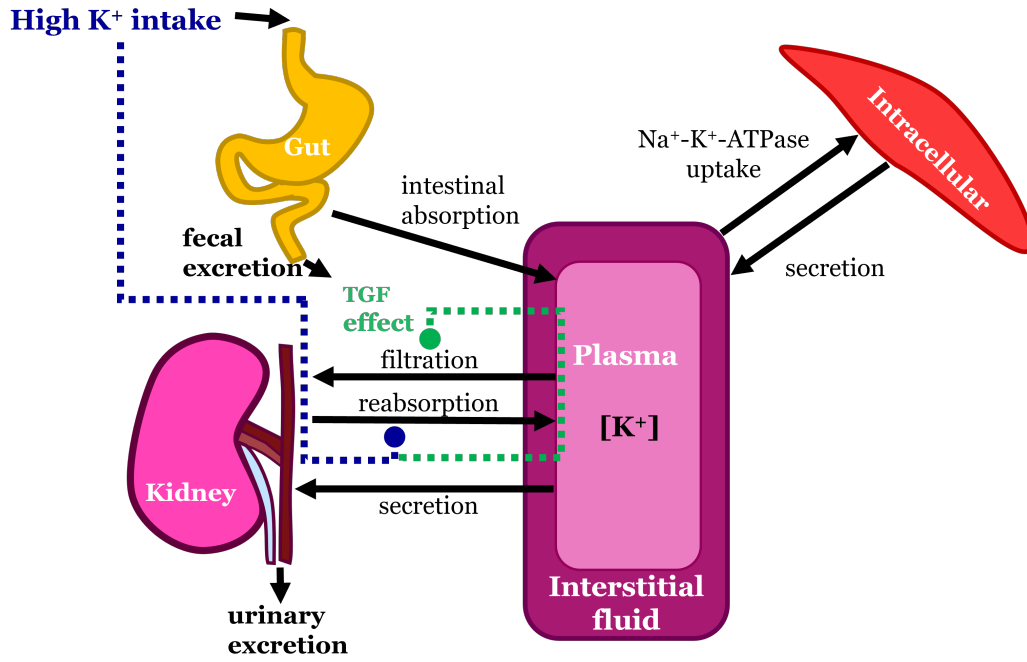


Figure 4.1: Minimal model schematic including renal impact of high K^+ intake. Black arrows represent transport of K^+ between the compartments (gut, plasma, interstitial fluid, and intracellular fluid). The blue arrow represents the inhibitory impacts of high K^+ intake on renal reabsorption. The green arrow represents tubuloglomerular feedback. Note that not all regulatory mechanisms included in the model are represented in this figure. See Fig. C.1 for a full model schematic. TGF: tubuloglomerular feedback.

4.2.1 High K^+ diet simulation

To conduct simulations of a control or chronic high K^+ diet, we added a fixed amount of K^+ to the gut compartment at meal times for 50 days. We simulated 3 meals of equal content per day at the simulation hours 6, 12, and 18. For the control K^+ diet, the 3 meals total 78 mmol per day, typical of an average modern Western diet [142]. For the high K^+ diet, we increased the K^+ intake per meal by four folds, totaling 312 mmol per day. This diet protocol is proportional to the control and high K^+ diet experimental protocols conducted in rats in Ref. [230]. We chose to simulate for 50 days to capture a chronic high K^+ diet while remaining computationally feasible for analysis that requires a large number of simulations such as the global sensitivity analysis (discussed in Section 4.2.3). Indeed, longer or shorter time frames for the simulation can be conducted with our model code.

4.2.2 Impact of high K^+ intake on proximal segment K^+ reabsorption

In a recent experimental study, Wang et al. [230] showed that rats fed a high K^+ diet for 7 days exhibited decreased PT fractional K^+ reabsorption and reduction in GFR. This is associated with a significant increase in urinary K^+ excretion to balance the high K^+ load. Specifically, Wang et al. [230] reported that fractional PT K^+ reabsorption in rats on a high K^+ diet was 37% versus 66% in control diet rats. Similar changes occurred for PT fractional Na^+ reabsorption [230]. Additionally, the authors reported the GFR decreased by 29% likely as a result of TGF activated by the increased delivery of Na^+ , K^+ and Cl^- to the macula densa [230]. Increased delivery of these electrolytes, in particular K^+ , is driven by decreased Na^+ reabsorption along the PT.

To investigate the impacts of a high K^+ diet on whole-body K^+ regulation, we incorporate into our model the effects of a high K^+ diet on fractional PT K^+ reabsorption and TGF. Let $\eta_{pt-Kreab}$ be a parameter that denotes fractional K^+ reabsorption along the PT. Under normal K^+ intake this is about 67% [81, 214, 230]. Fractional reabsorption of K^+ along the loop of Henle is denoted by $\eta_{LoH-Kreab} = 25\%$. Let “proximal segment” refer to the segments before the macula densa (i.e., the PT and loop of Henle) so that

$$\eta_{ps-Kreab} = \eta_{pt-Kreab} + \eta_{LoH-Kreab} \quad (4.1)$$

where $\eta_{ps-Kreab}$ is the fractional reabsorption of K^+ along the proximal segment. Note that since $\eta_{pt-Kreab}$ is a parameter, its value is set from the start of the simulation.

To simulate the TGF effect of altered fractional PT Na^+ and K^+ reabsorption, we determine GFR (Φ_{GFR}) by

$$\Phi_{\text{GFR}} = \Phi_{\text{GFR}}^{\text{base}} + \alpha_{\text{TGF}} \times (\eta_{\text{ps-Kreab}} - \eta_{\text{ps-Kreab}}^{\text{base}}) \quad (4.2)$$

where $\Phi_{\text{GFR}}^{\text{base}} = 0.125$ L/min is the baseline GFR, $\eta_{\text{ps-Kreab}}^{\text{base}} = 0.67 + 0.25$ is the baseline fractional proximal segment reabsorption, and α_{TGF} is a parameter representing the strength of the TGF signal. We refer to Eq. 4.2 as the ‘‘TGF effect’’. We compute the baseline value for α_{TGF} by letting $\Phi_{\text{GFR}} = (1 - 0.29)\Phi_{\text{GFR}}^{\text{base}}$ and $\eta_{\text{pt-Kreab}} = 0.37$ as reported in Ref. [230] for the GFR and fractional PT K^+ reabsorption under a high K^+ diet. Therefore, plugging these values in and solving Eq. 4.2 we get that

$$\alpha_{\text{TGF}}^{\text{base}} = \frac{(1 - 0.29) \times \Phi_{\text{GFR}}^{\text{base}} - \Phi_{\text{GFR}}^{\text{base}}}{(0.37 + 0.25) - (0.67 + 0.25)} \approx 0.12 \quad (4.3)$$

where $\alpha_{\text{TGF}}^{\text{base}}$ denotes the baseline value for α_{TGF} . Unless otherwise noted, this is the parameter value used for simulations involving the TGF effect (i.e., Eq. 4.2).

Since K^+ is freely filtered through the glomerulus, filtered K^+ load (Φ_{filK}) is proportional to Φ_{GFR} and plasma $[\text{K}^+]$ (K_{plasma}) so that

$$\Phi_{\text{filK}} = \Phi_{\text{GFR}} \times K_{\text{plasma}}. \quad (4.4)$$

Net proximal segment K^+ reabsorption ($\Phi_{\text{ps-Kreab}}$) is then given by

$$\Phi_{\text{ps-Kreab}} = \eta_{\text{ps-Kreab}} \times \Phi_{\text{filK}}. \quad (4.5)$$

After the proximal segment, the filtrate enters the distal segment. No changes were made in this study to the mathematical modeling of distal segment K^+ handling. A model schematic for the renal compartment is shown in Fig. 4.2. We direct readers to Ref. [214] (Chap. 2) or Appendix B.1 for details on the model representation of these segments.

4.2.3 Sensitivity analysis

Like many models of complex physiological systems, the present whole-body K^+ regulation model involves a large number of parameters, some of which are not directly measurable. Sensitivity analysis is a computational method that allows us to evaluate the sources of uncertainty, and to unravel the relationship between the model parameters and results [176, 182, 191].

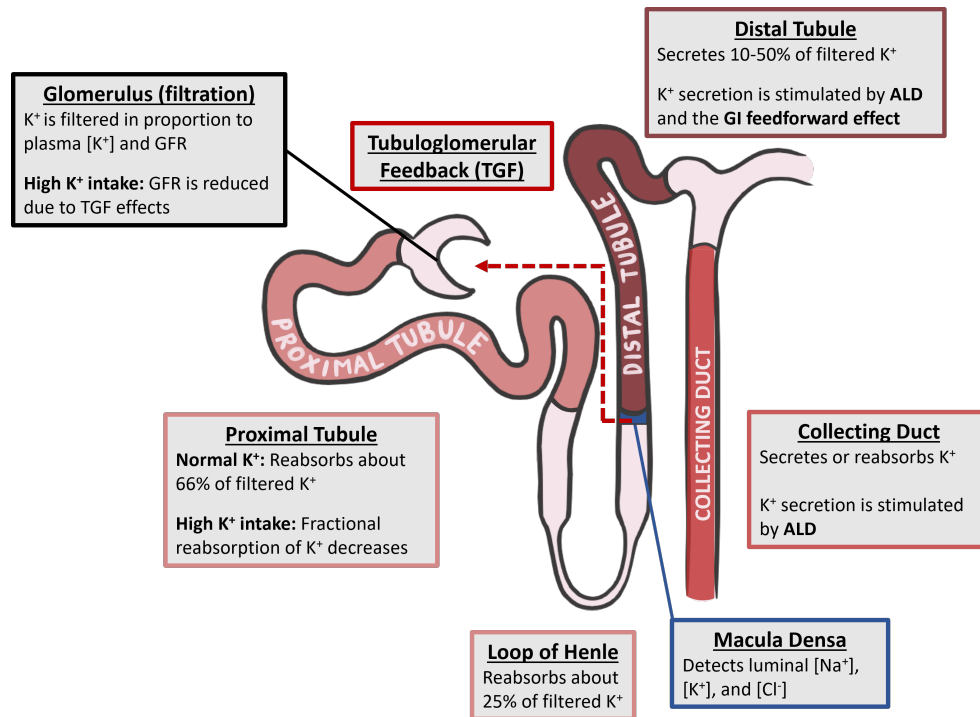


Figure 4.2: Schematic of renal K^+ handling along various nephron segments in the kidneys. This figure is adapted from Ref. [214]. ALD: aldosterone; GFR: glomerular filtration rate; GI: gastrointestinal; TGF: tubuloglomerular feedback

There are two primary types of sensitivity analysis: local and global methods. Local methods examine sensitivity of model behaviors to one parameter at a specific point in the parameter space. This is done by changing one parameter, fixing all other parameters at the baseline value, and evaluating the change in the model output. While this method is easy to implement and, relatively speaking, not computationally expensive, it is limited in that it does not consider interactions with other parameter changes or different points in the parameter space.

In contrast, global methods compute sensitivities at multiple points and involve changing multiple parameters, thus capturing the model sensitivity to individual parameter changes, interactions with other parameters, and evaluates the model at many points in the parameter space. Global sensitivity analysis gives a more comprehensive understanding of how each of the parameters in the system impact the model output. A major challenge of global methods is they are computationally expensive, especially for models with lots of

parameters. For more details about sensitivity analysis (both local and global methods), see Refs. [176, 182, 191].

To evaluate how changes to an individual parameter value impact model predictions, we first conduct a local sensitivity analysis. Specifically, we increase and decrease individual parameters by 10%, and compute the resulting plasma and intracellular $[K^+]$ at the end of the 50 day simulation with that individual parameter change. All other parameters remain at their baseline value. The local sensitivity is determined by

$$\Delta K_{c,i}^{+10\%}(\%) = \frac{K_{c,i}^{+10\%} - K_c^{\text{baseline}}}{K_c^{\text{baseline}}} \times 100\% \quad (4.6)$$

for an increase in parameter i by 10% and

$$\Delta K_{c,i}^{-10\%}(\%) = \frac{K_{c,i}^{-10\%} - K_c^{\text{baseline}}}{K_c^{\text{baseline}}} \times 100\% \quad (4.7)$$

for a decrease in parameter i by 10%. The net local sensitivity is given by

$$|\Delta K_{c,i}|(\%) = |\Delta K_{c,i}^{+10\%} - \Delta K_{c,i}^{-10\%}| \quad (4.8)$$

where c denotes the compartment (i.e., c = plasma or intracellular) and i is the parameter number. K_c^{baseline} denotes the baseline $[K^+]$ in compartment c at the end of the 50 day simulation for no changes in any of the parameter values from baseline. $K_{c,i}^{+10\%}$ denotes the $[K^+]$ in compartment c at the end of the 50 day simulation for parameter i increased by 10% from its baseline value, and conversely $K_{c,i}^{-10\%}$ is the $[K^+]$ at the end of the 50 day simulation for parameter i decreased by 10%. These metrics (Eqs. 4.6 – 4.8) allow us to consider the impacts of increasing and decreasing individual parameter values.

We use the Morris method to evaluate the global sensitivity of the whole-body K^+ regulation model under high K^+ intake [152]. The Morris method is known to be more computationally efficient than other global sensitivity methods [176]. The method is useful for parameter ranking of complex systems. We first use our local sensitivity analysis to identify parameters that the model is sensitive to, and include those in our global sensitivity analysis. By reducing the number of parameters included in the analysis, we reduce computation time. Specifically, parameters that result in $|\Delta K_{c,i}|$ (Eq. 4.8) greater than 2% are included in the global sensitivity analysis.

The Morris method involves computing elementary effects for each of the parameters, which quantify how much a change in that parameter impacts the model output. Each parameter has r elementary effects computed at different points in the parameter space.

After computing the r elementary effects for each parameter, denoted as parameter i , we compute the average value (μ_i), average of the absolute values (μ_i^*), the standard deviation (σ_i), and Morris Index (MI_i) in the following ways:

$$\mu_i = \frac{\sum_{k=1}^r EE_i^k}{r} \quad (4.9)$$

$$\mu_i^* = \frac{\sum_{k=1}^r |EE_i^k|}{r} \quad (4.10)$$

$$\sigma_i = \sqrt{\frac{\sum_{k=1}^r (EE_i^k - \mu_i)^2}{r - 1}} \quad (4.11)$$

$$MI_i = \sqrt{\mu_i^2 + \sigma_i^2} \quad (4.12)$$

where EE_i^k denotes the elementary effect of the i -th parameter during the k -th model evaluation. The greater the value of μ_i^* for parameter i , the more the i -th parameter affects the model output. The greater the σ_i value, the more the parameter is nonlinear or involved with interactions with other parameters; a low σ_i , by contrast, indicates a linear, additive factor. We will refer to our sensitivity analysis using the Morris method as a “Morris analysis” in short. A more detailed description of the Morris method is given in Chap. 3 (Section 3.2.4).

We conduct the Morris analysis for the whole-body K^+ regulation by sampling over the parameter space, integrating the system for a 50 day simulation under high K^+ intake with the PT and TGF effects, and computing the final plasma and intracellular $[K^+]$. The model output used for the computation of the elementary effects is the final plasma and intracellular $[K^+]$. We compute $r = 100$ elementary effects for each parameter that is considered in the Morris analysis. The ranges for the parameters were determined by the physiological range for those that have a direct physiological meaning or were varied by $\pm 50\%$ of their baseline value. The parameter ranges used are listed in Table C.2 (Appendix C.2).

4.2.4 Parameters

The parameters used in this study were taken from Ref. [214] (Chap. 2) where they were fit to clinical data investigating meals of various types (i.e., including or excluding K^+ and glucose). The baseline parameter values are listed in Table C.2 (Appendix C).

4.2.5 Software

The simulations were conducted using MATLAB R2022a. Numerical solutions of the ODE system were solved using `ode15s`, a variable-step, variable-order ODE solver. Steady-state solutions were obtained using the root finding function `fsolve`. For the Morris analysis we used the packages `sensitivity` and `desolve` in R [104, 202]. All code for the model simulations and sensitivity analysis is available at:

<https://github.com/Layton-Lab/highK>.

4.3 Results

4.3.1 High K^+ intake results in high intracellular K^+

How does a high K^+ diet impact whole-body K^+ regulation? To investigate this question, we conducted simulations of 50 days of a normal and a high K^+ diet (see Section 4.2.1) with and without different PT and TGF effects (see Section 4.2.2). Specifically, the high K^+ diet simulations were conducted with:

- (i) no PT and TGF effects, i.e., $\eta_{pt-Kreab} = 0.67$ (baseline value) and $\alpha_{TGF} = 0$ in Eq. 4.2. This is labeled as “High K^+ - no PT/TGF effect” in the corresponding figures.
- (ii) with the PT and TGF effect found in Ref. [230], i.e., $\eta_{pt-Kreab} = 0.37$ and $\alpha_{TGF} = \alpha_{TGF}^{base}$ in Eq. 4.2. This is labeled as “High K^+ - PT + TGF effects” in the corresponding figures.
- (iii) only the decrease in PT fractional reabsorption, i.e., $\eta_{pt-Kreab} = 0.37$ and $\alpha_{TGF} = 0$ in Eq. 4.2. This is labeled as “High K^+ - only PT effect” in the corresponding figures.

The simulations that represent the experiments conducted in Wang et al. [230] are the control K^+ and high K^+ diet with PT and TGF effects (i.e., “High K^+ - PT + TGF effects”). We note that the “High K^+ - only PT effect” and “High K^+ - no PT/TGF effect” simulations cannot easily be done experimentally, thus no data is available. Wang et al. [230] reported that fractional urinary K^+ excretion was 1–50% on a control diet and increased to 40–130% on a high K^+ diet (see Fig. 1 of Ref. [230]). The control K^+ simulations predict an average fractional urinary K^+ excretion of 11%, whereas the high K^+ diet simulations predict 54% (Fig. 4.3B); both within the respective ranges reported by Wang et al. [230]. Additionally, rats on the control K^+ diet maintained a stable plasma

$[K^+]$ over their experiment, while rats on a high K^+ diet plasma $[K^+]$ increased by about 15% on average [230]. In our simulations, control K^+ diet plasma $[K^+]$ remained stable while the high K^+ diet increased by 16% on average (Fig. 4.4A). Since we are comparing different species (i.e., humans in the mathematical model vs. rat experiments) we do not compare the exact values, but rather compare relative changes. The relative changes for our control K^+ and “High K^+ - PT + TGF effects” simulations are within the ranges of fractional urinary K^+ excretion and plasma $[K^+]$ reported by Wang et al. [230]. Wang et al. [230] did not report intracellular $[K^+]$.

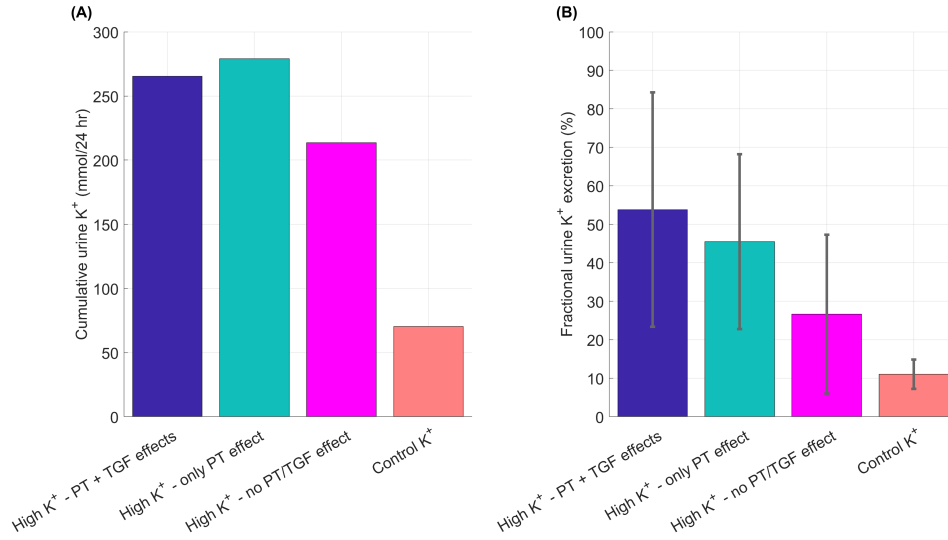


Figure 4.3: (A) Mean cumulative urine K^+ excretion per day and (B) mean and standard deviation fractional urine K^+ excretion over the full simulation time for control and high K^+ diet with both proximal tubule and tubuloglomerular feedback effects (“PT + TGF effects”), only the PT effect (“only PT effect”), and no proximal tubule or tubuloglomerular feedback effects (“no PT/TGF effect”). Wang et al. [230] reported fractional K^+ excretion of 1–50% in control K^+ rats and 40 – 130% in high K^+ diet rats. PT: proximal tubule; TGF: tubuloglomerular feedback

For the control K^+ diet simulations, total daily K^+ intake is 78 mmol. The model assumes that 10% of the K^+ intake is excreted through feces (or other mechanisms such as sweat) [214]. Daily cumulative urinary K^+ excretion is predicted to be 70.2 mmol (Fig. 4.3A), which is equal to 90% of the total daily K^+ intake. Therefore, the control K^+ diet results in balance between daily K^+ intake and excretion. This balance between daily intake and excretion results in stable total body K^+ so that plasma and intracellular $[K^+]$

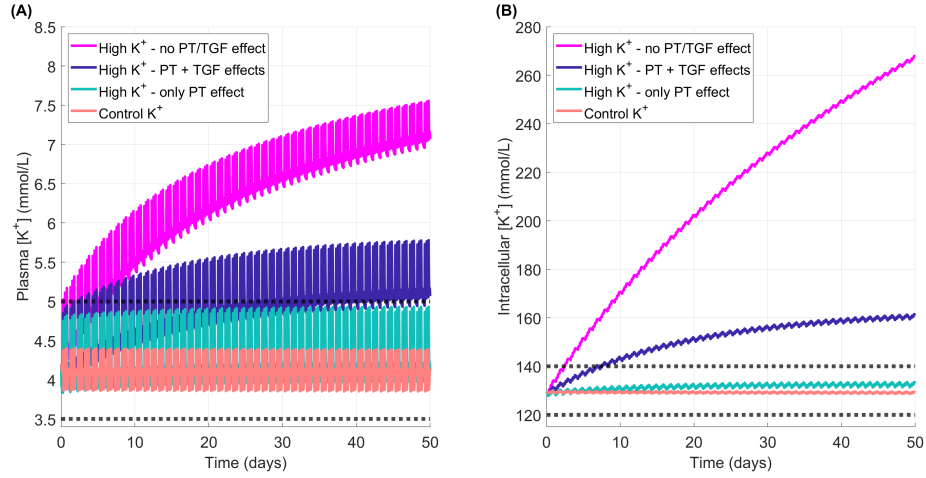


Figure 4.4: Predicted (A) plasma $[K^+]$ and (B) intracellular $[K^+]$ for control and high K^+ diet with no proximal tubule or tubuloglomerular feedback effects, both proximal tubule and tubuloglomerular feedback effects, and only proximal tubule effect. Wang et al. [230] reported a 15% increase of plasma $[K^+]$ in rats on a high K^+ diet. Dark gray dotted lines indicate normal ranges for plasma $[K^+]$ (3.5-5.0 mmol/L) and intracellular $[K^+]$ (120-140 mmol/L). PT: proximal tubule; TGF: tubuloglomerular feedback

levels remain stable throughout the full simulation (Fig. 4.4).

For the high K^+ diet simulation, total daily K^+ intake is 312 mmol, 10% of which is assumed to be excreted via feces. To achieve K^+ balance, the remaining 281 mmol should be excreted via urine. Without PT K^+ reabsorption adaptation to a high K^+ diet (“High K^+ - no PT/TGF effect”), daily urinary K^+ excretion is about 213 mmol, resulting in a 68 mmol per day deficit for K^+ balance (Fig. 4.3A). Predicted fractional urinary K^+ excretion for the simulation of a high K^+ diet with no PT or TGF effects is on average 27% (Fig. 4.3B; “High K^+ - no PT/TGF effect”), below the 40%–130% range reported for rats on a high K^+ diet [230]. The predicted lower fractional and daily urine K^+ excretions results in an imbalance between K^+ intake and excretion; as a result, total body K^+ increases. By day 3 of the simulation time, predicted intracellular $[K^+]$ levels are above normal range (>140 mmol/L) and by day 5 of simulation time predicted plasma $[K^+]$ is above normal range (>5.0 mmol/L) (Fig. 4.4; “High K^+ - no PT/TGF effect”). As the simulation time continues, plasma and intracellular $[K^+]$ levels reach over 7 mmol/L and 260 mmol/L, respectively. Notably, intracellular $[K^+]$ of 260 mmol/L is almost certainly incompatible with mammalian life.

With the addition of the PT and TGF effects, predicted fractional urinary K^+ excretion is on average 54% (Fig. 4.3B; “High K^+ - PT + TGF effects”), within the range found by Wang et al. [230] for a high K^+ diet. This results in daily cumulative urine K^+ excretion of 265 mmol (Fig. 4.3A; “High K^+ - PT + TGF effects”), only 5% below the excretion needed for perfect balance with K^+ intake. Predicted plasma and intracellular $[K^+]$ levels do increase as a result of this slight imbalance, but at a much slower rate than when there are no PT and TGF effects (Fig. 4.4; “High K^+ - PT + TGF effects” vs. “High K^+ - no PT/TGF effect”). By day 10, intracellular $[K^+]$ is above normal range and plasma $[K^+]$ is consistently above normal range by day 20 (Fig. 4.4). At the end of the simulation, plasma $[K^+]$ is 5.1 mmol/L and intracellular $[K^+]$ is 162 mmol/L (Fig. 4.4).

With a high K^+ diet, the reduction in PT K^+ reabsorption increases K^+ excretion, whereas TGF lowers GFR and, when taken in isolation, reduces K^+ excretion. To uncouple the effects of these two opposing responses, we consider the hypothetical situation with only the reduction in PT K^+ reabsorption, but no TGF effect, i.e., GFR is kept at baseline level. The model simulation predicts that daily cumulative urine K^+ excretion is nearly at exact balance at 279 mmol – 90% of K^+ intake of 312 mmol is 280.8 mmol (Fig. 4.3A; “High K^+ - only PT effect”). This results in nearly steady intracellular $[K^+]$ levels (Fig. 4.4B; “High K^+ - only PT effect”). At the end of the simulation time, plasma $[K^+]$ is 4.2 mmol/L and intracellular $[K^+]$ is 133.4 mmol/L, both well within normal range (Fig. 4.4). Note that while removing the TGF increases net urinary K^+ excretion, it also decreases fractional urine K^+ excretion (Fig. 4.3B; “High K^+ - only PT effect”). This apparent paradox is due to the GFR-lowering effect of TGF (Eq. 4.2), which decreases K^+ filtration. A further analysis of the dynamics of filtered K^+ revealed that, in the first 10 days of the simulation, filtered K^+ is on average 24% lower with the TGF effect (compared “High K^+ - PT + TGF effects” with “High K^+ - only PT effect” high K^+ simulation in Fig. 4.5A). With the TGF effect represented, filtered K^+ increases over time due to the increasing plasma $[K^+]$ levels (Fig. 4.4A; Fig. 4.5A). Despite that increase, average cumulative filtered K^+ per day is 17% lower for the “High K^+ - PT + TGF effects” simulation than the “High K^+ - only PT effect” simulation over the 50 days (Fig. 4.5B).

In summary, adaptive PT K^+ reabsorption to a high K^+ diet found in Ref. [230] is sufficient for maintaining plasma and intracellular $[K^+]$ levels within normal range, if it were the only mechanism activated. However, the TGF effect caused by altered Na^+ , K^+ , and Cl^- delivery to the macula densa reduces K^+ filtration. This results in lower urinary K^+ excretion causing significant K^+ imbalance. Ultimately, the K^+ retention leads to a high total body K^+ content as shown by increased plasma and intracellular $[K^+]$ levels on a high K^+ diet.

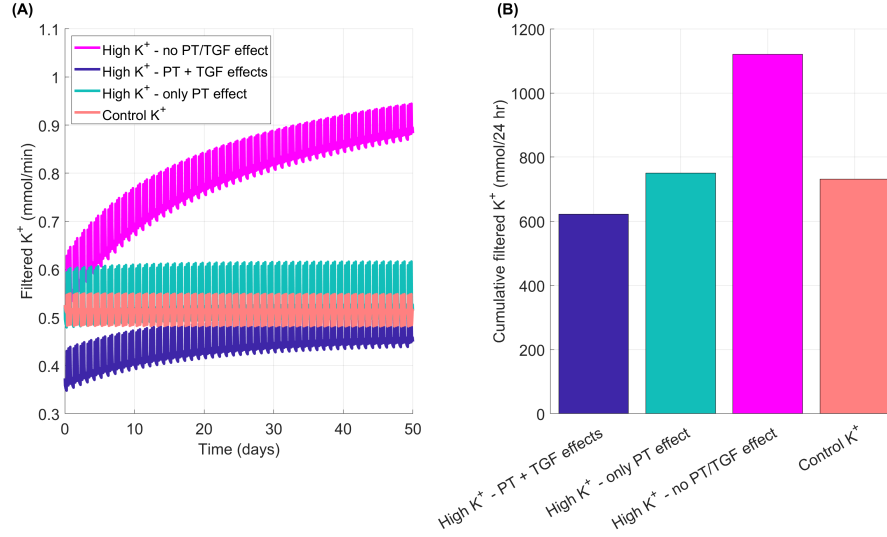


Figure 4.5: Filtered K⁺ into the nephrons (A) over the full simulation time and (B) mean cumulative filtered K⁺ per day. Simulation types correspond to Fig. 4.4.

4.3.2 Predicted effects of varying fractional proximal tubule K⁺ reabsorption and tubuloglomerular feedback

The extent to which PT K⁺ reabsorption is suppressed under a high K⁺ diet or the TGF signal may vary due to many factors such as hormone levels, Na⁺ intake, acid-base balance, as well as pathological conditions such as diabetes or CKD. How does variations in PT K⁺ reabsorption or TGF signalling impact whole body K⁺ handling under a high K⁺ diet? To investigate this question, we conducted high K⁺ intake simulations by varying

- (i) fractional PT K⁺ reabsorption ($\eta_{\text{pt-Kreab}}$) individually,
- (ii) TGF signal strength (α_{TGF}) individually,
- (iii) both $\eta_{\text{pt-Kreab}}$ and α_{TGF} simultaneously.

In each of these simulations the only parameters that are changed are $\eta_{\text{pt-Kreab}}$ and α_{TGF} . All other parameter values remain fixed at their baseline values.

The simulations for varied $\eta_{\text{pt-Kreab}}$ were conducted with the α_{TGF} at the baseline value ($\alpha_{\text{TGF}}^{\text{base}}$, Eq. 4.3). The results are shown in Fig. 4.6. Recall that, for no change

in $\eta_{\text{pt-Kreab}}$ under high K^+ intake (i.e., $\eta_{\text{pt-Kreab}} = 0.67$), plasma and intracellular $[\text{K}^+]$ quickly exceed normal range (Fig. 4.4; Fig. 4.6A1-B1). Decreasing $\eta_{\text{pt-Kreab}}$ by 0.06 from baseline leads to a 7.5 and 14% decrease in plasma and intracellular $[\text{K}^+]$ at the end of the simulation, respectively (Fig. 4.6A2-B2, $\eta_{\text{pt-Kreab}} = 0.61$). A similar decrease in plasma and intracellular $[\text{K}^+]$ is predicted for another decrease by 0.06 (Fig. 4.6A2-B2, $\eta_{\text{pt-Kreab}} = 0.55$). However, as we continue to decrease $\eta_{\text{pt-Kreab}}$ by 0.06, the model predicts diminishing responses, such that when decreasing $\eta_{\text{pt-Kreab}}$ from 0.24 to 0.18, there is a negligible difference in the end point plasma and intracellular $[\text{K}^+]$ (Fig. 4.6A2-B2). This is due to the TGF signal, which reduces GFR when delivery of K^+ to the macula densa is lowered (Eq. 4.2).

To investigate the impact of a differing TGF signal strength, we varied the parameter α_{TGF} from 0.5 to 1.5 times the baseline value ($\alpha_{\text{TGF}}^{\text{base}}$, Eq. 4.3) and conducted simulations for a high K^+ diet with $\eta_{\text{pt-Kreab}} = 0.36$ (fractional PT K^+ reabsorption under a high K^+ diet as reported in Ref. [230]). The results are shown in Fig. 4.7. Increasing α_{TGF} results in a larger impact of reduced proximal segment K^+ reabsorption on a high K^+ diet, thus lowering the GFR (Eq. 4.2). The lower filtration reduces urinary K^+ excretion and ultimately results in a faster increase in plasma and intracellular $[\text{K}^+]$ levels than lower α_{TGF} values (Fig. 4.7A1-B1). Conversely, when decreasing α_{TGF} , the GFR is decreased less by the reduced proximal segment K^+ reabsorption, resulting in a slower increase in plasma and intracellular $[\text{K}^+]$ levels due to higher K^+ filtration than when α_{TGF} is equal to the baseline value (Fig. 4.7A1-B1). When varying α_{TGF} , there is a linear response in the final plasma and intracellular $[\text{K}^+]$ levels (Fig. 4.7A2-B2). For $\alpha_{\text{TGF}} \leq 0.875 \times \alpha_{\text{TGF}}^{\text{base}}$, the plasma $[\text{K}^+]$ stays within normal range for the full simulation (Fig. 4.7A1-A2). However, for all α_{TGF} values simulated, intracellular $[\text{K}^+]$ is higher than normal range by day 20 (Fig. 4.7B1).

To investigate the combined impact of varying $\eta_{\text{pt-Kreab}}$ and α_{TGF} , we conducted high K^+ intake simulations for each combination of the varied values of $\eta_{\text{pt-Kreab}}$ and α_{TGF} in Fig. 4.6 and Fig. 4.7, respectively. The resulting final plasma and intracellular $[\text{K}^+]$ at the end of the 50 day simulation are shown in Fig. 4.8. Note that the results in the columns with $\alpha_{\text{TGF}}/\alpha_{\text{TGF}}^{\text{base}} = 1$ are the same as in Fig. 4.6A2-B2 and rows with $\eta_{\text{pt-Kreab}} = 0.36$ are the same as in Fig. 4.7A2-B2.

When $\eta_{\text{pt-Kreab}} = 0.67$ there is no change for changes in α_{TGF} since $\eta_{\text{pt-Kreab}}$ is at the baseline value, i.e., $\eta_{\text{ps-Kreab}} - \eta_{\text{ps-Kreab}}^{\text{base}} = 0$, so there is no impact on the GFR (see Eq. 4.2). Similarly, when $\eta_{\text{pt-Kreab}}$ is slightly reduced to $\eta_{\text{pt-Kreab}} = 0.61$, there is little effect of α_{TGF} since the difference between the fractional proximal segment K^+ reabsorption and baseline value ($\eta_{\text{ps-Kreab}}^{\text{base}}$) is small. However, as $\eta_{\text{pt-Kreab}}$ is further reduced, varying α_{TGF} has a much larger impact. For $\eta_{\text{pt-Kreab}} = 0.18$, there is a difference of 78 mmol/L or a 38%

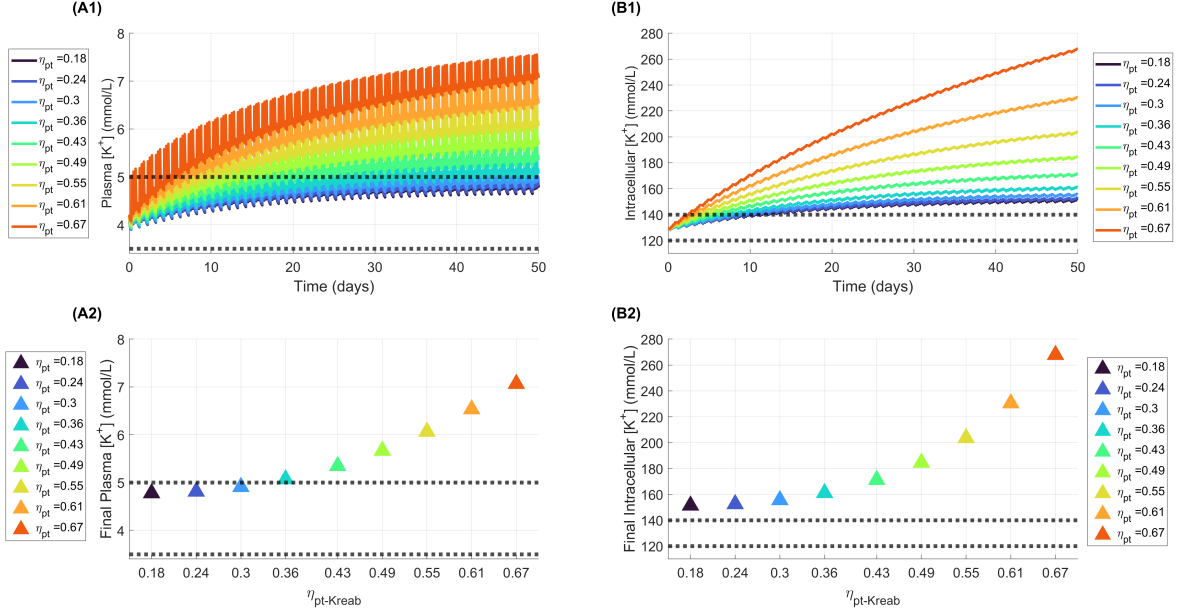


Figure 4.6: Predicted impact of varying fractional proximal tubule K^+ reabsorption ($\eta_{pt-Kreab}$, shortened to η_{pt} in the legends). Model predictions of plasma $[K^+]$ (A1) and intracellular $[K^+]$ (B1) for 50 days of a high K^+ diet with varied values for the $\eta_{pt-Kreab}$ parameter, which represents fractional proximal tubule K^+ reabsorption. The plasma and intracellular $[K^+]$ at the end of the 50 day simulations are plotted in panels A2 and B2, respectively. In all simulations shown here the tubuloglomerular feedback effect is included with $\alpha_{TGF} = \alpha_{TGF}^{base}$. Note that the baseline high K^+ intake fractional proximal tubule reabsorption is 0.36 as found by Wang et al. [230] and baseline normal K^+ intake fractional proximal tubule reabsorption is 0.67. $\eta_{pt} = \eta_{pt-Kreab}$: fractional proximal tubule K^+ reabsorption

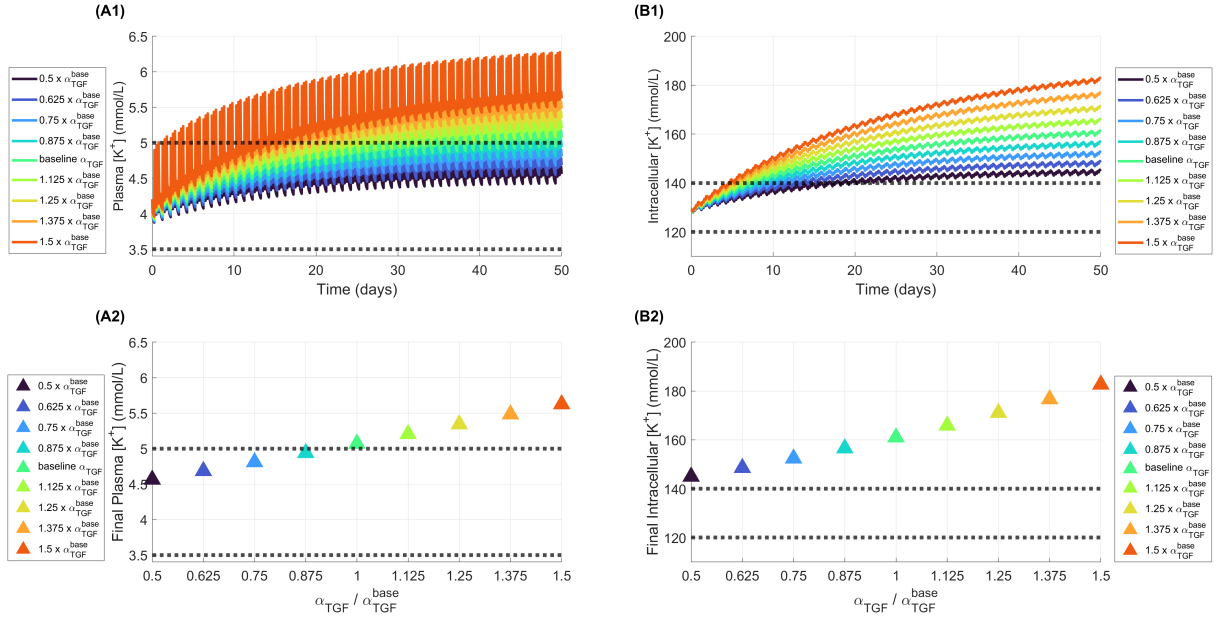


Figure 4.7: Predicted effects of varying the tubuloglomerular feedback effect (α_{TGF}). Model predictions of plasma $[K^+]$ (A1) and intracellular $[K^+]$ (B1) for 50 day of high K^+ diet with varied values for the α_{TGF} parameter, which represents the strength of the tubuloglomerular feedback effect. The plasma and intracellular $[K^+]$ at the end of the 50 day simulations are plotted in panels A2 and B2, respectively. Proximal tubule K^+ reabsorption $\eta_{pt-Kreab} = 0.36$ in all cases. α_{TGF}^{base} : baseline α_{TGF} parameter value (see Eq. 4.3). TGF: tubuloglomerular feedback

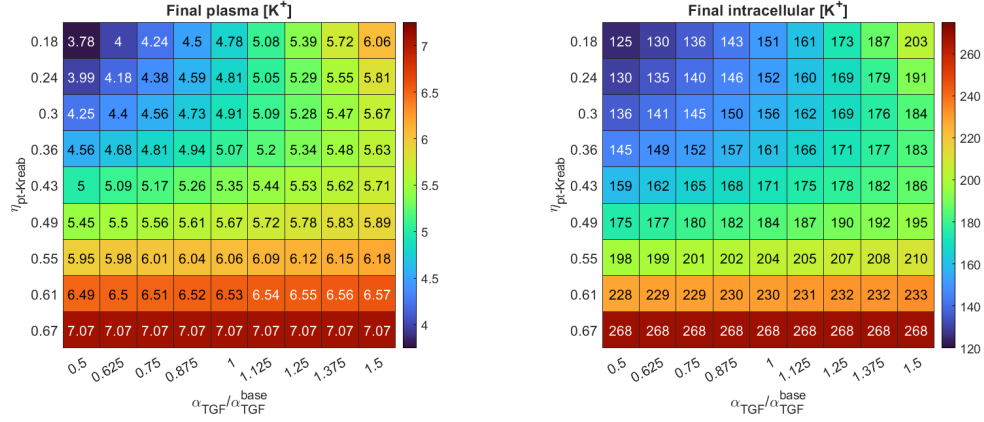


Figure 4.8: Model predictions of final plasma and intracellular $[K^+]$ at the end of the 50 day simulations are shown for varied values of α_{TGF} and $\eta_{pt-Kreab}$. The simulations were conducted for the same parameter variations as in Fig. 4.6 and Fig. 4.7. The units for the plasma and intracellular $[K^+]$ are in mmol/L. α_{TGF}^{base} : baseline α_{TGF} parameter value, $\eta_{pt-Kreab}$: fractional proximal tubule reabsorption (Eq. 4.3).

reduction in intracellular $[K^+]$ at the end of the simulations between $\alpha_{TGF} = 1.5 \times \alpha_{TGF}^{base}$ and $\alpha_{TGF} = 0.5 \times \alpha_{TGF}^{base}$. Similarly, there is a 38% reduction in plasma $[K^+]$ between these two values of α_{TGF} (Fig. 4.8).

Decreasing $\eta_{pt-Kreab}$ under a high K^+ diet in isolation decreases plasma $[K^+]$ and intracellular $[K^+]$ nonlinearly so that at the end of the 50 day simulation the final plasma and intracellular $[K^+]$ levels remain high (Fig. 4.6). However, when α_{TGF} is also decreased, the resulting final plasma and intracellular $[K^+]$ levels decrease as well (Fig. 4.8). Specifically, when α_{TGF} is $0.75 \times \alpha_{TGF}^{base}$ and $\eta_{pt-Kreab} = 0.24$, final plasma and intracellular $[K^+]$ are predicted to be 4.38 and 139.8 mmol/L, respectively, both within normal range (Fig. 4.8). However, we note that this low of fractional proximal tubule reabsorption and TGF effect may not be physiological.

In summary, decreasing $\eta_{pt-Kreab}$ under a high K^+ diet improves plasma and intracellular $[K^+]$ levels nonlinearly so that there is a limiting effect on the impact of lowering $\eta_{pt-Kreab}$. This nonlinearity is due to the TGF effect, which lowers GFR in response to high delivery of K^+ to the macula densa (Eq. 4.2). Similarly, decreasing the TGF effect parameter, α_{TGF} , results in a linear improvement in plasma and intracellular $[K^+]$ dependent on how much $\eta_{pt-Kreab}$ is decreased from its baseline value. These simulations quantify how each of these parameters impact long term K^+ regulation on a high K^+ diet.

4.3.3 Sensitivity analysis

Regulation of K^+ can be impacted by many different physiological changes. Such changes can be represented by varying parameter values. Sensitivity analysis is an approach used to quantify how changes in parameter values impact model predictions (see Section 4.2.3). We conducted a local sensitivity analysis by increasing and decreasing parameters by 10% and computing the resulting changes in the final plasma and intracellular $[K^+]$ at the end of a 50 day simulation of a high K^+ diet with PT and TGF effects. The sensitivities were determined using Eq. 4.6 and Eq. 4.7. We conducted the local sensitivity analysis for all parameters in the model (see Table C.1). Local sensitivity analysis results for parameters that had a more than 1% change in plasma or intracellular $[K^+]$ are shown in Fig. 4.9.

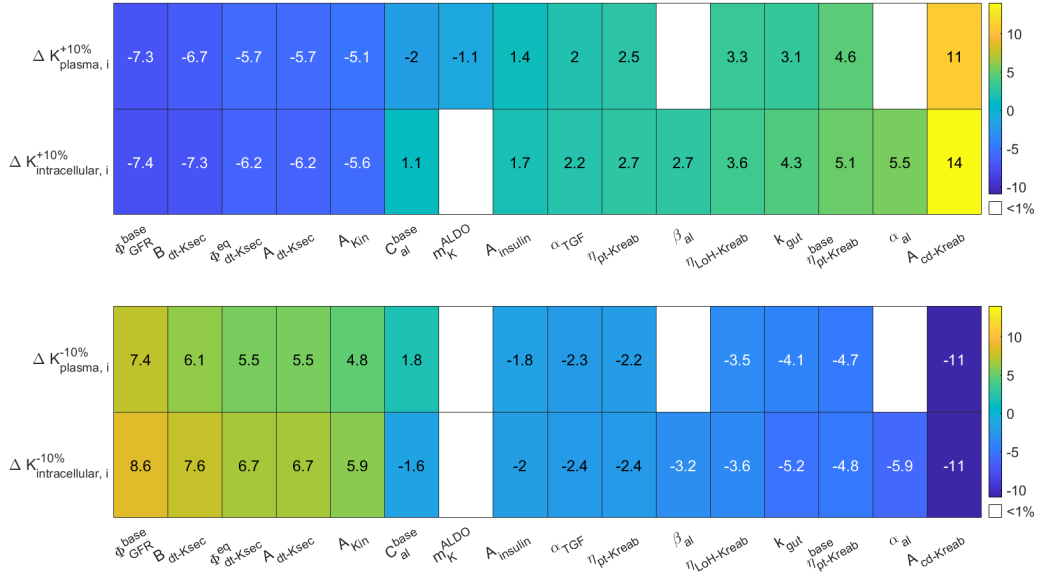


Figure 4.9: Local sensitivity analysis of the impact of individual parameters on final plasma and intracellular $[K^+]$ at the end of the 50 day simulation of a high K^+ diet with proximal tubule and tubuloglomerular feedback effects. Sensitivity is determined by increasing and decreasing individual parameters by 10% and computing $\Delta K_{c,i}^{+10\%}$ and $\Delta K_{c,i}^{-10\%}$ as given by Eq. 4.6 and Eq. 4.7, respectively. The results for an increase by 10% and decrease by 10% are shown in the top and bottom panels, respectively for each parameter. Parameters that did not result in a greater than $\pm 1\%$ change for either K_{plasma} or $K_{\text{intracellular}}$ are not shown. PT: proximal tubule, TGF: tubuloglomerular feedback

The parameters that impact the final plasma and intracellular $[K^+]$ the most are $A_{cd-Kreab}$ and Φ_{GFR}^{base} , which represent collecting duct K^+ reabsorption and baseline GFR, respectively. Indeed, altered filtration directly impacts urinary K^+ due to more or less K^+ entering the nephrons. Changing $A_{cd-Kreab}$ directly by 10% increases collecting duct K^+ reabsorption proportionally, significantly lowering or increasing K^+ excretion. This leads to a direct impact on intracellular and plasma $[K^+]$. The parameters involved in distal tubule K^+ secretion also have a large impact on model results ($B_{dt-Ksec}$, $\Phi_{dt-Ksec}^{eq}$, $A_{dt-Ksec}$) both when increasing and decreasing the individual parameters by 10%. From a local sensitivity perspective, renal handling of K^+ has the largest impact on long-term plasma and intracellular $[K^+]$ on a high K^+ diet.

We used the net local sensitivity (Eq. 4.8) to determine which parameters to include in the Morris analysis. Specifically, the parameters that had a less than 2% change in K_{plasma} or $K_{intracellular}$ were excluded from the analysis. This reduced computation time by reducing the number of parameters in the analysis. The 16 parameters included in the Morris analysis are shown in Fig. 4.9 and the ranges for each parameter used are listed in the Table C.2. Parameter ranges for parameters that have measurable physiological values (e.g., Φ_{GFR}^{base} , $\Phi_{dt-Ksec}^{eq}$, C_{al}^{base}) were chosen based on normal physiological ranges. Parameters with unknown physiological ranges (e.g., A_{Kin} , α_{al} , m_K^{ALDO}) were given ranges of $\pm 50\%$ of their baseline value.

The μ^* and σ values (Eq. 4.10; Eq. 4.11) for each parameter included in the Morris analysis with respect to the final plasma and intracellular $[K^+]$ are plotted in Fig. 4.10. This type of plot is often referred to as a “Morris plot” [176]. The Morris indices (MI_i , Eq. 4.12) are plotted in Fig. 4.11.

The μ^* and σ values for the intracellular $[K^+]$ are much larger than the plasma $[K^+]$ (Fig. 4.10). This is due to intracellular $[K^+]$ having a larger baseline value than plasma $[K^+]$ (130 mmol/L vs. 4.2 mmol/L) and intracellular $[K^+]$ having a larger tolerance for changes in K^+ levels. Hence, it is expected that there should be more variance in the final intracellular $[K^+]$ than plasma $[K^+]$ as reflected in the Morris analysis results.

The parameters with the three largest μ^* values and Morris indices for both plasma and intracellular $[K^+]$ are $A_{cd-Kreab}$, $B_{dt-Ksec}$, and $A_{dt-Ksec}$ (Fig. 4.10; Fig. 4.11), which represent collecting duct K^+ reabsorption and the effect of ALD on distal tubule K^+ secretion. Additionally, the parameter with the fifth largest Morris index was $\Phi_{dt-Ksec}^{eq}$, which represents baseline distal tubule K^+ secretion. These parameters are all involved in renal handling of K^+ .

The parameter with the fourth largest Morris index for plasma and intracellular $[K^+]$ is A_{Kin} . This parameter determines stimulation of distal tubule K^+ secretion based on oral

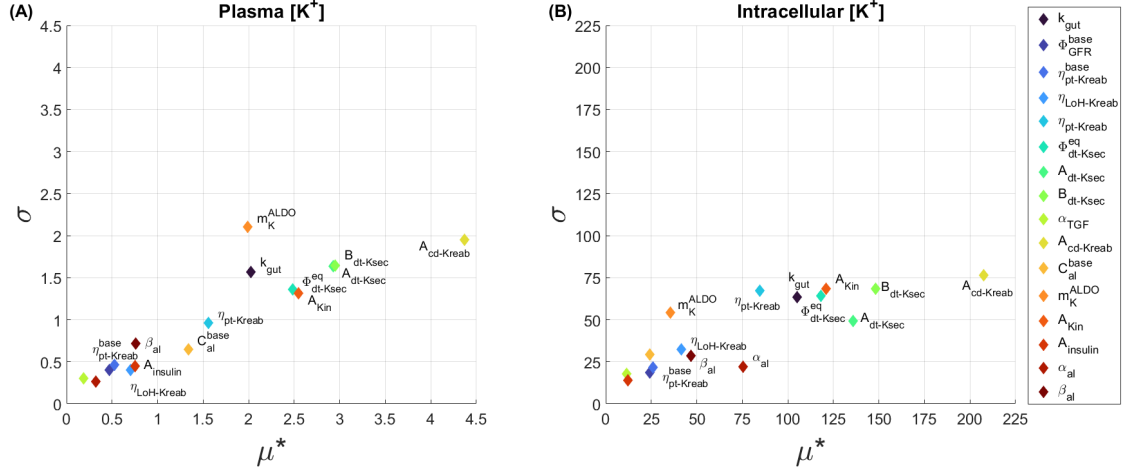


Figure 4.10: Morris plots for μ^* (Eq. 4.10) and σ (Eq. 4.11) values for individual parameters values in the Morris analysis as measured by impact on the final (A) plasma $[K^+]$ and (B) intracellular $[K^+]$ at the end of a 50 day simulation of high K^+ intake.

K^+ intake. In our previous study the gastrointestinal feedforward effect, which is driven by A_{Kin} , was the feedforward effect that had the largest impact on whole body K^+ levels [214]. This feedforward mechanism impacts whole body K^+ homeostasis by altering distal tubule K^+ secretion. Therefore, these effects are due to altered renal K^+ handling.

In this study, the primary model extension was the renal effects on a high K^+ diet, by changing $\eta_{pt-Kreab}$ and adding Eq. 4.2 to include TGF effects. Indeed, in the Morris analysis we found that both plasma and intracellular $[K^+]$ are highly sensitive to changes in $\eta_{pt-Kreab}$ (Fig. 4.10; Fig. 4.11) on a high K^+ diet. In contrast, α_{TGF} had a smaller impact on the model output (Fig. 4.10; Fig. 4.11). This difference shows that PT K^+ reabsorption has a larger impact on model results when combined with other parameters from a global sensitivity analysis perspective.

We note that in the Morris analysis, Φ_{GFR}^{base} did not have a high Morris Index, despite physiologically GFR having a large impact on renal handling and being one of the most sensitive parameters in the local sensitivity analysis (Fig. 4.9). This is because the GFR used in the model is based on Eq. 4.2, which depends not only on Φ_{GFR}^{base} , but, α_{TGF} , $\eta_{pt-Kreab}$, $\eta_{LoH-Kreab}$ and $\eta_{pt-Kreab}^{base}$, all of which are also changed in the Morris analysis. This is in contrast to the local sensitivity analysis where only one parameter is changed and Φ_{GFR}^{base} was one of the most sensitive parameters (Fig. 4.9). Notably, $\eta_{pt-Kreab}$ has a large impact on both intracellular and plasma $[K^+]$ levels based on the Morris analysis,

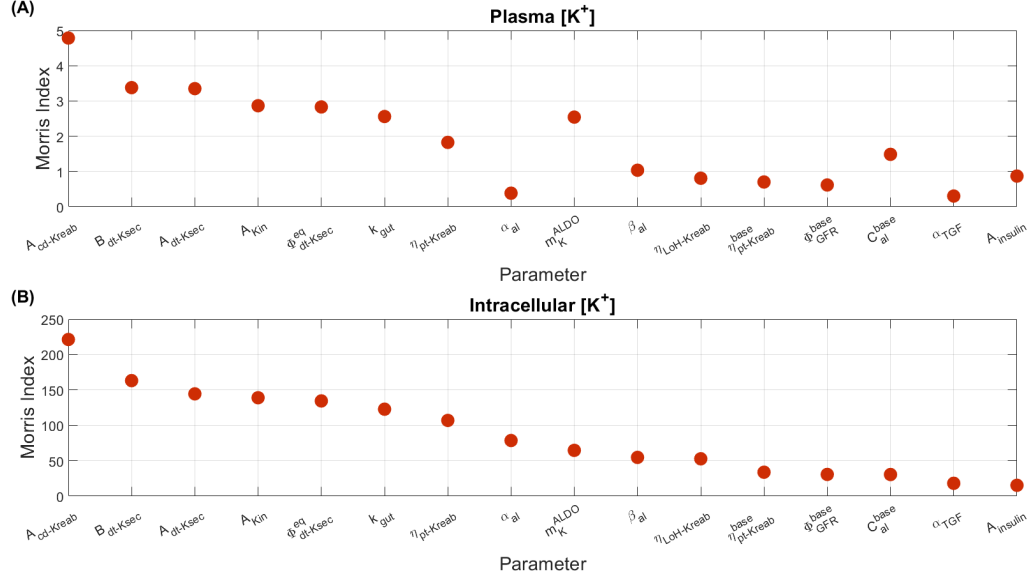


Figure 4.11: Morris indices (MI_i ; Eq. 4.12) for individual parameter values in the Morris analysis as measured by the impact on the final (A) plasma $[K^+]$ and (B) intracellular $[K^+]$ at the end of a 50 day simulation of high K^+ intake.

which directly impacts GFR (Eq. 4.2).

In summary, our sensitivity analysis results quantify the impacts of each parameter and its interactions with other parameters on the final plasma and intracellular $[K^+]$ after 50 days of high K^+ intake. Our Morris analysis results show that final plasma and intracellular $[K^+]$ levels depend primarily on parameters that characterize renal K^+ handling.

4.4 Discussion

The mathematical model presented in this study includes a comprehensive description of known regulatory mechanisms involved in K^+ handling, as well as the newly discovered impacts of high K^+ intake on PT K^+ reabsorption and the resulting TGF effects [230]. We conducted model analysis to:

- Predict the impact of recently reported PT and TGF effects of a high K^+ diet on whole body potassium homeostasis (Fig. 4.3; Fig. 4.4; Fig. 4.5).

- Unravel the interconnected effects of decreased PT K^+ reabsorption with the TGF signal under a high K^+ diet (Fig. 4.3; Fig. 4.4; Fig. 4.5).
- Quantify the impact of varied amounts of fractional PT K^+ reabsorption ($\eta_{pt-Kreab}$) and TGF signaling (α_{TGF}) under a high K^+ diet in isolation (Fig. 4.6 and Fig. 4.7) as well as combined (Fig. 4.8).
- Evaluate model sensitivity to changes in parameter values with both a local (Fig. 4.9) and global approach (Fig. 4.10; Fig. 4.11).

Overall, our model analysis provides insights into the impacts of renal adaptations to a modified K^+ diet, and provides a better understanding of the complex regulation of K^+ homeostasis under high K^+ intake.

The kidneys play a key role in long-term K^+ homeostasis by ensuring that total daily K^+ excretion matches daily K^+ intake. Detailed mathematical models of renal epithelial transport have been developed and applied to K^+ handling by the kidney, albeit without explicitly considering extrarenal K^+ balance. The model of rat kidney function by Weinstein [233] indicated that a K^+ load attenuates PT fluid reabsorption and increases Na^+ delivery to the distal nephron, despite a TGF-lowered GFR. As previously reported in Ref. [125], our group’s model simulations of nephron function following an acute K^+ load indicated that K^+ secretion along the connecting tubule is increased due to elevated expression and activity of $Na^+-K^+-ATPase$, epithelial Na^+ channels, and renal outer medullary K^+ channels, together with downregulated Na^+-Cl^- cotransporters. This increase in K^+ secretion resulted in a >6 -fold increase in urinary K^+ excretion, substantially exceeding the filtered K^+ load.

Unlike the studies discussed above, the kidney component of the present model is much simpler and does not represent detailed epithelial transport [125, 233]. Without altering nephron transport under a high K^+ diet, urinary K^+ excretion is too low to maintain K^+ balance (Fig. 4.3A). By reducing the fractional PT K^+ reabsorption, average fractional urine K^+ excretion nearly doubles, resulting in near K^+ balance (Fig. 4.3B). However, changes to electrolyte transport in the kidneys does not occur in isolation. Notably, the decrease in PT K^+ reabsorption in rats found by Wang et al. [230] is driven by reduced Na^+ reabsorption. That results in a higher Na^+ and K^+ load to the macula densa, where Na^+ , K^+ , and Cl^- levels are detected for TGF signalling [223, 224]. The TGF signal reduces flow to the glomerulus, lowering the single nephron GFR, and therefore directly decreasing renal K^+ filtration into each nephron. The resulting reduction in urinary K^+ excretion leads to a slight K^+ imbalance (Fig. 4.3A) and a gradual rise in total body K^+ levels (Fig. 4.4). Previous studies have also shown that GFR decreases in adrenalectomized dogs under a

high K^+ diet, thus showing this phenomenon occurs in other species independent of ALD effects [246]. Our model simulations unravel the complex interplay of K^+ transport along the PT with TGF effects.

In our previous study [214] (Chap. 2), we conducted “what-if” simulations of a hypothesized muscle-kidney cross talk under short-term K^+ loading or depletion. In this study, we also conducted high K^+ diet simulations with this signal incorporated. Due to the absence of direct supporting experimental evidence for this signal, those results are described in Appendix C.3.

Heterogeneity in individual biology impacts how the body responds to different physiological stimuli. To explore the functional implications of individual differences, we conducted simulations with different decreases in PT K^+ reabsorption under a high K^+ diet by changing the relevant parameter value ($\eta_{pt-Kreab}$; Eq. 4.1). We found that due to the TGF signal, decreasing PT K^+ reabsorption has a diminishing impact on plasma and intracellular $[K^+]$ balance (Fig. 4.6). Specifically, we simulated changes in $\eta_{pt-Kreab}$ to as low as 50% of the reported fractional PT K^+ reabsorption in Ref. [230] and found that at the end of the 50 day simulation, plasma $[K^+]$ was on the high end of normal range in between meals and intracellular $[K^+]$ was above normal range by day 20 when there were no other changes to the model (Fig. 4.6). While in this case plasma $[K^+]$ is within the normal range, a person with high intracellular $[K^+]$ levels may be more susceptible to hyperkalemia because of impaired intracellular K^+ buffering. Additionally, we note that this low fractional PT K^+ reabsorption may be outside of physiological range.

The TGF response is also impacted by heterogeneity in physiology. To investigate this, we conducted high K^+ intake simulations with varied TGF strength characterized by α_{TGF} (Eq. 4.2). When α_{TGF} is increased, the plasma and intracellular $[K^+]$ levels increase as a result of decreased filtration to the nephrons (Fig. 4.7). Conversely, when α_{TGF} is decreased, the filtration is not as strongly affected by reduced PT fractional K^+ reabsorption, which yields a better balance of urinary K^+ excretion. When α_{TGF} is reduced by 12.5% or more, final plasma $[K^+]$ is within normal range (Fig. 4.7A2), but even when α_{TGF} is half of its baseline value (i.e., $\alpha_{TGF} = 0.5 \times \alpha_{TGF}^{base}$), intracellular $[K^+]$ levels rise to higher than normal range within the 50 day simulation (Fig. 4.7B1-B2). This is because even with a smaller reduction in K^+ filtration, the change is enough to result in insufficient urinary K^+ excretion so that some K^+ is retained within the body. Since the intracellular fluid uptakes extra K^+ to keep plasma $[K^+]$ within normal range, intracellular $[K^+]$ levels are increased. Therefore, the TGF effect lowers K^+ filtration leading to reduced urinary K^+ excretion so that excess K^+ remains within the body.

The TGF signal relies on how much Na^+ , Cl^- , and K^+ is delivered to the macula densa

to determine how to adjust GFR (Eq. 4.2). Clearly, PT reabsorption and the TGF effect are interconnected. To investigate the synergy of PT K^+ reabsorption and TGF effect, we conducted simulations for each combination of the varied parameter values (Fig. 4.8). For all values of α_{TGF} , the impact of lowering $\eta_{pt-Kreab}$ had a diminishing effect because of the lowered GFR. These simulations quantify how altered TGF and PT K^+ reabsorption impact K^+ regulation on a high K^+ diet. We note that the TGF effect and PT K^+ reabsorption is also impacted by diseases such as diabetes and CKD as well as certain medications [26,74]; therefore varying the TGF effect and PT K^+ reabsorption may provide insights into these conditions as well.

As with many mathematical models of physiological systems, the present model contains (i) epistemic uncertainty, which comes from an incomplete knowledge of the physiological system, and (ii) aleatory uncertainty, which refers to randomness and heterogeneity that arise in biological systems [183]. For example, in humans, it is impossible to (ethically) measure the exact rate of how K^+ secretion along the distal tubule changes with varied [ALD]. Instead, these parameters must be estimated based on the data that we do have. This is an example of epistemic uncertainty in this K^+ regulation model. Aleatory uncertainty comes in that even if we could measure every parameter exactly in a person, this will not represent the whole population, and may not even represent that person on a different diet or at a different time. For this reason, studying the effects of varied parameters using global sensitivity analysis can help us further understand uncertainties that arise when modeling complex systems as done in this study.

To elucidate the relationship of the parameters and the predicted plasma and intracellular $[K^+]$, we conducted a Morris analysis to quantify how much each parameter impacts final plasma and intracellular $[K^+]$ after 50 days of a high K^+ diet (Fig. 4.10; Fig. 4.11). This analysis showed that for both plasma and intracellular $[K^+]$, the parameters that determine renal K^+ transport have the largest impact on final $[K^+]$. In particular, the parameter with the second highest impact was the PT K^+ reabsorption, showing that this change under a high K^+ diet is critical for maintaining K^+ balance even when other parameters are altered. Additionally, this Morris analysis showed that our model predictions were driven primarily by renal transport. This shows that our model captures what is happening physiologically since long-term K^+ regulation is primarily regulated by renal K^+ handling via urinary K^+ excretion.

Model limitations and future work

The whole-body K^+ regulation model used in this study represents K^+ homeostasis in humans. However, the addition of the PT K^+ reabsorption adaptations under a high K^+

diet is based on an experimental study in rats [230]. Indeed, there are species differences in renal electrolyte handling [127, 210]. However, due to the invasive experiments required to get renal transport data as done in Ref. [230], human data is not possible at this time. Therefore, we assumed that the same percent change in fractional PT K^+ reabsorption and TGF effects occurred in the human model, but did not change other renal parameters. It is likely that there may be variation between the species, which is why studying differences in parameters such as done in Fig. 4.6 and Fig. 4.7 is useful for studying heterogeneity that may occur in populations and between the species.

Another limitation and opportunity is that this model is built on data that is not sex-specific. Specifically, this model is formulated to represent a healthy, average-body, man. It is known that there are sex differences in renal K^+ transport [100, 143, 210], Na^+ balance [143, 210], and ALD concentration [148], which all affect K^+ homeostasis. It has also been hypothesized that males and females have different plasma $[K^+]$ set points for handling high K^+ loads [162]. Indeed, this whole-body K^+ model may be extended to consider sex differences as well as unique physiological states in females that undergo major changes in K^+ regulation such as during pregnancy and lactation [46, 215, 236].

A significant limitation of our model is that it is well known that regulation of renal K^+ transport as well as the TGF signal is indeed coupled to other solutes such as Na^+ . Additionally, osmotic equilibrium would change how K^+ is regulated in the body. Indeed, future work can extend this model to include other electrolytes for a more accurate representation of electrolyte homeostasis. For example, this model could be combined with a model of Na^+ regulation (e.g., Refs. [90, 131]) to investigate the impacts of Na^+ intake on K^+ excretion and vice versa [247]. An extension of a Na^+/K^+ homeostasis model would also be able to include blood pressure control as in Refs. [90, 131]. Since the combination of dietary Na^+ and K^+ has been shown to impact blood pressure [111, 248], a worthwhile study would be to investigate the impacts of dietary K^+ on blood pressure control by integrating the K^+ model presented here blood pressure regulation models. (Update: We have published a study coupling the K^+ model with the blood pressure model in Ref. [207] since the publication of this study.)

Circadian rhythms impact many physiological systems, including renal electrolyte handling [52, 82, 126, 208]. A recent QSP model of K^+ homeostasis in patients with hyperkalemia included the impact of the circadian rhythm on GFR in their study [42]. Indeed, circadian rhythms in K^+ handling likely have a role in whole-body K^+ homeostasis. A similar approach to this current study may include circadian effects on renal K^+ transport along the nephrons [82, 208] to investigate how this impacts the full body.

Understanding K^+ homeostasis is critical due to the risks and potentially severe con-

sequences of hyperkalemia and hypokalemia. Our current study considers K^+ regulation in a healthy person. Indeed, future work may consider pathological states such as CKD, diabetes, or persons taking medications that can affect K^+ balance. To simulate impaired kidney function or medications that target the kidney, the renal component of the present model may be adapted to represent the changes that occur from renal impairment.

Chapter 5

Mathematical modeling of calcium homeostasis in female rats: An analysis of sex differences and maternal adaptations

Abstract

Calcium plays a vital role in various biological processes, including muscle contractions, blood clotting, skeletal mineralization, and cell signaling. While extracellular calcium makes up less than 1% of total body calcium, it is tightly regulated since too high or too low extracellular calcium concentration can have dangerous effects on the body. Female bodies differ from male bodies in that during their lifetime they may undergo massive adaptations during pregnancy and lactation. Indeed, maternal adaptations impact calcium regulation in all mammals. In pregnant rodents, intestinal absorption of calcium is massively increased in the mother's body to meet the needs of the developing fetus. In a lactating rodent, much of the calcium needs of milk are met by bone resorption, intestinal absorption, and renal calcium reabsorption. Given these observations, the goal of this project is to develop mathematical models of calcium homeostasis that represents (1) how sex differences impact calcium homeostasis in female vs. male rats and (2) how a female body adapts to support the excess demands brought on by pregnancy and lactation. We used these models to quantify the impact of individual sex differences as well as maternal adaptations during

pregnancy and lactation. Additionally, we conducted “what if” simulations to test whether sex differences in calcium regulation may enable females to better undergo maternal adaptations required in pregnancy and lactation than males.

5.1 Introduction

Calcium, the most abundant mineral in the human body, is essential for many physiological and cellular processes. Together with phosphate, calcium plays a critical component of the skeleton by forming a calcium-phosphate compound called hydroxyapatite, the main structural component of the bone and where approximately 99% of the total body calcium is stored [32, 175, 184]. The remaining 1% of body calcium plays an important role in various other physiological processes such as cell signalling, both skeletal and smooth muscle contraction, and blood clotting [29, 32, 175, 184]. Tight regulation of the serum calcium concentration ($[\text{Ca}^{2+}]_p$) is essential since too much or too little calcium (i.e., hypercalcemia and hypocalcemia) can have dangerous, potentially fatal consequences [58, 91].

To maintain calcium balance, it is crucial to regulate the fluxes of calcium among the primary organs involved in calcium regulation, namely the intestine, bone, and kidneys. Along the intestine, approximately 40–80% of ingested calcium is absorbed via paracellular and transcellular pathways [36, 60, 64]. Subsequently, calcium enters the bloodstream, and eventually unbound calcium is filtered by the kidneys. Along the nephrons, specific calcium transporters control the reabsorption of calcium back into the bloodstream, while the remaining is excreted through urine [4, 50, 174]. Calcium reabsorption primarily occurs in the proximal tubule, thick ascending limb, and distal tubule [4, 50, 174]. Additionally, there is a constant exchange of calcium between the bones and blood. Most of this exchanged calcium goes through a process called accretion, where it forms hydroxyapatite, which makes up the skeleton. Hydroxyapatite can later be broken down and the calcium returned to the bloodstream through a process called resorption. The regulation of bone formation and resorption is carried out by bone cells called osteoblasts and osteoclasts.

Calcium fluxes between the intestines, kidneys, and bone are mediated by several factors, including PTH, calcitriol ($1,25(\text{OH})_2\text{D}_3$), and the CaSR. PTH is secreted by the parathyroid glands and binds to receptors on bone and kidney cells, stimulating bone resorption and renal calcium reabsorption, respectively [4, 15, 50]. The CaSR is predominantly expressed in the parathyroid glands, kidneys, and mammary gland (in lactating females) [91]. It plays a role in regulating PTH secretion, urinary calcium excretion, skeletal development, and lactation. $1,25(\text{OH})_2\text{D}_3$ is synthesized in the proximal tubules of the kidneys from its precursor vitamin D via the enzyme 1α -hydroxylase [15, 174].

About two-thirds of total intestinal absorption is mediated by $1,25(\text{OH})_2\text{D}_3$ by binding to the vitamin D receptor [13, 64]. When bound, the vitamin D receptor- $1,25(\text{OH})_2\text{D}_3$ complex enhances intestinal calcium absorption by increasing tight junction permeability and upregulating the activity and expression of calcium transport proteins [4, 50, 64].

Along the nephrons of the kidneys, PTH, $1,25(\text{OH})_2\text{D}_3$, and CaSR regulate renal calcium reabsorption. The CaSR is expressed along the nephrons and detects changes in calcium concentration in the extracellular fluid [91]. Along the proximal tubule, both CaSR and PTH regulate $1,25(\text{OH})_2\text{D}_3$ synthesis [5, 91]. PTH impacts proximal tubule calcium reabsorption via altered Na^+/H^+ exchanger activity, which has an impact on calcium reabsorption due to the tight connection between sodium and calcium reabsorption along this segment [5, 6, 50]. Along the distal segments (cortical thick ascending limb and distal convoluted tubule) the CaSR regulates calcium reabsorption by altering the paracellular permeability of calcium [4, 50, 91]. PTH and $1,25(\text{OH})_2\text{D}_3$ also impact calcium reabsorption along these segments [5, 50].

The CaSR regulates systemic calcium homeostasis by detecting changes in $[\text{Ca}^{2+}]_p$ and triggering a cascade of signaling mechanisms that regulate secretion of PTH. Additionally, in lactating mammals, the CaSR in the mammary gland detects changes in extracellular calcium concentrations to regulate bone resorption, facilitating the release of bone calcium for milk production [91]. Essentially, the CaSR acts as a key signaling mechanism, ensuring the tight regulation of $[\text{Ca}^{2+}]_p$ by modulating plasma concentrations of PTH ($[\text{PTH}]_p$) and $1,25(\text{OH})_2\text{D}_3$ ($[1,25(\text{OH})_2\text{D}_3]_p$) as well as direct effects on the kidneys.

In summary, maintaining calcium balance relies on the highly coupled regulation of various processes, including intestinal absorption, renal filtration and reabsorption, and bone remodeling. Given the multitude of interconnected physiological processes involved in calcium homeostasis, mathematical modeling proves valuable in comprehending its complexities. Figure 5.1 provides a schematic representation of the fundamental fluxes and hormones involved in calcium homeostasis.

Sex differences in calcium homeostasis

Across many species, sex differences have been found in most organ systems beyond the reproductive system. In particular, sex differences have been reported in mammals in their systems involved in calcium regulation. First, it is worth noting that for some mammals, such as rats, females are on average smaller than male rats and thus have a smaller plasma volume [129]. Additionally, female rats generally consume less food compared to males. Despite these overall differences, no significant differences in $[\text{PTH}]_p$ and $[\text{Ca}^{2+}]_p$ have been

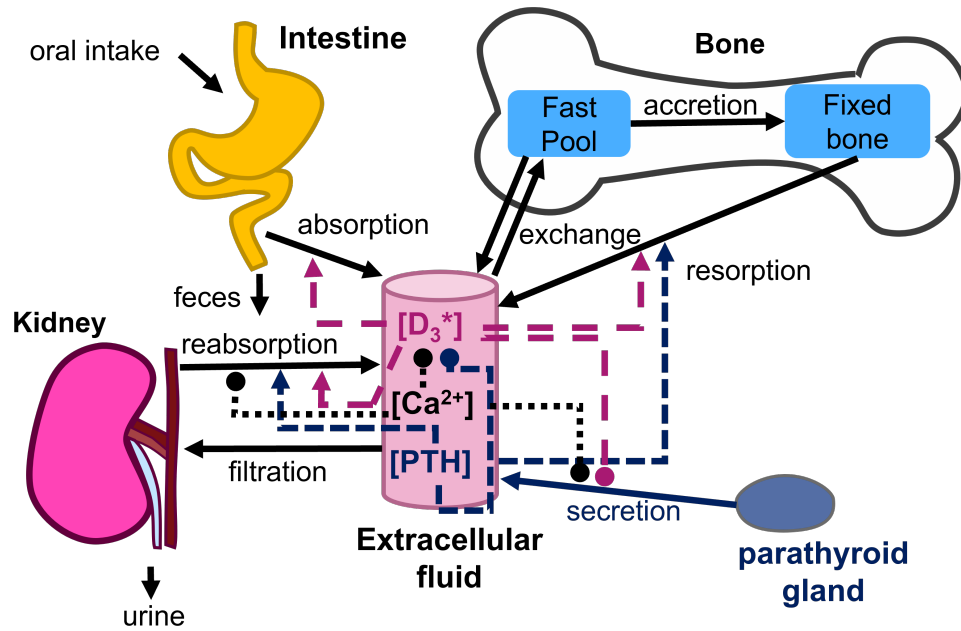


Figure 5.1: Schematic representation of primary organs (intestines, bone, kidneys) and hormones (calcitriol and PTH) involved in regulating calcium homeostasis. Solid black arrows show calcium fluxes. Dashed black line show the impact of the calcium sensing receptor (CaSR). Dashed blue and pink lines indicate hormonal impacts of parathyroid hormone and calcitriol, respectively. Pointed arrows indicate activation and blunted arrows indicate inhibition. PTH: parathyroid hormone, D_3^* : calcitriol ($1,25(OH)_2D_3$)

found in male versus female rats [158]. However, $[1,25(OH)_2D_3]_p$ levels in female rats are about 50-60% that of male levels despite similar inactive vitamin D levels [158]. While lower $[1,25(OH)_2D_3]_p$ in isolation should decrease intestinal absorption of calcium, female mice have a higher baseline fractional intestinal absorption than male mice [205]. Sex differences also occur in renal electrolyte handling along the nephrons in male and female rats [97, 143, 225]. Specifically, sex steroids have been found to have an impact on calcium transporters, thereby altering renal calcium handling [97, 112, 113].

Maternal adaptations during pregnancy and lactation

Pregnancy. During pregnancy, the maternal body must significantly adapt to support the rapidly growing fetoplacental unit [1, 155]. In rats, the gestation period is about 21-22 days

[235]. Notably, during the final four days of gestation, around 80% of the total fetal calcium is transported into the fetoplacental unit, thus requiring major changes in calcium handling in the maternal body [43, 118]. Given this observation, we will focus on late pregnancy, during which the largest changes in maternal calcium homeostasis occur. Furthermore, most experimental studies have focused on this gestational period so most literature data is from late pregnancy. Also, notable in pregnancy is that plasma volume rapidly expands largely due in part to major changes in renal function [235]. Without adaptations in calcium regulation, increased plasma volume as well as excess calcium demands of the fetus would lead to severe maternal hypocalcemia.

PTH and $1,25(\text{OH})_2\text{D}_3$ both increase $[\text{Ca}^{2+}]_p$ and their plasma concentrations are greatly increased during pregnancy [25, 87, 118, 193]. However, the elevated $[1,25(\text{OH})_2\text{D}_3]_p$ does not fully account for the increases in intestinal absorption observed during pregnancy [38, 39, 153]. The extra intestinal absorption is likely accounted for by the high levels of prolactin and estrogen during pregnancy [17, 38, 39, 118]. The resulting enhanced intestinal absorption during pregnancy contributes to most of the calcium that is delivered to the fetus [118]. See Fig. 5.2 for a schematic on adaptations during late pregnancy on calcium homeostasis in rats.

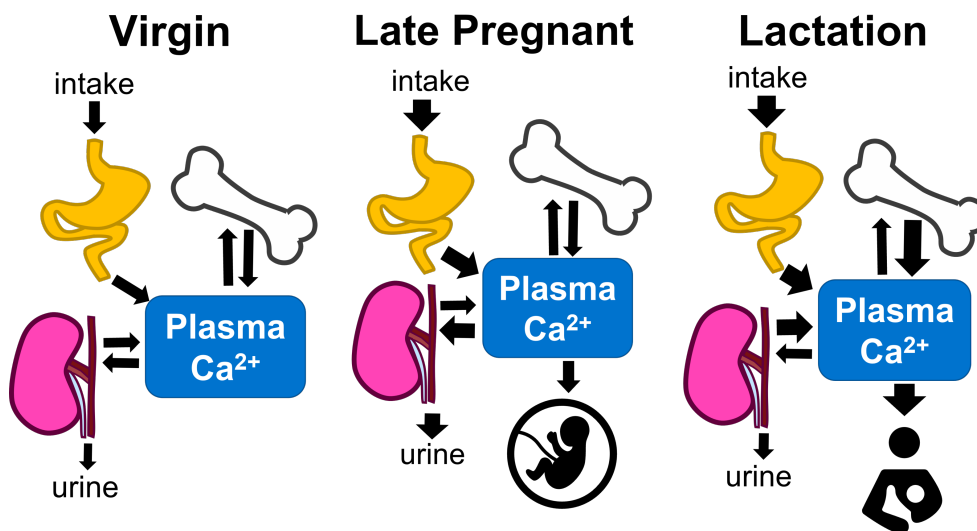


Figure 5.2: Schematic diagram of maternal adaptations in late pregnancy and lactation in rats. Black arrows represent fluxes of calcium between organs (intestines, bone, kidney, fetus, mammary glands). Thickness of the arrows indicates (approximate) relative increase or decrease with respect to virgin rat levels in late pregnancy and lactation.

Lactation. More calcium is delivered to the milk during lactation than to the fetus during pregnancy; therefore lactation imposes an even higher demand on calcium regulation in the maternal body [118]. Post-parturition, plasma volume in the maternal body decreases from late pregnancy levels, but remains elevated from pre-pregnancy plasma volume [23]. $[PTH]_p$ and $[1,25(OH)_2D_3]_p$ levels remain at or above pregnancy values during lactation [87, 118, 193], as well as intestinal absorption [88, 118]. What differs the most in calcium adaptations between pregnancy and lactation (in rats) is that the latter is a state of excessive bone resorption [116, 118, 134, 189]. See Fig. 5.2 for a diagram on the adaptations during lactation on calcium homeostasis in rats.

Vitamin D deficiency

Maintaining adequate vitamin D levels is important for overall health. Vitamin D is obtained through exposure to sunlight, diet, and (when needed) supplements. It plays a crucial role in maintaining calcium homeostasis as the precursor to $1,25(OH)_2D_3$. When a person or rodent becomes deficient in vitamin D, intestinal calcium absorption decreases, causing a decrease in $[Ca^{2+}]_p$. In turn, the CaSR detects the drop in $[Ca^{2+}]_p$, triggering the secretion of PTH. Increased $[PTH]_p$ levels stimulate $1,25(OH)_2D_3$ production in order to enhance the activity of osteoclasts (bone resorbing cells), increase renal reabsorption of calcium, and stimulate intestinal absorption to increase calcium into the bloodstream. For small decreases in vitamin D levels, this is sufficient to return levels of $[Ca^{2+}]_p$ back to baseline. However, as vitamin D deficiency becomes more severe, compensatory mechanisms cannot make up for this loss, resulting in low $[Ca^{2+}]_p$.

In late pregnant rodents, or third trimester pregnant women, there is an increased demand for calcium, and maintaining optimal vitamin D levels becomes crucial for the health of the mother and the developing fetus. In women, vitamin D deficiency during pregnancy is a global health issue, with reported prevalence varying between 18 and 84%, depending on the environment and local customs (diet, clothing, sun exposure, etc.) [153]. In the United States, an estimated 5-50% of pregnant women experience vitamin D deficiency with African American women facing a higher risk compared to women of other races due to increased skin pigmentation and lower dietary intake [21, 153].

Low vitamin D levels during pregnancy and infancy have been associated with various adverse health outcomes, including neonatal hypocalcemia, poor postnatal growth, increased bone fragility in both mother and neonate, rickets in children, pre-eclampsia, low birth weight, and a higher incidence of autoimmune diseases throughout their lifetime [153]. Similarly, vitamin D deficiency occurs at a high rate in breastfeeding mothers, in turn resulting in a higher risk of vitamin D deficiency in breastfed infants [153]. In

fact, the American Academy of Pediatrics recommends supplementation of vitamin D for breastfed infants (note that formula is typically supplemented with vitamin D) [227, 228].

Mathematical modeling of calcium homeostasis

In the past couple of decades, there have been ongoing efforts in developing multi-scale computational models of calcium regulation for human and rodent data (e.g., Refs. [78, 79, 165, 180]). However, all published mathematical models are gender neutral; and, other than a brief section in Ref. [78], sex differences have not been explicitly addressed. Also, despite the marked adaptations in calcium handling in pregnancy and lactation, no mathematical models have considered these unique physiological states.

In this study, we develop the first series of mathematical models that describe calcium homeostasis in a male rat, a virgin female rat, a pregnant rat, and a lactating rat. We then conduct model simulations to better understand how calcium handling differs between the two sexes, and how the multitude of physiological adaptations in pregnancy and lactation impact calcium homeostasis. Additionally, we use the models to predict how vitamin D deficiency will impact calcium homeostasis for the sexes as well as pregnancy and lactation.

5.2 Methods

The mathematical models used in this study are based on the mathematical model of calcium homeostasis for a rat developed by Granjon et al. [78]. The model represents $[\text{Ca}^{2+}]_p$, $[\text{PTH}]_p$, and $[1,25(\text{OH})_2\text{D}_3]_p$, as well as the flux of calcium between the plasma and relevant organ systems (intestine, bones, and kidneys). By refitting key model parameters, we have created separate models that capture calcium homeostasis in a male rat, female (virgin) rat, pregnant rat, and lactating rat.

5.2.1 Mathematical model

Here we briefly describe the model equations; see Ref. [78] for more details on baseline model development. A model schematic is provided in Fig. 5.1. How the model was developed for the female rat, pregnant rat, and lactating rat is described in Section 5.2.2 and Section 5.2.3, respectively.

Parathyroid hormone

Parathyroid gland PTH concentration, denoted by $[\text{PTH}]_g$, is modeled as

$$\frac{d[\text{PTH}]_g}{dt} = \frac{k_{\text{prod}}^{\text{PTHg}}}{1 + \gamma_{\text{prod}}^{1,25(\text{OH})_2\text{D}_3} [1, 25(\text{OH})_2\text{D}_3]_p} - F([Ca^{2+}]_p) [\text{PTH}]_g - k_{\text{deg}}^{\text{PTHg}} [\text{PTH}]_g \quad (5.1)$$

where $k_{\text{prod}}^{\text{PTHg}}$ is the basal rate of PTH production in the gland, $\gamma_{\text{prod}}^{1,25(\text{OH})_2\text{D}_3}$ represents inhibition of PTH_g by $[1,25(\text{OH})_2\text{D}_3]_p$, $k_{\text{deg}}^{\text{PTHg}}$ is the first-order degradation rate of PTH_g , and

$$F([Ca^{2+}]_p) = \beta_{\text{exo}}^{\text{PTHg}} - \frac{\gamma_{\text{exo}}^{\text{PTHg}} ([Ca^{2+}]_p)^n}{([Ca^{2+}]_p)^n + (K_{Ca}^{\text{CaSR}})^n} \quad (5.2)$$

represents PTH_g exocytosis where $\beta_{\text{exo}}^{\text{PTHg}}$, $\gamma_{\text{exo}}^{\text{PTHg}}$ are the maximal rate of PTH secretion from the parathyroid gland and maximal inhibition of the CaSR on PTH secretion, respectively, with K_{Ca}^{CaSR} representing the CaSR binding affinity factor and n is the steepness.

Next, we have that change in $[\text{PTH}]_p$ is given as

$$\frac{d[\text{PTH}]_p}{dt} = F([Ca^{2+}]_p) \frac{V_g}{V_p} [\text{PTH}]_g - k_{\text{deg}}^{\text{PTHp}} [\text{PTH}]_p \quad (5.3)$$

where $k_{\text{deg}}^{\text{PTHp}}$ is the first order degradation rate constant for PTH_p , V_g is the volume of the parathyroid gland, and V_p is plasma volume.

Calcitriol

$1,25(\text{OH})_2\text{D}_3$ is the activated form of vitamin D. We let inactive vitamin D plasma concentration (25-hydroxyvitamin D) be a constant denoted by $[D_3]_p^{\text{inact}}$ as in Ref. [78]. $[1,25(\text{OH})_2\text{D}_3]_p$ is modeled by [78]

$$\begin{aligned} \frac{d[1, 25(\text{OH})_2\text{D}_3]_p}{dt} &= (k_{\text{conv}}^{\text{min}} + \eta_{\text{PTHp}} \times \eta_{\text{CaSR}} \times \eta_{1,25(\text{OH})_2\text{D}_3} \times \delta_{\text{conv}}^{\text{max}}) [D_3]_p^{\text{inact}} \\ &\quad - \eta_{\text{deg}} \times k_{\text{deg}}^{1,25(\text{OH})_2\text{D}_3} [1, 25(\text{OH})_2\text{D}_3]_p \end{aligned} \quad (5.4)$$

where $k_{\text{conv}}^{\text{min}}$ denotes the minimum rate of conversion of inactive vitamin D, $\delta_{\text{conv}}^{\text{max}}$ is the maximal increased in $1,25(\text{OH})_2\text{D}_3$ synthesis, $k_{\text{deg}}^{1,25(\text{OH})_2\text{D}_3}$ is the baseline degradation rate

of $1,25(\text{OH})_2\text{D}_3$, and

$$\eta_{\text{PTHp}} = \frac{([\text{PTH}]_p)^{n_{\text{conv}}}}{([\text{PTH}]_p)^{n_{\text{conv}}} + \left(K_{\text{conv}}^{\text{PTHp}}\right)^{n_{\text{conv}}}} \quad (5.5)$$

$$\eta_{\text{CaSR}} = \frac{1}{1 + \gamma_{\text{conv}}^{\text{Ca}} [\text{Ca}^{2+}]_p} \quad (5.6)$$

$$\eta_{1,25(\text{OH})_2\text{D}_3} = \frac{1}{1 + \gamma_{\text{conv}}^{1,25(\text{OH})_2\text{D}_3} [1, 25(\text{OH})_2\text{D}_3]_p} \quad (5.7)$$

represent the inhibition of $[\text{PTH}]_p$, $[\text{Ca}^{2+}]_p$, and $[1,25(\text{OH})_2\text{D}_3]_p$ on the synthesis of vitamin D into $1,25(\text{OH})_2\text{D}_3$, respectively, and

$$\eta_{\text{deg}} = \frac{1}{1 + \gamma_{\text{deg}}^{\text{PTHp}} [\text{PTH}]_p} \quad (5.8)$$

represents the inhibition of $1,25(\text{OH})_2\text{D}_3$ degradation by $[\text{PTH}]_p$.

Calcium

Plasma calcium concentration is modeled by

$$\frac{d[\text{Ca}^{2+}]_p}{dt} = \frac{(1 - \kappa_b)}{V_p} (\Gamma_{\text{abs}} + \Gamma_{\text{res}} + \Gamma_{\text{f-p}} - \Gamma_{\text{p-f}} - \Gamma_{\text{u}}) \quad (5.9)$$

where κ_b denotes that fraction of calcium bound to proteins. Net intestinal calcium absorption is denoted by Γ_{abs} and is given by

$$\Gamma_{\text{abs}} = I_{\text{Ca}} \left(\Gamma_{\text{abs0}} + \delta_{\text{abs}}^{1,25(\text{OH})_2\text{D}_3} \times \rho_{1,25(\text{OH})_2\text{D}_3} \right) \quad (5.10)$$

where I_{Ca} is oral calcium intake, Γ_{abs0} is the baseline fractional intestinal absorption, $\delta_{\text{abs}}^{1,25(\text{OH})_2\text{D}_3}$ is the maximal stimulation of intestinal absorption by $1,25(\text{OH})_2\text{D}_3$, and the impact of $[1,25(\text{OH})_2\text{D}_3]_p$ is given by

$$\rho_{1,25(\text{OH})_2\text{D}_3} = \frac{[1, 25(\text{OH})_2\text{D}_3]_p^2}{[1, 25(\text{OH})_2\text{D}_3]_p^2 + \left(K_{\text{abs}}^{1,25(\text{OH})_2\text{D}_3}\right)^2} \quad (5.11)$$

where $K_{\text{abs}}^{1,25(\text{OH})_2\text{D}_3}$ is the binding affinity for $1,25(\text{OH})_2\text{D}_3$ to the vitamin D receptor [78].

Bone resorption is denoted by Γ_{res} and is given by

$$\Gamma_{\text{res}} = \Gamma_{\text{res}}^{\min} + \delta_{\text{res}}^{\max} \left(\frac{\gamma_{\text{res}}^{\text{PTH}_p} \times [\text{PTH}]_p^2}{[\text{PTH}]_p^2 + \left(K_{\text{res}}^{\text{PTH}_p}\right)^2} + \frac{\gamma_{\text{res}}^{1,25(\text{OH})_2\text{D}_3} \times [1, 25(\text{OH})_2\text{D}_3]_p^2}{[1, 25(\text{OH})_2\text{D}_3]_p^2 + \left(K_{\text{res}}^{1,25(\text{OH})_2\text{D}_3}\right)^2} \right)$$

where $\Gamma_{\text{res}}^{\min}$ is baseline bone resorption, $\delta_{\text{res}}^{\max}$ denotes maximal stimulation of resorption with the fractional impact of $[\text{PTH}]_p$ and $[1,25(\text{OH})_2\text{D}_3]_p$ determined by $\gamma_{\text{res}}^{\text{PTH}_p}$ and $\gamma_{\text{res}}^{1,25(\text{OH})_2\text{D}_3}$. $K_{\text{res}}^{\text{PTH}_p}$ and $K_{\text{res}}^{1,25(\text{OH})_2\text{D}_3}$ are the respective binding affinities for PTH and $1,25(\text{OH})_2\text{D}_3$. Exchange of calcium from the bone fast pool to the plasma and vice versa are denoted by $\Gamma_{\text{f-p}}$ and $\Gamma_{\text{p-f}}$, respectively, where

$$\Gamma_{\text{p-f}} = k_{\text{p-f}}^{\text{Ca}} [\text{Ca}^{2+}]_p V_p \quad (5.12)$$

where $k_{\text{p-f}}^{\text{Ca}}$ is a parameter that determines the rate of calcium uptake by the fast pool in the bone, and

$$\Gamma_{\text{f-p}} = k_{\text{f-p}}^{\text{Ca}} N_{\text{Ca}_f} \quad (5.13)$$

where $k_{\text{f-p}}^{\text{Ca}}$ is a parameter that determines the rate a calcium exchange and N_{Ca_f} is the amount of calcium in the rapidly exchangeable pool given by

$$\frac{dN_{\text{Ca}_f}}{dt} = \Gamma_{\text{p-f}} - \Gamma_{\text{f-p}} - \Gamma_{\text{ac}} N_{\text{Ca}_f} \quad (5.14)$$

with Γ_{ac} denoting the rate of accretion into the slow bone pool.

Last, the renal compartment is divided into calcium reabsorption in the PT, thick ascending limb (TAL) and distal tubule (DT). Total fractional reabsorption of calcium along the full nephron is given by

$$\lambda_{\text{reab}} = \lambda_{\text{PT}} + \lambda_{\text{TAL}} + \lambda_{\text{DT}} \quad (5.15)$$

where the details for reabsorption in the individual segments λ_{PT} , λ_{TAL} , λ_{DT} are impacted by PTH, CaSR, $1,25(\text{OH})_2\text{D}_3$ as detailed in Ref. [78]. Finally, urinary excretion of calcium, Γ_{u} , is given by

$$\Gamma_{\text{u}} = \Phi_{\text{GFR}} \times [\text{Ca}^{2+}]_p \times (1 - \lambda_{\text{reab}}) \quad (5.16)$$

where Φ_{GFR} denotes the GFR.

5.2.2 Modeling sex differences in calcium homeostasis

To develop sex-specific models of calcium homeostasis, we incorporated known sex differences, including those describing plasma composition, intestinal absorption, and renal transport. The parameter changes made are listed in Table 5.1 and are described below. All other parameters are the same for the male and female models.

Table 5.1: Female-to-male (F-to-M) indicates the ratio between the value for the female model parameter to the male model parameter value. Parameters not listed are the same for both the male and female models.

Parameter	Units	F-to-M Ratio	Description
V_p	mL	0.65	plasma volume
Φ_{GFR}	mL/min	0.70	glomerular filtration rate
$\gamma_{\text{conv}}^{1,25(\text{OH})_2\text{D}_3}$	(pmol/L) ⁻¹	2.11	inhibition of 1,25(OH) ₂ D ₃ on its own production
$\delta_{\text{max}}^{\text{conv}}$	min ⁻¹	0.58	maximal increase in 1,25(OH) ₂ D ₃ production rate
$k_{\text{min}}^{\text{conv}}$	min ⁻¹	0.57	minimum production rate constant for 1,25(OH) ₂ D ₃
$\gamma_{\text{prod}}^{1,25(\text{OH})_2\text{D}_3}$	(pmol/L) ⁻¹	1.80	inhibition of PTH _g synthesis by 1,25(OH) ₂ D ₃
I_{Ca}	μmol/min	0.80	calcium intake
Γ_{abs0}	%	1.20	basal fractional absorption
$\delta_{\text{abs}}^{1,25(\text{OH})_2\text{D}_3}$	%	1.22	maximal effect of absorption by [1,25(OH) ₂ D ₃] _p
$K_{\text{abs}}^{1,25(\text{OH})_2\text{D}_3}$	pmol/L	0.53	stimulation of absorption by [1,25(OH) ₂ D ₃] _p
$K_{\text{res}}^{1,25(\text{OH})_2\text{D}_3}$	pmol/L	0.53	stimulation of bone resorption by [1,25(OH) ₂ D ₃] _p
Λ_{PT}^0	%	0.90	minimal PT fractional reabsorption
$\delta_{\text{PT}}^{\text{max}}$	%	0.90	maximal increase in PT fractional reabsorption
Λ_{TAL}^0	%	1.14	minimal TAL fractional reabsorption
$\delta_{\text{TAL}}^{\text{max}}$	%	1.67	maximal increase in TAL fractional reabsorption
Λ_{DT}^0	%	1.34	minimal DT fractional reabsorption
$\delta_{\text{DT}}^{\text{max}}$	%	1.20	maximal increase in DT fractional reabsorption
$K_{\text{DT}}^{1,25(\text{OH})_2\text{D}_3}$	pmol/L	0.53	stimulation of DT reabsorption by [1,25(OH) ₂ D ₃] _p

Plasma concentrations

Female rats are on average smaller than male rats and have a smaller plasma volume [129]. Additionally, female rats in general consume less than male rats. We reduced the parameters V_p and I_{Ca} in the female model to represent the difference in plasma volume and calcium intake, respectively (Table 5.1).

There are no significant differences in $[\text{PTH}]_p$ and $[\text{Ca}^{2+}]_p$ between male and female rats [158]. However, $[1,25(\text{OH})_2\text{D}_3]_p$ levels in females are about 50-60% that of male levels

despite similar inactive vitamin D concentrations [158]. To capture this difference, we decreased synthesis of $1,25(\text{OH})_2\text{D}_3$ by reducing the parameters $k_{\text{conv}}^{\min}$ and $\delta_{\text{conv}}^{\max}$ to determine the minimum and maximal synthesis of $1,25(\text{OH})_2\text{D}_3$ (Eq. 5.4). Additionally we scaled $\gamma_{\text{conv}}^{1,25(\text{OH})_2\text{D}_3}$ so that at baseline steady-state $[1,25(\text{OH})_2\text{D}_3]_{\text{p}}$, the value of $\eta_{1,25(\text{OH})_2\text{D}_3}$ is the same as the male value at male baseline $[1,25(\text{OH})_2\text{D}_3]_{\text{p}}$ (Eq. 5.7). Additionally, since $1,25(\text{OH})_2\text{D}_3$ impacts PTH synthesis in the parathyroid gland, we scaled $\gamma_{\text{prod}}^{1,25(\text{OH})_2\text{D}_3}$ so that at the female baseline steady-state $[1,25(\text{OH})_2\text{D}_3]_{\text{p}}$ levels there is the same percent inhibition in the female model as in the male model (Eq. 5.1). Similarly, $K_{\text{abs}}^{1,25(\text{OH})_2\text{D}_3}$, $K_{\text{res}}^{1,25(\text{OH})_2\text{D}_3}$, $K_{\text{DT}}^{1,25(\text{OH})_2\text{D}_3}$, the binding affinity constants for $[1,25(\text{OH})_2\text{D}_3]_{\text{p}}$ on absorption, resorption, and distal tubule reabsorption, respectively, were scaled to account for the female baseline $[1,25(\text{OH})_2\text{D}_3]_{\text{p}}$ (Table 5.1).

Intestinal calcium absorption

Despite lower $[1,25(\text{OH})_2\text{D}_3]_{\text{p}}$, female mice have been shown to have a higher baseline fractional intestinal absorption than male mice [205]. While analogous studies have not been found in rats, we assume that the same sex difference applies. Accordingly, we increased $\Gamma_{\text{abs}0}$ and $\delta_{\text{abs}}^{1,25(\text{OH})_2\text{D}_3}$ in the female model so that at baseline, fractional absorption of calcium is 22% higher in female rats than male rats (see Table 5.1; Eq. 5.10). Note that because of their lower calcium intake, a higher fractional absorption does not necessarily yield a higher net calcium absorption in females.

Renal calcium handling

Female rats have a lower GFR than males [98, 154, 181]. Given similar $[\text{Ca}^{2+}]_{\text{p}}$, less calcium is being filtered to the nephrons of the kidneys of female rats. Furthermore, sex difference in renal electrolyte handling along the nephrons in male and females differ [97, 143, 225]. Specifically, females have a lower fractional sodium reabsorption in the proximal tubules, driven by altered sodium transporter activity as well as shorter proximal tubules relative to the whole kidney [98, 133, 143, 225]. Since calcium transport in the proximal tubules is tightly regulated to sodium proximal tubule transport, a similar shift to the distal segments in renal calcium handling is likely. Additionally, sex steroids have been shown to impact the calcium transporters [112]. Specifically, females have a higher expression of calcium transporter proteins when compared to males [97]. In the end, urinary excretion of calcium is lower in females than when compared to males [97]. We set renal parameters based on a recent modeling analysis of sex-specific calcium transport [85] so that (i) baseline proximal

tubule fractional reabsorption in the female model is about 90% of male and (ii) baseline thick ascending limb and distal tubule fractional reabsorption is higher in female so that total nephron fractional reabsorption (Eq. 5.15) is the same for both sexes (see Table 5.1).

5.2.3 Modeling calcium homeostasis in pregnancy and lactation

We developed models for pregnant and lactating rats based on known adaptations that occur in these unique physiological states. The female rat model, described in Section 5.2.2, is used as a virgin control. It is important to note that both pregnancy and lactation are dynamic processes that adapt to the changing needs of the growing fetus or neonate. The pregnancy model represents a late pregnant rat (about 19-21 days gestation). The lactation model represents rats in mid-lactation (about 12 days post-parturition). This choice was based on the most data available. Certainly, similar models may be developed to capture the various stages of lactation and pregnancy. The parameter changes made for the pregnant and lactating rat models are listed in Table 5.2 and described in the following sections. Parameters not listed in Table 5.2 are the same as the virgin control (i.e., female) model.

Fetal and milk calcium delivery

To represent fetal calcium delivery in pregnancy or milk delivery in lactation, we add a sink term, Γ_x , to the conservation equation for maternal calcium (Eq. 5.9)

$$\frac{d[Ca^{2+}]_p}{dt} = \frac{(1 - \kappa_b)}{V_p} (\Gamma_{\text{abs}} + \Gamma_{\text{res}} + \Gamma_{\text{f-p}} - \Gamma_{\text{p-f}} - \Gamma_{\text{u}} - \Gamma_x). \quad (5.17)$$

To simulate pregnancy, we set $\Gamma_x = \Gamma_{\text{Fetus}}$ based on these observations. In the last four days of gestation, about 90% of the total fetal calcium is transported into the fetus in rats [43, 118]. Just after birth, each pup has about 20 mg of calcium [27]. This means that in the last day of gestation about 5 mg of calcium is delivered per pup. Normal litter size is 6-12 pups [118]. Based on this we have that the estimated range for Γ_{Fetus} is 0.26-0.52 $\mu\text{mol}/\text{min}$. We chose average for about 9 pups so that $\Gamma_{\text{Fetus}} = 0.39 \mu\text{mol}/\text{min}$.

During lactation, approximately 125 mg of calcium is delivered to the milk per day [27]. Thus, we set $\Gamma_x = \Gamma_{\text{Milk}} = 1.08 \mu\text{mol}/\text{min}$ to simulate lactation.

Table 5.2: Pregnant-to-virgin (P-to-V) and lactation-to-virgin (L-to-V) parameter values ratios. Parameters not listed are the same as the baseline virgin (female) model.

Parameter	P-to-V	L-to-V	Description
V_p	1.69	1.35	plasma volume
Γ_{res}^{min}	1.00	1.20	Minimum resorption rate
δ_{res}^{max}	1.00	1.20	Maximum resorption increase
Φ_{GFR}	1.20	1.05	glomerular filtration rate
$\gamma_{conv}^{1,25(OH)_2D_3}$	0.29	0.28	inhibition of $1,25(OH)_2D_3$ production by itself
δ_{conv}^{max}	4.14	5.56	maximal increase in $1,25(OH)_2D_3$ production rate
k_{conv}^{min}	4.14	5.69	minimum production rate of $1,25(OH)_2D_3$
$[D_3]_p^{inact}$	0.60	0.60	inactive vitamin D concentration
$\gamma_{prod}^{1,25(OH)_2D_3}$	0.33	0.29	inhibition of PTH synthesis in gland by $[1,25(OH)_2D_3]_p$
I_{Ca}	1.05	1.20	calcium intake
Γ_{abs0}	1.83	2.25	minimum fractional absorption
$\delta_{abs}^{1,25(OH)_2D_3}$	0.77	0.59	maximum $[1,25(OH)_2D_3]_p$ regulated gut absorption
$K_{abs}^{1,25(OH)_2D_3}$	3.12	2.82	stimulation of absorption by $[1,25(OH)_2D_3]_p$
$K_{res}^{1,25(OH)_2D_3}$	3.12	3.35	stimulation of resorption by $[1,25(OH)_2D_3]_p$
$K_{DCT}^{1,25(OH)_2D_3}$	3.12	3.41	stimulation of DCT reabsorption by $[1,25(OH)_2D_3]_p$
K_{Ca}^{CaSR}	1.07	1.07	binding of Ca^{2+} to CaSR
K_{conv}^{PTH}	2.00	2.00	activation of $1,25(OH)_2D_3$ production by PTH
$k_{prod}^{PTH_g}$	1.30	1.30	production of PTH in gland
$K_{res}^{PTH_p}$	4.08	1.08	stimulation of bone resorption by PTH
$\gamma_{deg}^{PTH_p}$	0.55	0.55	inhibition of $1,25(OH)_2D_3$ synthesis by PTH
PTH_p^{ref}	1.67	1.67	reference PTH value for PT reabsorption
$K_{TAL}^{PTH_p}$	2.00	1.25	reference PTH value for TAL reabsorption
$K_{DT}^{PTH_p}$	2.00	1.38	reference PTH value for DT reabsorption
$\beta_{exo}^{PTH_g}$	1.65	1.65	maximal PTH exocytosis
$\gamma_{exo}^{PTH_g}$	1.67	1.67	maximal CaSR inhibition of PTH exocytosis

Plasma volume

During pregnancy, plasma volume markedly expands, to 70% higher than virgin values by late pregnancy in rats [12, 235]. This volume decreases post-parturition but remains elevated from pre-pregnancy values [23]. To capture this change we increased the parameter representing plasma volume, V_p , by 69% and 35% in the pregnant and lactation models, respectively (Table 5.2).

Parathyroid hormone

PTH concentrations are about double virgin levels in both pregnancy and lactation [25, 193]. Additionally, Schultz et al. [193] hypothesized an altered set point for PTH exocytosis from the parathyroid glands. To capture this in the model we increased the parameter for inhibition of PTH secretion by $[Ca^{2+}]_p$, K_{Ca}^{CaSR} , to a higher set point. Additionally, to increase $[PTH]_p$ levels, parameters β_{exo}^{PTHg} and γ_{exo}^{PTHg} were increased and decreased, respectively. PTH degradation has been shown to be the same in lactating and virgin rats so k_{deg}^{PTHp} was kept the same for all models [70]. Parameters involved in feedback from PTH signalling that depend on $[PTH]_p$ were chosen so that the setpoint for corresponding models was adjusted to the new baseline $[PTH]_p$ (i.e., K_{conv}^{PTH} , K_{res}^{PTH} , γ_{deg}^{PTH} , K_{TAL}^{PTH} , K_{DT}^{PTH} , PTH_p^{ref} ; Table 5.2).

Calcitriol

Inactive vitamin D ($[D_3]_p^{inact}$) is decreased by about the same amount from virgin values during pregnancy and lactation [87]. As such we decreased the corresponding parameter by 40% from the virgin value in both models. This decrease in inactive vitamin D levels may be caused by increased synthesis of $1,25(OH)_2D_3$ because $[1,25(OH)_2D_3]_p$ is greatly increased in both pregnancy and lactation from virgin values [87]. Indeed, lactation $[1,25(OH)_2D_3]_p$ levels are even higher than pregnancy [87]. It has been observed that increased 1α -hydroxylase activity occurs in pregnancy and lactation [118, 119, 153]. To model this, we increased parameters k_{conv}^{min} and δ_{conv}^{max} to increase synthesis of $1,25(OH)_2D_3$ in the pregnancy and lactation models. Additionally, parameters that depend on $[1,25(OH)_2D_3]_p$ (i.e., $K_{res}^{1,25(OH)_2D_3}$, $K_{DCT}^{1,25(OH)_2D_3}$, $\gamma_{conv}^{1,25(OH)_2D_3}$, $\gamma_{prod}^{1,25(OH)_2D_3}$) were adjusted to the new baseline $[1,25(OH)_2D_3]_p$ (Table 5.2).

Intestinal absorption

Although $[1,25(\text{OH})_2\text{D}_3]_p$ is increased in pregnancy and lactation, its increase does not fully account for the increases in intestinal absorption observed in both pregnancy and lactation [38]. In fact, pregnancy-induced increases in fractional calcium absorption persist even under severe vitamin D-deficiency in rats [28, 88, 118]. To account for the enhanced intestinal calcium absorption in both pregnancy and lactation, we increased baseline fractional intestinal absorption $\Gamma_{\text{abs}0}$ in these models. Additionally, we had to decrease the intestinal absorption impact of calcitriol ($\delta_{\text{abs}}^{1,25(\text{OH})_2\text{D}_3}$) to ensure that fractional intestinal absorption could not be higher than 1.0. Net intestinal absorption is also impacted directly by calcium intake. During both pregnancy and lactation, calcium intake is increased from pre-pregnancy values [27, 132]. Notably this is higher in lactation than in pregnancy [132]. As such, I_{Ca} was increased to account for this difference (Table 5.2).

Bone remodeling

Lactation is a state of rapid bone loss via enhanced resorption [68, 118, 189]. To capture this in the model we increased the parameters representing calcium resorption, $\Gamma_{\text{res}}^{\text{min}}$ and $\delta_{\text{res}}^{\text{max}}$ (Table 5.2). During pregnancy, there is inconclusive evidence about how bone remodeling may change [118]. For this reason we did not change bone-specific parameters in the pregnancy model.

Kidney function

GFR during pregnancy and lactation is increased from virgin values [11, 12]. To capture this, we increased the GFR parameter by 20% and 5% in the late pregnant and lactation models, respectively.

5.2.4 Numerical simulations

Model equations were programmed on MATLAB software. The model steady-state was solved using `fsolve` (algorithm: trust-region-dogleg), which finds the root of a system of nonlinear equations. Model code and simulation data is publicly available at https://github.com/Layton-Lab/CaReg_FemPregLact.

5.3 Results

5.3.1 Baseline steady-state results

The steady-state $[\text{PTH}]_p$, $[\text{Ca}^{2+}]_p$, and $[1,25(\text{OH})_2\text{D}_3]_p$ for the male, female (virgin), late pregnancy, and lactation models are shown in Fig. 5.3. $[\text{PTH}]_p$ is about the same in males and virgin females, but doubles during pregnancy and lactation (Fig. 5.3a). $[\text{Ca}^{2+}]_p$ is approximately the same between males and females, and remains steady during pregnancy and lactation (Fig. 5.3b). The most notable difference among the four models is in $[1,25(\text{OH})_2\text{D}_3]_p$. Steady-state female rat model $[1,25(\text{OH})_2\text{D}_3]_p$ is about 55% of male value, whereas the pregnancy and lactation models exhibit a 3.2- and 3.9-fold increase, respectively, when compared to the virgin value (Fig. 5.3c). These concentrations are within reported ranges [25,87,193]. The functional implications of these different plasma hormone levels are analyzed below.

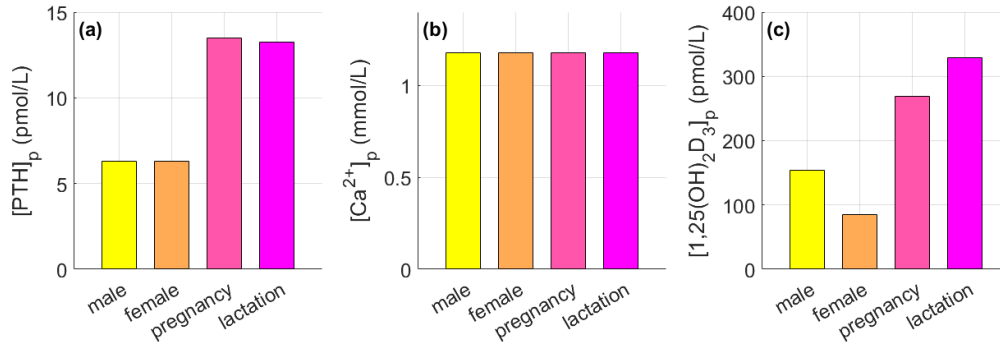


Figure 5.3: Steady-state plasma concentrations of (a) parathyroid hormone, (b) calcium, and (c) $1,25(\text{OH})_2\text{D}_3$ for male, virgin female, pregnancy, and lactation models. $[\text{Ca}^{2+}]_p$: plasma calcium concentration; $[\text{PTH}]_p$: plasma parathyroid hormone concentration; $[1,25(\text{OH})_2\text{D}_3]_p$: plasma calcitriol concentration.

Steady-state calcium fluxes (see Eq. 5.17) for the male, female, pregnancy, and lactation models are shown in Fig. 5.4. Note that Fig. 5.4a-b are fluxes that go into the plasma and Fig. 5.4c-e are calcium fluxes that go out of the plasma (see Eq. 5.17). Between the male and (virgin) female models, there is little difference between the calcium fluxes other than urinary excretion (Fig. 5.4c), which is lower in females, consistent with Ref. [97,158]. Despite lower calcium intake, fractional intestinal absorption of calcium is higher in females (see Table 5.1), which together results in a similar net intestinal absorption as males (Fig. 5.4a).

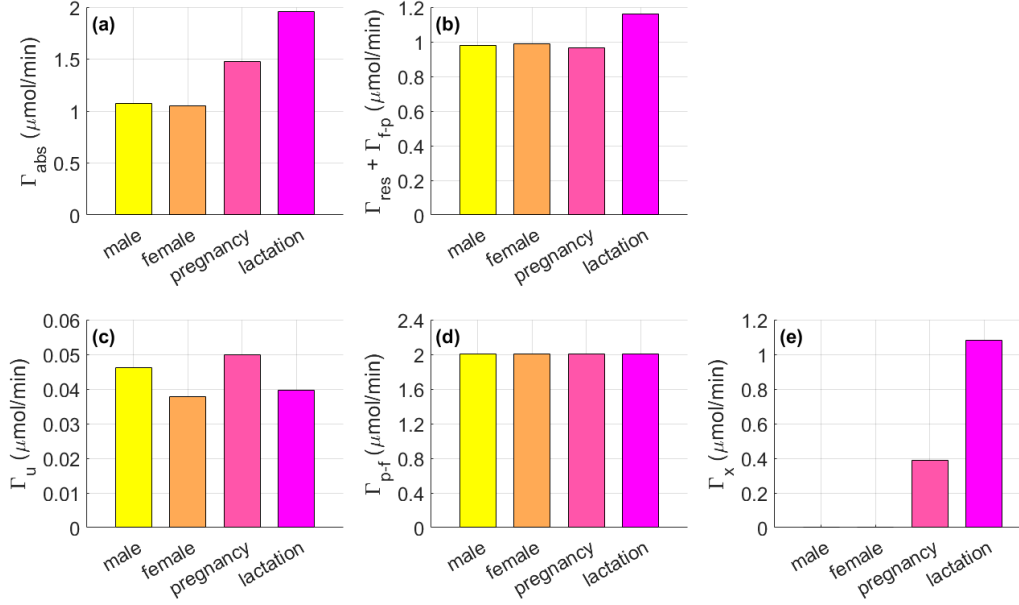


Figure 5.4: Steady-state calcium fluxes of (a) intestinal absorption, (b) bone into plasma, (c) urine excretion, (d) plasma to bone, and (e) fetus or milk flux for male, female, pregnancy, and lactation models. Γ_{abs} : intestinal absorption; Γ_{res} : bone resorption; $\Gamma_{\text{f-p}}$: bone fast pool to plasma flux; Γ_{u} : urinary excretion; $\Gamma_{\text{p-f}}$: plasma to bone fast pool flux; Γ_{x} : fetus delivery (Γ_{Fetus} ; pregnancy) or milk delivery (Γ_{Milk} ; lactation).

Major adaptations must occur during pregnancy to support the excess calcium being delivered to the fetus as well as calcium needs of an expanded plasma volume. To enable a steady maternal $[\text{Ca}^{2+}]_{\text{p}}$ level, there is a 34% increase in fractional intestinal absorption. Combined with increased calcium intake, net intestinal absorption increases by 40% from virgin values (Fig. 5.4a). Together these fluxes balance with the calcium loss to the fetoplacental unit and increased urinary excretion to maintain a stable plasma calcium concentration (Fig. 5.3b).

Similarly, during lactation, major adaptations must occur to support the substantial amount of calcium being delivered to milk. To achieve that, increased intestinal absorption from virgin levels along with increased calcium intake results in net intestinal absorption that is 85% above virgin values (Fig. 5.4a). Bone turnover is altered so that the flux of calcium from the bones to plasma increased by 18% from virgin and pregnancy values (Fig. 5.4b). Urinary excretion during lactation returns to near virgin levels (Fig. 5.4c).

Together these changes support the massive demands of milk calcium along with a stable $[\text{Ca}^{2+}]_p$ despite increased plasma volume (Fig. 5.3b).

5.3.2 Local sensitivity analysis

We conducted a local sensitivity analysis for the male, female, pregnancy, and lactation models by increasing individual parameters by 5% and computing the model steady-state with that individual parameter perturbation. The percent change in $[\text{PTH}]_p$, $[\text{Ca}^{2+}]_p$, and $[1,25(\text{OH})_2\text{D}_3]_p$ for the each of the models is shown in Fig. 5.5. A similar analysis with a 5% decrease in individual parameters is also given in Appendix D (Fig. D.1).

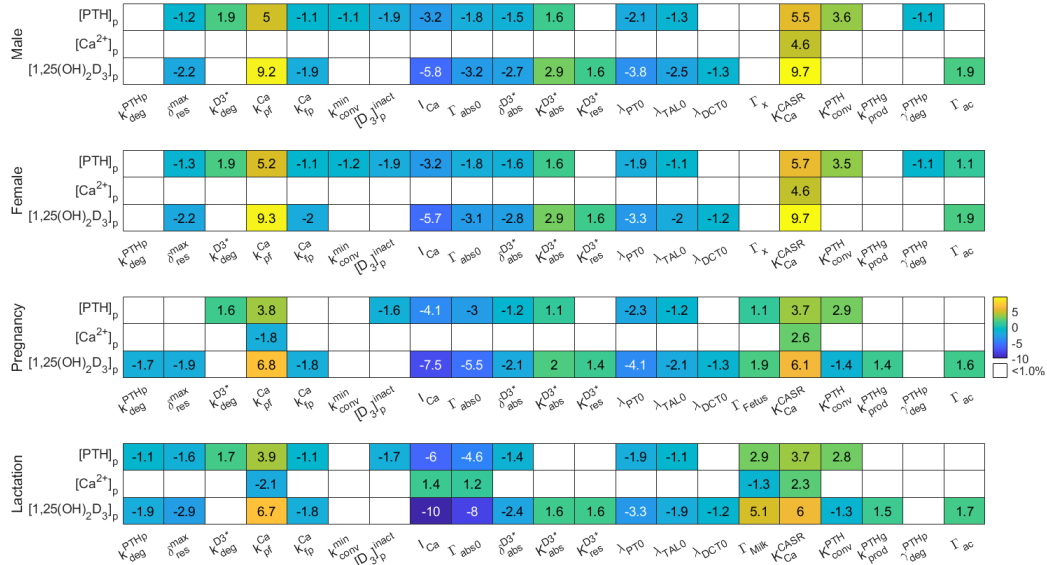


Figure 5.5: Local sensitivity analysis for the male, female, pregnancy, and lactation models. Sensitivity analysis was conducted by increasing individual parameters by 5%. The resulting percent change in model steady-state concentrations from baseline is presented here. PTH: parathyroid hormone; D_3^* : $1,25(\text{OH})_2\text{D}_3$ (calcitriol)

It is worth noting that for every perturbed parameter in this analysis, there is no resulting change in $[\text{Ca}^{2+}]_p$ that exceeds 5%. Considering that baseline $[\text{Ca}^{2+}]_p = 1.18$ mmol/L in all four models, a 5% increase would be around 1.24 mmol/L, while a 5%

decrease would be 1.12 mmol/L. These values still fall within the normal range for $[\text{Ca}^{2+}]_p$ (1.1-1.3 mmol/L), indicating that these individual parameter perturbations do not lead to hypercalcemia or hypocalcemia. However, perturbations in individual parameters do have a comparatively greater impact on $[\text{PTH}]_p$ and $[1,25(\text{OH})_2\text{D}_3]_p$ levels across all four models.

The male and female models exhibit a similar response to perturbations in individual parameters (Fig. 5.5). Both the male and female model are most sensitive to the parameter $K_{\text{Ca}}^{\text{CaSR}}$, which determines the inhibition of PTH exocytosis from the parathyroid gland by the CaSR (see Eq. 5.2). Increasing this parameter results in a higher set point for PTH exocytosis inhibition, resulting in more PTH secretion into the bloodstream. $[1,25(\text{OH})_2\text{D}_3]_p$ levels also rise due to reduced degradation and increased synthesis from higher $[\text{PTH}]_p$ levels (see Eq. 5.5 and 5.8). Together these changes result in higher $[\text{Ca}^{2+}]_p$ by stimulating intestinal absorption, renal reabsorption, and bone resorption. The pregnancy and lactation models are less sensitive to a change in $K_{\text{Ca}}^{\text{CaSR}}$ because of their higher baseline $[\text{PTH}]_p$ and $[1,25(\text{OH})_2\text{D}_3]_p$ levels as well as a higher baseline value for $K_{\text{Ca}}^{\text{CaSR}}$ than the (virgin) female model (Fig. 5.3; Table 5.2).

In each of the four models, $[1,25(\text{OH})_2\text{D}_3]_p$ exhibits the highest sensitivity to parameter changes. In the lactation model, $[1,25(\text{OH})_2\text{D}_3]_p$ is most sensitive to changes that directly impact $[\text{Ca}^{2+}]_p$. For instance, increasing the delivery of calcium to the milk, Γ_{Milk} , results in a direct change in calcium dynamics by removing more calcium from the plasma compartment (see Eq. 5.17). However, as a compensatory response, $[1,25(\text{OH})_2\text{D}_3]_p$ levels rise, resulting in increased intestinal absorption while $[\text{PTH}]_p$ levels increase, primarily enhancing bone resorption and renal reabsorption. Similarly, changes to the intestinal absorption parameters, calcium intake (I_{Ca}), and baseline fractional intestinal absorption (Γ_{abs0}), would typically result in a substantial increase in $[\text{Ca}^{2+}]_p$. However, $[1,25(\text{OH})_2\text{D}_3]_p$ levels adapt by decreasing their levels, reducing intestinal absorption to limit the amount of calcium entering the bloodstream, thereby maintaining $[\text{Ca}^{2+}]_p$ levels within normal range (Fig. 5.5).

Together, individual changes to the parameters in each of the models leads to larger fractional changes in $[\text{PTH}]_p$ and $[1,25(\text{OH})_2\text{D}_3]_p$ levels than $[\text{Ca}^{2+}]_p$. Recall that calcium homeostasis is achieved via the balance of fluxes between the bones, intestines, and kidneys which are regulated by calcitropic hormones, primarily PTH and $1,25(\text{OH})_2\text{D}_3$. While $[\text{Ca}^{2+}]_p$ must remain within a tight normal range (1.1-1.3 mmol/L; up to 18% increase), normal range for $[\text{PTH}]_p$ (1.5-13 pmol/L; up to 8.6-fold change) and $[1,25(\text{OH})_2\text{D}_3]_p$ (80-250 pmol/L; up to 3.13-fold change) can vary much more, thereby being able to change by a larger relative amount without having a dangerous impact on the body. By having calcitropic hormones that can respond to small perturbations in $[\text{Ca}^{2+}]_p$, calcium homeostasis

is maintained.

5.3.3 Impact of individual sex differences

Given the physiological differences between male and (virgin) female rats, including the substantially lower $[1,25(\text{OH})_2\text{D}_3]_p$, plasma volume, GFR, and calcium intake in females, sex differences are incorporated in a number of model parameters that characterize calcium handling in the intestines, kidneys, and bones (see Table 5.1). Together, these changes yield $[\text{Ca}^{2+}]_p$ and $[\text{PTH}]_p$ levels that are similar in the two sexes, and $[1,25(\text{OH})_2\text{D}_3]_p$ that is lower in females (Fig. 5.3). How important is each of these parameters in attaining the sex-specific hormones levels? To answer these questions, we set individual sex-specific parameter values in the male and female models to the respective value of the opposite sex, and computed the resulting steady-state. The results for the percent change in steady-state $[\text{PTH}]_p$, $[\text{Ca}^{2+}]_p$, and $[1,25(\text{OH})_2\text{D}_3]_p$ for each individual parameter change are shown in Fig. 5.6.

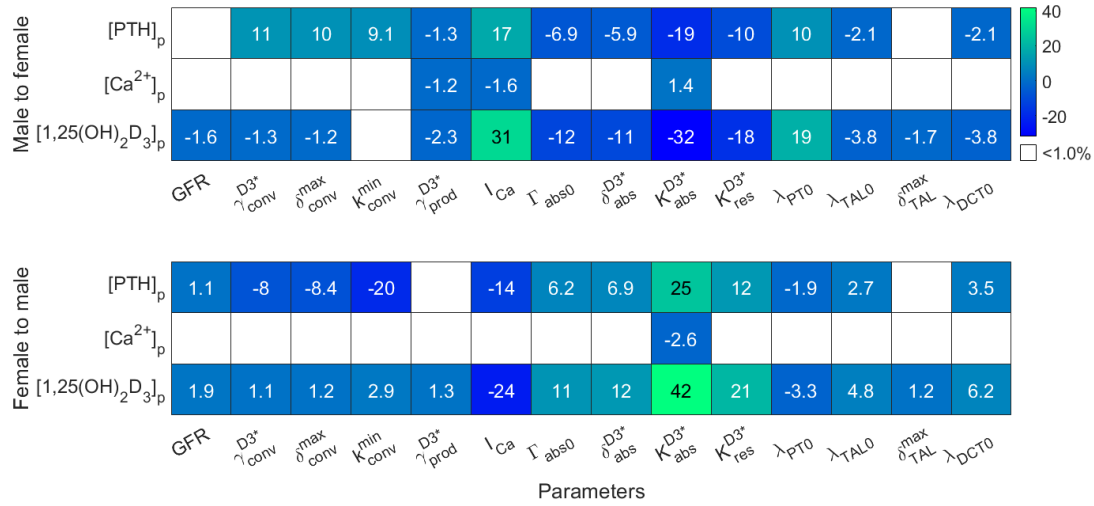


Figure 5.6: Impact of individual sex-specific parameters on plasma parathyroid hormone, calcium, and calcitriol concentrations in the male model (*top*) and female model (*bottom*). Reported values are percent changes in predicted variables (y-axis) from baseline values when the associated individual parameter values (x-axis) were changed to the corresponding values of the opposite sex. PTH: parathyroid hormone; D_3^* : $1,25(\text{OH})_2\text{D}_3$ (calcitriol). Model parameters are defined in Table 5.1.

The impact of male-to-female and female-to-male individual parameters are in the opposite direction for the two models, as expected. Note that no parameter change results in more than 3% change in $[\text{Ca}^{2+}]_p$ so that $[\text{Ca}^{2+}]_p$ still remains within normal range even when the parameters are perturbed from their baseline value. In contrast, impacts on $[\text{PTH}]_p$ and $[1,25(\text{OH})_2\text{D}_3]_p$ levels can be substantial (Fig. 5.6).

When decreasing calcium intake (I_{Ca}) in the male model by 20% to the female value, $1,25(\text{OH})_2\text{D}_3$ levels are predicted to increase by about 31%, which subsequently increases $1,25(\text{OH})_2\text{D}_3$ regulated intestinal absorption. Despite this increase, model steady-state net flux of intestinal absorption (Γ_{abs}) is about 9.7% lower than the baseline male flux due to the substantially lowered I_{Ca} (recall that net intestinal flux is a product of calcium intake with fractional intestinal absorption; see Eq. 5.10), resulting in a 1.6% decrease in $[\text{Ca}^{2+}]_p$.

The impact of $1,25(\text{OH})_2\text{D}_3$ on intestinal absorption is modeled using a Hill function with the parameter $K_{\text{abs}}^{1,25(\text{OH})_2\text{D}_3}$, which characterizes the stimulation of intestinal absorption by $[1,25(\text{OH})_2\text{D}_3]_p$ (see Eq. 5.11). In the male model, $K_{\text{abs}}^{1,25(\text{OH})_2\text{D}_3} = 160$ pmol/L, which is near the baseline value for the male model $[1,25(\text{OH})_2\text{D}_3]_p$ (steady-state male $[1,25(\text{OH})_2\text{D}_3]_p = 154$ pmol/L; Fig. 5.3c). This is so that when $[1,25(\text{OH})_2\text{D}_3]_p$ increases or decreases from its baseline value, stimulation of intestinal absorption by $[1,25(\text{OH})_2\text{D}_3]_p$ will increase or decrease accordingly. When decreasing $K_{\text{abs}}^{1,25(\text{OH})_2\text{D}_3}$ to the female value of 85 pmol/L, in isolation, this would result in a 59% increase in intestinal absorption stimulation by $1,25(\text{OH})_2\text{D}_3$ ($\rho_{1,25(\text{OH})_2\text{D}_3}$; Eq. 5.11). However, at steady-state there is a 32% decrease in $[1,25(\text{OH})_2\text{D}_3]_p$ so that intestinal absorption stimulation by $1,25(\text{OH})_2\text{D}_3$ is 25% higher than normal levels resulting in a net 12% increase in steady-state intestinal absorption flux. Additionally, to counteract the impacts of this enhanced intestinal absorption, $[\text{PTH}]_p$ decreases by 19%. With these adaptations, there is only a 1.4% increase in steady-state $[\text{Ca}^{2+}]_p$, well within normal range. The opposite occurs in the female model analysis. In all, sex differences in $[1,25(\text{OH})_2\text{D}_3]_p$ result in different set points for stimulation of intestinal calcium absorption between males and females.

5.3.4 Impact of individual maternal adaptations

To quantify how individual changes in the maternal body impact calcium homeostasis, we conducted the following analysis: We set individual parameters in the virgin (female) model to the respective pregnancy or lactation model parameter and computed the steady-state for the virgin model with that individual change (see Table 5.2 for pregnancy and lactation model parameter changes). The results for the percent change from virgin baseline $[\text{PTH}]_p$,

$[\text{Ca}^{2+}]_p$, and $[1,25(\text{OH})_2\text{D}_3]_p$ for each individual pregnancy and lactation parameter are shown in Fig. 5.7.

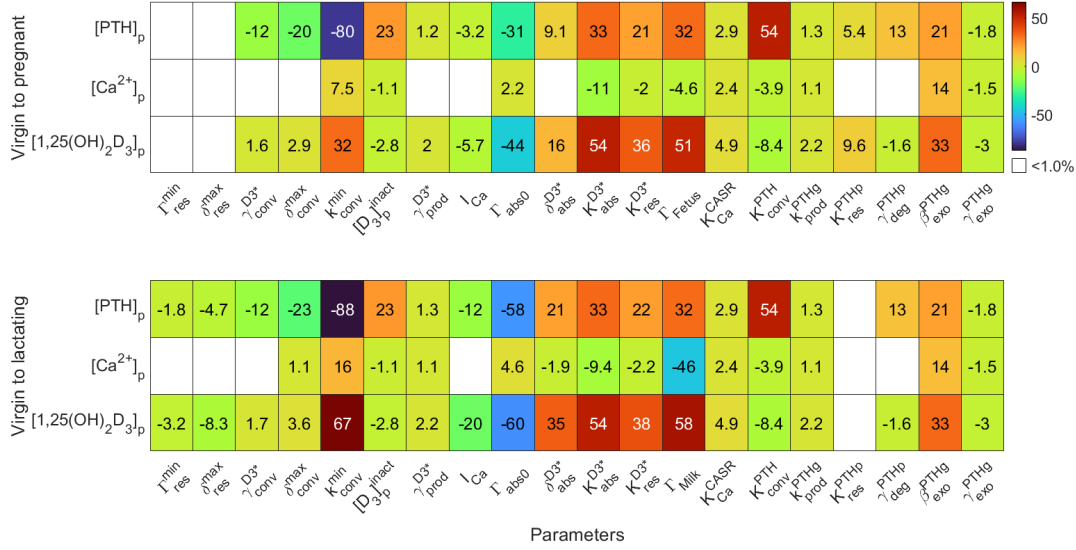


Figure 5.7: Impact of individual pregnancy (*top*) and lactation (*bottom*) parameters on plasma PTH, calcium, and calcitriol concentrations in the virgin rat model. Individual parameters in the virgin (female) rat model were set to the respective pregnancy/lactation parameter value and steady-state results computed. Percent change from baseline concentration is reported here. PTH: parathyroid hormone; D_3^* : $1,25(\text{OH})_2\text{D}_3$ (calcitriol). Model parameters are listed in Table 5.2.

The largest difference in maternal calcium homeostasis from virgin rats is the calcium demand by the fetus in pregnancy (Γ_{Fetus}) and by milk in lactation (Γ_{Milk}). In the virgin rat model, $\Gamma_x = 0 \mu\text{mol}/\text{min}$. When setting Γ_x to the pregnancy value, ($\Gamma_{\text{Fetus}} = 0.39 \mu\text{mol}/\text{min}$), $[\text{PTH}]_p$ and $[1,25(\text{OH})_2\text{D}_3]_p$ are increased to increase calcium flux from intestinal absorption, bones, and renal reabsorption. Notably, steady-state intestinal absorption flux increases by 18.8%, due to the increased $[1,25(\text{OH})_2\text{D}_3]_p$. In the end, $[\text{Ca}^{2+}]_p$ decreases by 4.6%. Since pups are larger than fetuses, milk demand of calcium is higher. When setting Γ_x to the lactation value ($\Gamma_{\text{Milk}} = 1.08 \mu\text{mol}/\text{min}$), $[\text{PTH}]_p$ and $[1,25(\text{OH})_2\text{D}_3]_p$ increases are not sufficient to maintain a normal $[\text{Ca}^{2+}]_p$, resulting in extremely low $[\text{Ca}^{2+}]_p = 0.64 \text{ mmol}/\text{L}$. In summary, additional pregnancy and lactation adaptations are essential for female rats to be able to support the extra calcium needs of a fetus or milk while maintaining a stable $[\text{Ca}^{2+}]_p$.

Both $[\text{PTH}]_p$ and $[1,25(\text{OH})_2\text{D}_3]_p$ levels are increased during pregnancy and lactation. To capture the increased synthesis of $1,25(\text{OH})_2\text{D}_3$ in the pregnancy and lactation models, we increased parameters $k_{\text{conv}}^{\min}$ and $\delta_{\text{conv}}^{\max}$ from virgin values (see Eq. 5.4; Table 5.2). Increasing the virgin model parameter to the lactation values for $k_{\text{conv}}^{\min}$, which represents the minimum production rate of $1,25(\text{OH})_2\text{D}_3$, increases $[1,25(\text{OH})_2\text{D}_3]_p$ by 67% (Fig. 5.7). This stimulates intestinal absorption by 23%. $[\text{PTH}]_p$ decreases massively (88%; Fig. 5.7) due to the inhibition of PTH_g production by high $[1,25(\text{OH})_2\text{D}_3]_p$ levels (Eq. 5.1), feedback from the CaSR resulting from the increase in $[\text{Ca}^{2+}]_p$ (Eq. 5.6), and inhibition of $\text{PTH}_{\text{exocytosis}}$ by high $[1,25(\text{OH})_2\text{D}_3]_p$ (Eq. 5.7). In all, this increase in $1,25(\text{OH})_2\text{D}_3$ production alone results in a 16% increase in steady-state $[\text{Ca}^{2+}]_p$ (Fig. 5.7). Note that the $[1,25(\text{OH})_2\text{D}_3]_p$ resulting from this change is still not nearly as high as the levels found in lactation (see Fig. 5.3c).

We conducted another similar analysis on the pregnancy and lactation models: We set individual parameters in the pregnant and lactation models to the respective virgin model parameter and computed the steady-state with that individual change. The results for the percent change in $[\text{PTH}]_p$, $[\text{Ca}^{2+}]_p$, and $[1,25(\text{OH})_2\text{D}_3]_p$ are given in Fig. 5.8.

Baseline intestinal absorption and calcium intake are increased in pregnant and lactating rats (see Table 5.2; Fig 5.4a). When decreasing baseline fractional intestinal absorption (Γ_{abs0}) to the virgin value in the pregnancy model, steady-state intestinal absorption decreases by 29% despite an 11% increase in $[1,25(\text{OH})_2\text{D}_3]_p$. Additionally, PTH exocytosis increases by 33% to increase $[\text{PTH}]_p$. However, despite these changes, $[\text{Ca}^{2+}]_p$ reduces by 25% (Fig. 5.8). Similar changes occur in the lactation model with a decrease in Γ_{abs0} as well as for calcium intake (I_{Ca}). Model predictions reveal that without enhanced baseline intestinal absorption, the maternal body is unable to support the needs of the maternal plasma calcium along with fetal and milk calcium delivery.

Maternal $[1,25(\text{OH})_2\text{D}_3]_p$ are more than double virgin values (Fig. 5.3c). To capture this in our pregnancy and lactation models, we increased synthesis of vitamin D into $1,25(\text{OH})_2\text{D}_3$. Notably, it has been found that vitamin D levels are lower in pregnant and lactating rats when compared to virgins, likely due to the increased synthesis of vitamin D into $1,25(\text{OH})_2\text{D}_3$ [87]. Increasing baseline inactive vitamin D ($[D_3]_p^{\text{inact}}$) to virgin levels results in increased $[1,25(\text{OH})_2\text{D}_3]_p$ by about 6% in both the pregnancy and lactation models (Fig. 5.8). In the lactation model, this yields 1.0% increase in net intestinal absorption and 2.2% increase in bone resorption, while urinary excretion decreases by 2%. In all, $[\text{Ca}^{2+}]_p$ increases by 2.2% (Fig. 5.8).

$1,25(\text{OH})_2\text{D}_3$ inhibits PTH production within the parathyroid gland (see Eq. 5.1). The parameter $\gamma_{\text{prod}}^{1,25(\text{OH})_2\text{D}_3}$ was decreased in the pregnancy and lactating rat models due to the

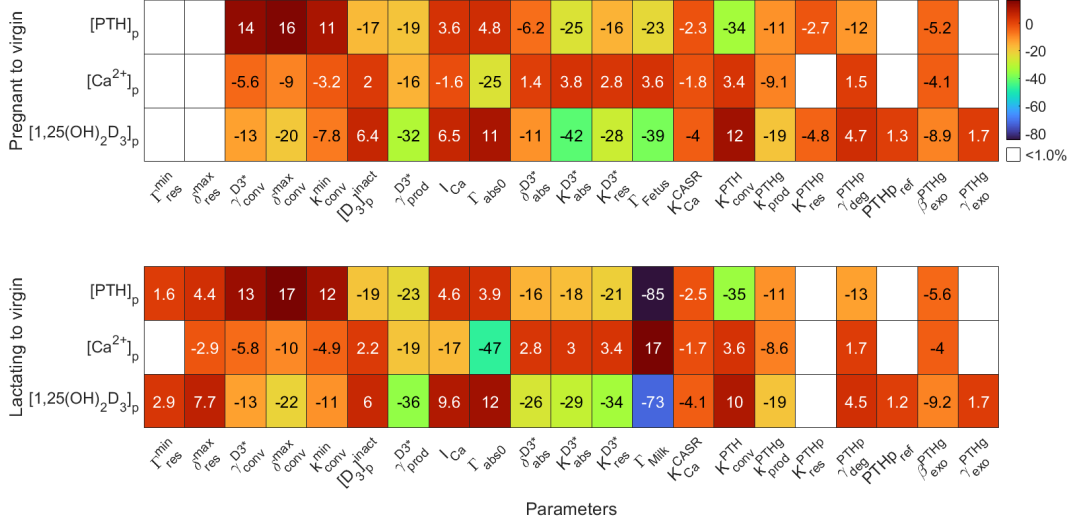


Figure 5.8: Impact of individual virgin parameters on plasma PTH, calcium, and calcitriol concentrations in the pregnant (*top*) and lactating (*bottom*) rat models. Individual parameters were set to the respective female parameter value and steady-state results computed. Percent change from baseline is reported here. PTH: parathyroid hormone; D_3^* : $1,25(\text{OH})_2\text{D}_3$ (calcitriol). Model parameters are defined in Table 5.2.

higher baseline $[1,25(\text{OH})_2\text{D}_3]_p$ levels (Table 5.2). Changing this inhibition back to the virgin value in the lactation model, resulted in a 23% decrease in $[\text{PTH}]_p$ due to lowered PTH production in the parathyroid gland from the high baseline $[1,25(\text{OH})_2\text{D}_3]_p$ levels in lactation (Fig. 5.8; Fig. 5.3). Since $[\text{PTH}]_p$ impacts $1,25(\text{OH})_2\text{D}_3$ synthesis (see Eq. 5.5), this is reduced, resulting in a 26% decrease in $1,25(\text{OH})_2\text{D}_3$ synthesis which contributes to the 36% decrease in $[1,25(\text{OH})_2\text{D}_3]_p$ (Fig. 5.8). Both PTH and $1,25(\text{OH})_2\text{D}_3$ are potent calcitropic hormones which together alter intestinal absorption, bone remodeling, and urinary excretion. The resulting predicted $[\text{Ca}^{2+}]_p$ is 19% lower than baseline (Fig. 5.8). A similar impact occurs in the pregnancy model when setting $\gamma_{\text{prod}}^{1,25(\text{OH})_2\text{D}_3}$ to the corresponding virgin parameter value.

This model analysis unravels how a failure to achieve individual maternal adaptations can cause multi-factorial dysregulation of calcium balance.

5.3.5 Differential pregnancy and lactation maternal adaptations

In the previous section, we unraveled how individual pregnancy and lactation adaptations differ from calcium homeostasis in a virgin rat. However, we also know that, due to the different hormone levels that impact calcium regulation (e.g., estrogen, prolactin) and the differing calcium needs of a fetus versus milk, calcium regulation during these two maternal states differ (Fig. 5.2). To compare calcium homeostasis between pregnancy and lactation, we set individual parameters in the pregnancy model to the corresponding value in the lactating rat model and vice versa. We then computed the steady-state for the model with each individual parameter change. The resulting fractional changes in $[PTH]_p$, $[Ca^{2+}]_p$, and $[1,25(OH)_2D_3]_p$ from baseline are shown in Fig. 5.9.

The major difference between lactation and pregnancy is the calcium demands of the fetus or milk. Recall that the calcium needs of the fetus is about $\Gamma_{Fetus} = 0.39 \mu\text{mol}/\text{min}$ while milk demands are 2.8-fold this value at $\Gamma_{Milk} = 1.08 \mu\text{mol}/\text{min}$. Setting the value for Γ_x in the pregnancy model to Γ_{Milk} results in a dangerously low $[Ca^{2+}]_p = 0.74 \text{ mmol}/\text{L}$ despite compensatory increases in $[1,25(OH)_2D_3]_p$ and $[PTH]_p$. Other changes to lactation adaptations result in a less than 3% increase or decrease in $[Ca^{2+}]_p$, i.e., calcium levels within normal range.

Intestinal absorption in the lactation model is 32% higher in the lactation model than the pregnancy model (Fig. 5.4a). This is driven by a 14% higher calcium intake (I_{Ca}) and 23% increase in baseline fractional intestinal absorption (Γ_{abs0}) (Table 5.2). Reducing either of these parameters to the corresponding pregnancy value results in a 12% and 14% decrease in $[Ca^{2+}]_p$, respectively, despite increased $[1,25(OH)_2D_3]_p$ and $[PTH]_p$.

From this analysis we conclude that maternal adaptations in pregnancy are not sufficient to support the calcium demands of milk during lactation.

5.3.6 Vitamin D deficiency

To study the effects of vitamin D deficiency on each of the four models, we reduced the concentration of inactive vitamin D ($[D_3]_p^{inact}$; Eq. 5.4) by a factor of 1 to 100% and computed resulting steady-state with this change (as done by Granjon et al. [78]). The relative change in $[PTH]_p$, $[Ca^{2+}]_p$, and $[1,25(OH)_2D_3]_p$ for each simulation of vitamin D deficiency for virgin, pregnant, and lactating females are shown in Fig. 5.10 and relative change in calcium fluxes is shown in Fig. 5.11. Our model predictions did not show a difference in relative impact in female and male rats, so these results are given in Appendix D (Fig. D.2).

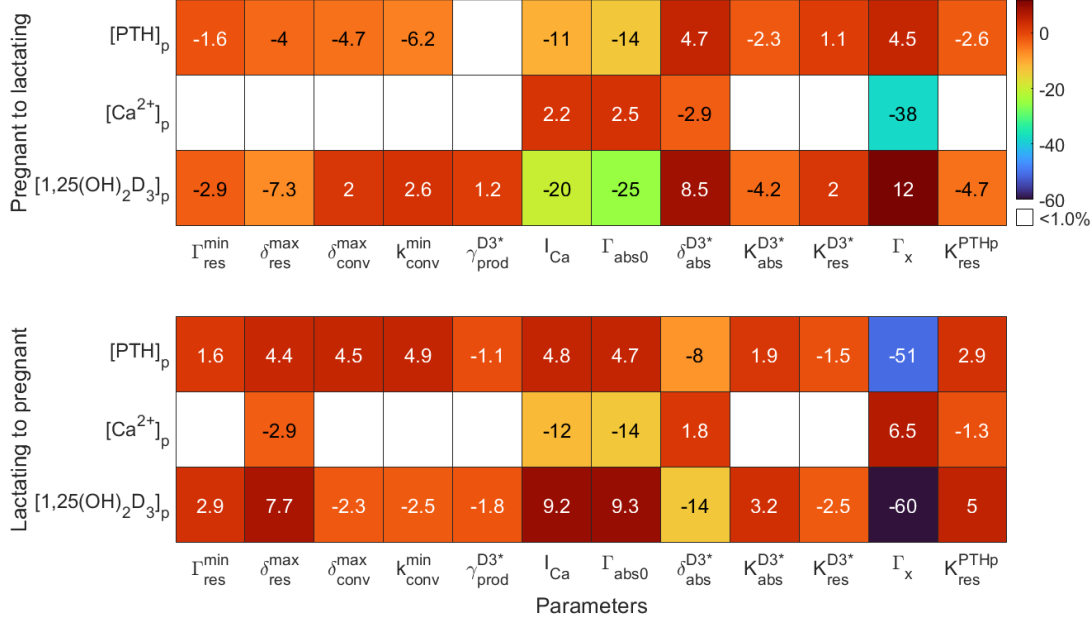


Figure 5.9: Impact of individual pregnancy and lactation parameters on plasma concentrations of parathyroid hormone, calcium, and calcitriol in the pregnancy and lactating rat models. *Top*. Individual parameters in the pregnancy model were set to respective lactation model parameter and steady-state results computed. *Bottom*. Individual parameters in the lactation model were set to respective pregnancy model parameter and steady-state results computed. Percent change from the respective baseline model steady-state is reported here. PTH: parathyroid hormone; D_3^* : 1,25(OH) $_2D_3$ (calcitriol). Model parameters are listed in Table 5.2.

In each of the models, inhibiting $[D_3]_p^{inact}$ diminished 1,25(OH) $_2D_3$ synthesis, decreasing $[1,25(OH)_2D_3]_p$, which has a direct impact on the rate of intestinal calcium absorption and bone resorption (Fig. 5.11a), ultimately resulting in a lower $[Ca^{2+}]_p$ (Fig. 5.10). Simultaneously, the decreased $[1,25(OH)_2D_3]_p$ attenuates the inhibitory effects of 1,25(OH) $_2D_3$ on PTH production in the parathyroid gland (Eq. 5.1) and PTH exocytosis (Eq. 5.7). This lack of inhibition leads to an elevation in $[PTH]_p$ (Fig. 5.10). Together decreased $[Ca^{2+}]_p$ and increased $[PTH]_p$ enhance 1,25(OH) $_2D_3$ synthesis to mitigate the effects of the decreasing concentration of $[D_3]_p^{inact}$.

In the virgin model, these adaptations are sufficient to maintain a stable $[Ca^{2+}]_p$ until $[D_3]_p^{inact}$ is inhibited by over 50%. At 50% $[D_3]_p^{inact}$, $[Ca^{2+}]_p$ is about 1.8% lower than

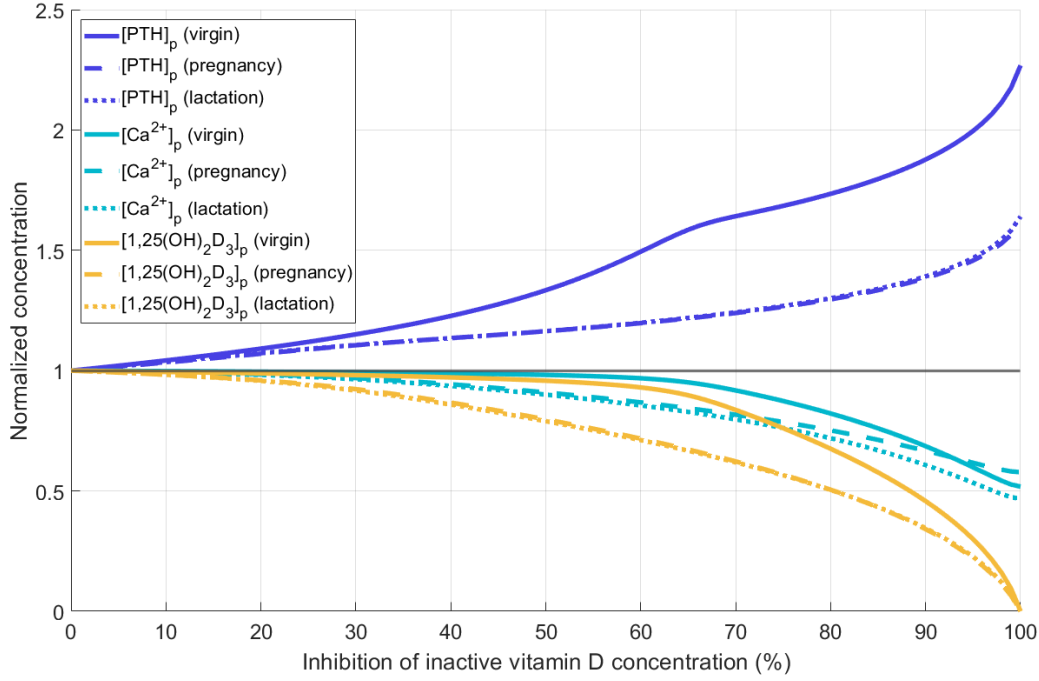


Figure 5.10: Predicted normalized steady-state plasma concentrations of parathyroid hormone, calcium (Ca^{2+}), and calcitriol ($1,25(\text{OH})_2\text{D}_3$) in virgin, pregnant, and lactating rats under decreased inactive vitamin D concentration ($[\text{D}_3]_p^{\text{inact}}$). PTH: parathyroid hormone

baseline, within normal range ($[\text{Ca}^{2+}]_p = 1.15 \text{ mmol/L}$; normal range is 1.1-1.3 mmol/L). However, as $[\text{D}_3]_p^{\text{inact}}$ is further inhibited, at 70% inhibition, $[\text{Ca}^{2+}]_p$ drops below normal range ($[\text{Ca}^{2+}]_p = 1.08 \text{ mmol/L}$) and continues to drop as $[\text{D}_3]_p^{\text{inact}}$ is further inhibited (Fig. 5.10). The counterbalancing effects of $[\text{Ca}^{2+}]_p$ and PTH on $1,25(\text{OH})_2\text{D}_3$ synthesis are unable to make up for the extremely low $[1,25(\text{OH})_2\text{D}_3]_p$ driven by nearly fully inhibited $[\text{D}_3]_p^{\text{inact}}$ (Fig. 5.10).

The pregnancy and lactation models are predicted to be less able to adapt to decreased $[\text{D}_3]_p^{\text{inact}}$ levels than the virgin model (Fig. 5.10). At 50% $[\text{D}_3]_p^{\text{inact}}$, $[1,25(\text{OH})_2\text{D}_3]_p$ is predicted to be about 21% lower than baseline in both the pregnancy and lactation models, compared to about 4% lower in the virgin model. Additionally, $[\text{PTH}]_p$ does not increase as much as the virgin model predictions. This results in a faster decline in $[\text{Ca}^{2+}]_p$: $[\text{Ca}^{2+}]_p$ at 50% inhibition of $[\text{D}_3]_p^{\text{inact}}$ is about 1.07 mmol/L in the pregnancy and lactation models, below normal range, compared to 1.15 mmol/L for the virgin model.

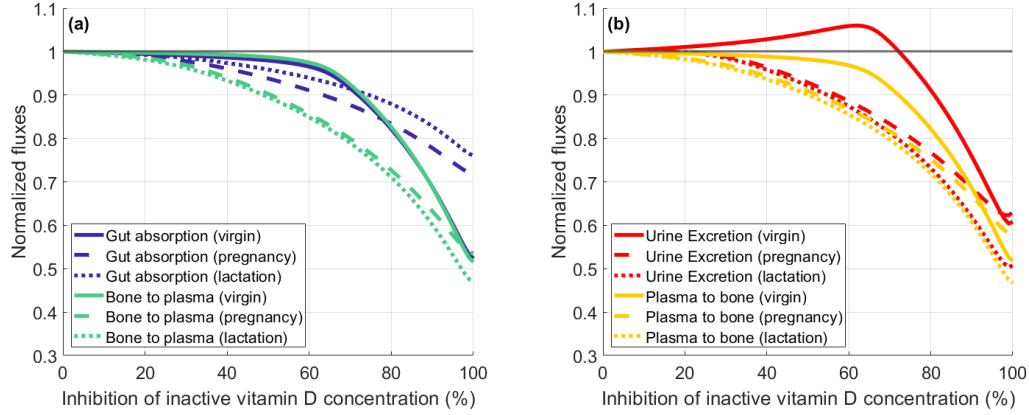


Figure 5.11: Predicted normalized steady-state calcium fluxes for virgin, pregnant, and lactating rats under decreased inactive vitamin D ($[D_3]_p^{\text{inact}}$) concentrations. (a) Calcium fluxes in the direction going into plasma. Bone to plasma flux of calcium from the bone compartments into the plasma given by the sum of resorption (Γ_{res}) and fast pool to plasma flux (Γ_{f-p}). (b) Calcium fluxes in the direction going out of the plasma. Plasma to bone indicates the total flux of calcium from the plasma to the bone given by the plasma to fast pool flux (Γ_{p-f}).

At 100% inhibition of $[D_3]_p^{\text{inact}}$, $1,25(\text{OH})_2\text{D}_3$ is no longer in the system, $[\text{Ca}^{2+}]_p$ is at severely low levels for each of the virgin, pregnant, and lactating rat models (Fig. 5.10). The lactating rat has the largest decline in $[\text{Ca}^{2+}]_p$ with predicted levels to be about 53% of baseline.

In summary, our vitamin D deficiency simulations show that for vitamin D levels inhibited by up to 70% in virgin rats, compensatory mechanisms are able to sustain $[\text{Ca}^{2+}]_p$ levels within normal range. However, our predictions show that pregnancy and lactating rats are more sensitive to changes in vitamin D levels and begin to show hypocalcemic $[\text{Ca}^{2+}]_p$ levels at around 50% inhibition.

5.3.7 What if simulations: Impact of pregnancy and lactation on male model

The main difference between male and female calcium homeostasis is that female rat $[1,25(\text{OH})_2\text{D}_3]_p$ is about half of the male values (Fig. 5.3c). Natural selection prioritizes traits that improve reproductive success. For females, this includes the ability to adapt

to the significant changes required to support a healthy pregnancy, as well as the ability to nourish offspring through lactation. It is plausible that through the process of natural selection, female bodies have evolved to maintain lower levels of $[1,25(\text{OH})_2\text{D}_3]_p$ to allow for a more efficient elevation of $[1,25(\text{OH})_2\text{D}_3]_p$ when necessary (i.e., during pregnancy and lactation).

To investigate this idea, we performed “what if” simulations by modifying the parameters of the male model by the pregnancy-to-virgin and lactation-to-virgin ratios as in Table 5.2. We will refer to these simulations as the “male-pregnancy” and “male-lactation” simulations while pregnancy and lactation will refer to the female pregnant and lactating rat models described in the previous sections. The steady-state solutions from these simulations are shown in Fig. 5.12.

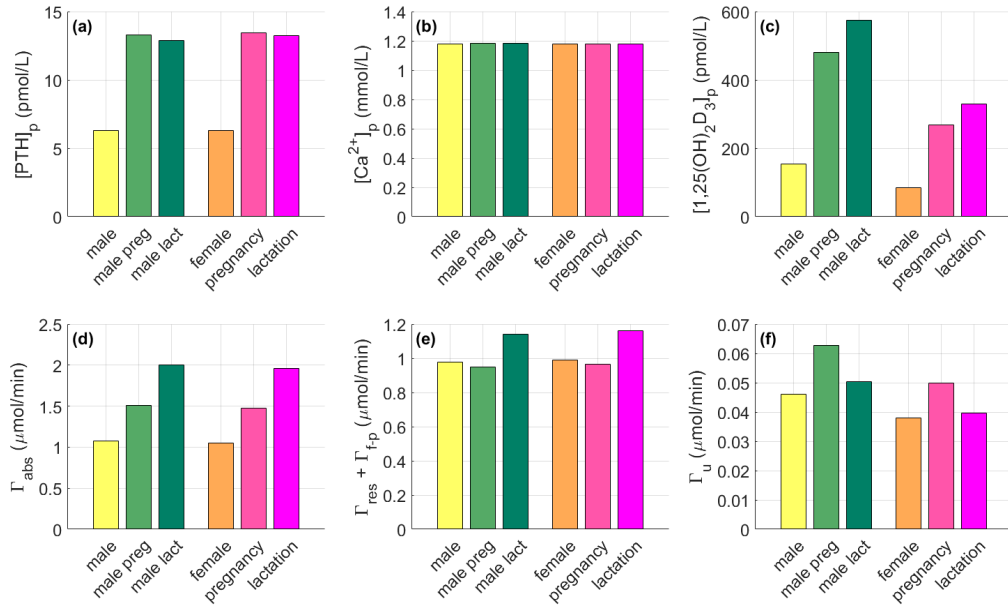


Figure 5.12: Steady-state concentrations (a)-(c) and calcium fluxes (d)-(f) for male and female models with pregnancy and lactation adaptations as listed in Table 5.2. male lact: “male-lactation”; male preg: “male-pregnancy”; PTH: parathyroid hormone; $1,25(\text{OH})_2\text{D}_3$: calcitriol; Γ_{abs} : intestinal absorption; Γ_{res} : bone resorption; $\Gamma_{\text{f-p}}$: bone fast pool to plasma flux; Γ_{u} : urinary excretion

In the same way as female pregnancy and lactation, the “male-pregnancy” and “male-lactation” models predicted $[\text{Ca}^{2+}]_p$ levels remain the same when compared to the baseline

male model (Fig. 5.12b). Additionally, the predicted increases in $[PTH]_p$, above the baseline male model, for the “male-pregnancy” and “male-lactation” models are similar to the female pregnancy and lactation models.

The largest difference between the “male-pregnancy” and “male-lactation” steady-state predictions versus the respective pregnancy and lactation models is the $[1,25(OH)_2D_3]_p$ levels (Fig. 5.12c). This is due to the difference in baseline non-pregnant $[1,25(OH)_2D_3]_p$ levels in males versus females. Calcium fluxes also show a similar relative increase in the “male-pregnancy” and “male-lactation” models as in the female pregnancy and lactation models (Fig. 5.12d-f). In all, these balance to maintain a stable $[Ca^{2+}]_p$ (Fig. 5.12b).

What do the higher $[1,25(OH)_2D_3]_p$ levels in the hypothetical “male-pregnancy” and “male-lactation” cases mean (Fig. 5.12c)? To understand that, we conducted simulations where we fixed $[1,25(OH)_2D_3]_p$ in those hypothetical male models at the female pregnancy and lactation levels (as in Fig. 5.3c) and then we solved for the other variables (PTH_g , $[PTH]_p$, N_{Ca_r} and $[Ca^{2+}]_p$) at steady-state. That is, we considered a subsystem of the calcium homeostasis model described in Section 5.2.1 without Eq. 5.4 and treated $[1,25(OH)_2D_3]_p$ as a fixed parameter rather than a dynamic variable. We will refer to these simulations as the “fixed $1,25(OH)_2D_3$ male-pregnancy” and “fixed $1,25(OH)_2D_3$ male-lactation” simulations. The assumption is that some parameters in Eq. 5.4 have taken on different values so that $[1,25(OH)_2D_3]_p$ becomes the corresponding female pregnancy or lactation values. The resulting steady-state plasma concentrations and fluxes are shown in Fig. 5.13.

The “fixed $1,25(OH)_2D_3$ male-pregnancy” and “fixed $1,25(OH)_2D_3$ male-lactation” predictions result in a compensatory increase in $[PTH]_p$ (Fig. 5.13a); however, this change is not enough to make up for the lost intestinal absorption and bone resorption from lowered $[1,25(OH)_2D_3]_p$ levels (Fig. 5.13d; Fig. 5.13e). In the end, these simulations result in hypocalcemic levels of $[Ca^{2+}]_p$ at about 0.92 mmol/L, significantly below normal range (Fig. 5.13b).

Predicted $[1,25(OH)_2D_3]_p$ levels for “male-pregnancy” and “male-lactation” are about double their respective female pregnancy and lactation values (Fig. 5.12c). To sustain this high $[1,25(OH)_2D_3]_p$ level, the predicted synthesis of vitamin D to $1,25(OH)_2D_3$ needs to be about 77% higher in the “male-pregnancy” model when compared to the female pregnancy model. Recall that inactive vitamin D is the precursor to $1,25(OH)_2D_3$. Notably, vitamin D concentrations lower by about 40% during pregnancy and lactation, likely due to the enhanced $1,25(OH)_2D_3$ synthesis that occurs to sustain high $[1,25(OH)_2D_3]_p$ levels. Recall in Section 5.3.6 that when inactive vitamin D levels are decreased by 40%, each of the models is able to sustain normal $[Ca^{2+}]_p$ (Fig. 5.10; Fig. D.2). However, as vitamin D

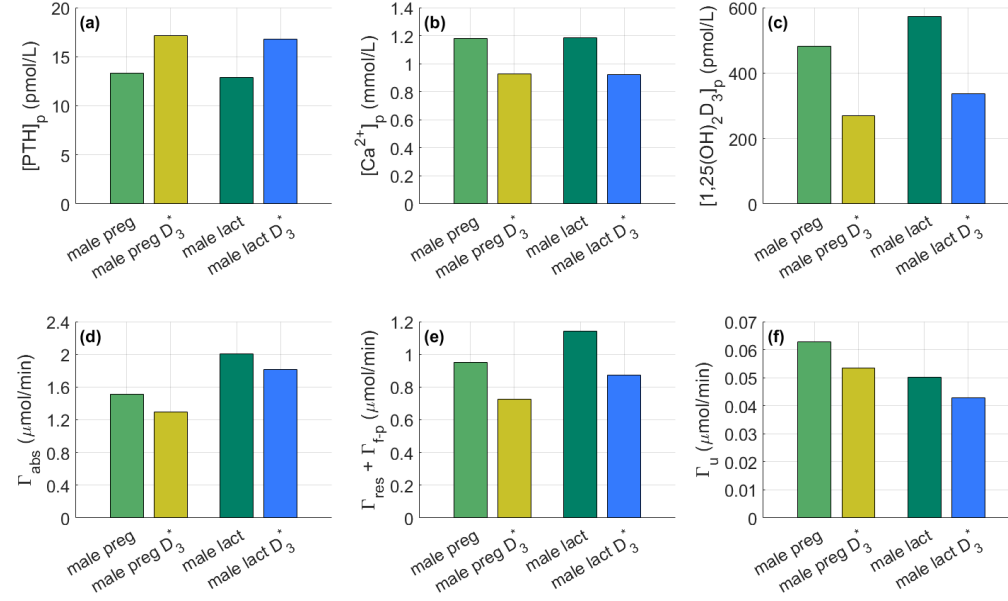


Figure 5.13: Predicted steady-state results for the “male-pregnancy” and “male-lactation” models with fixed $[1,25(OH)_2D_3]_p$ levels at female pregnancy and lactation values (i.e., “fixed $[1,25(OH)_2D_3]_p$ male-pregnancy” and “fixed $[1,25(OH)_2D_3]_p$ male-lactation” simulations). Plasma concentrations are given in (a)-(c) while fluxes are given in (d)-(f). male preg: “male-pregnancy”; male preg D_3^* : “fixed $[1,25(OH)_2D_3]_p$ male-pregnancy”; male lact: “male-lactation”; male lact D_3^* : “fixed $[1,25(OH)_2D_3]_p$ male-lactation”; PTH: parathyroid hormone; D_3^* : $1,25(OH)_2D_3$ (calcitriol); Γ_{abs} : intestinal absorption; Γ_{res} : bone resorption; Γ_{f-p} : bone fast pool to plasma flux; Γ_u : urinary excretion

levels decreased further, compensatory mechanisms were no longer able to sustain normal $[Ca^{2+}]_p$ levels. Noting that males and females have similar inactive vitamin D levels, it is likely that if during pregnancy or lactation, levels of $[1,25(OH)_2D_3]_p$ that are predicted in the “male-pregnancy” and “male-lactation” models were required, the vitamin D supply would be depleted further, leading to very low, potentially dangerous, concentrations.

5.4 Discussion

In this study we developed mathematical models that describe calcium exchanges between the intestine, plasma, slow and rapidly exchangeable bone pools, and the kidneys as well as their regulation by PTH and $1,25(\text{OH})_2\text{D}_3$. Model parameters were specified to simulate calcium homeostasis in a male versus (virgin) female rat, in a pregnant rat, and in a lactating rat. To our knowledge, this is the first mathematical model of rat calcium homeostasis that captures the maternal adaptations of pregnancy and lactation. We applied these models to:

- Assess the sensitivity of calcium regulation in each of the male, female, pregnancy, and lactation models (Fig. 5.5).
- Unravel how sex differences in $[1,25(\text{OH})_2\text{D}_3]_p$ result in different set points for stimulation of intestinal calcium absorption between male and female rats (Fig. 5.6).
- Quantify the impact of individual maternal adaptations versus virgin rat calcium homeostasis (Fig. 5.7; Fig. 5.8) and evaluate the differential effects of pregnancy and lactation on maternal calcium homeostasis (Fig. 5.9).
- Predict the effects of vitamin D deficiency on male, female, pregnant, and lactating rats (Fig. 5.10; Fig. 5.11).
- Explore how sex differences in $[1,25(\text{OH})_2\text{D}_3]_p$ may enable female rats to sustain the high levels of $[1,25(\text{OH})_2\text{D}_3]_p$ found in pregnancy and lactation more efficiently than male rats (Fig. 5.12; Fig. 5.13).

Overall, our model analysis provides insights into the multi-factorial impacts of individual maternal adaptations as well as sex differences to contribute a better understanding of the complex factors affecting regulation of calcium homeostasis.

Sensitivity analysis of calcium regulatory system. In our local sensitivity analysis of the male, female, pregnancy, and lactation models, we observed that no individual parameter perturbations resulted in a change in $[\text{Ca}^{2+}]_p$ levels by more than 5% (Fig. 5.5). Instead, the levels of $[\text{PTH}]_p$ and $[1,25(\text{OH})_2\text{D}_3]_p$ adapted in response to parameter perturbations to modulate calcium fluxes, ensuring the stability of $[\text{Ca}^{2+}]_p$ levels in each of the four models (Fig. 5.5). Furthermore, our analysis of individual sex differences on male and female model predictions, revealed that these changes in parameters (Table 5.1) did not

result in a change in predicted steady-state $[\text{Ca}^{2+}]_p$ exceeding 3% (Fig 5.6). Once again, the impact primarily affected the levels of $[\text{PTH}]_p$ and $[1,25(\text{OH})_2\text{D}_3]_p$.

In summary, calcium regulation is a robust process wherein hormonal changes compensate for (small) alterations that affect balance, effectively restoring $[\text{Ca}^{2+}]_p$ levels to baseline in healthy individuals.

Maternal adaptations of calcium homeostasis. During pregnancy, the maternal body undergoes significant adaptations to maintain calcium homeostasis and meet the increased calcium demands of the developing fetus (Fig. 5.2; Table 5.2). These adaptations involve hormonal changes as well as major adaptations to most physiological systems. One crucial adaptation is an increase in intestinal calcium absorption. However, while enhanced intestinal absorption is partially facilitated by the massive increase in $[1,25(\text{OH})_2\text{D}_3]_p$, studies have shown that the substantial rise cannot be solely attributed to $1,25(\text{OH})_2\text{D}_3$ [117, 119]. Other hormones such as prolactin and estrogen also play a major role in enhancing maternal intestinal absorption [38, 39, 153].

During lactation, maternal calcium homeostasis undergoes even more profound changes compared to pregnancy (Fig. 5.2; Table 5.2). Milk supply requires higher calcium levels to meet the needs of the neonates. In rodents, intestinal calcium absorption remains elevated during lactation [118]. Furthermore, there is an increase in renal calcium reabsorption, hence reducing calcium loss through urine [118]. Lastly, lactation is characterized by significant bone resorption, representing the most rapid period of bone loss in a (healthy) female’s lifetime [189, 239].

An advantage of computational modeling is that once the baseline model is created, we can conduct *in silico* experiments to test the impacts of individual changes, something that is not done easily in experimental models. To quantify the impact of each individual maternal adaptation on female rats, we set individual parameters in the virgin (female) model to the respective pregnancy or lactation model parameter value and predicted the resulting steady-state values (Fig. 5.7). The largest impact on $[\text{Ca}^{2+}]_p$ was setting Γ_x to the milk calcium demand, Γ_{Milk} . This resulted in a steady-state prediction of severe hypocalcemia despite massive increases in $[\text{PTH}]_p$ and $[1,25(\text{OH})_2\text{D}_3]_p$ levels (Fig. 5.7). This shows that compensatory mechanisms of PTH and $1,25(\text{OH})_2\text{D}_3$ are not sufficient to support the extra demands of calcium by the milk: lactation-specific adaptations are critical.

Due to elevated 1α -hydroxylase activity, $[1,25(\text{OH})_2\text{D}_3]_p$ is massively increased during pregnancy and lactation [118]. Increasing the relevant virgin model parameter ($k_{\text{conv}}^{\text{min}}$) to pregnancy and lactation values for $1,25(\text{OH})_2\text{D}_3$ synthesis resulted in massive increases in

steady-state $[1,25(\text{OH})_2\text{D}_3]_p$. This resulted in increased calcium fluxes into the bloodstream which drives up $[\text{Ca}^{2+}]_p$ levels (Fig. 5.7).

We also conducted an analysis on the pregnancy and lactation models by setting individual parameters to the respective virgin value (Fig. 5.8). Intestinal absorption is increased in both pregnancy and lactation (Γ_{abs0} ; Table 5.2). Without this increase, predicted steady-state $[\text{Ca}^{2+}]_p$ levels were far below normal range in both pregnancy and lactation despite compensatory changes in $[\text{PTH}]_p$ and $[1,25(\text{OH})_2\text{D}_3]_p$ levels. Our model predictions show how enhanced intestinal absorption, separate from $1,25(\text{OH})_2\text{D}_3$ -mediated absorption, is essential for supporting maternal calcium needs.

Differential impacts of pregnancy and lactation on calcium homeostasis. Pregnancy and lactation exhibit distinct calcium needs due to differing plasma volumes as well and the requirements of a fetus versus milk production. Hormone levels also differ. For example, estrogen peaks at the end of pregnancy, to the highest levels found in healthy females, then quickly drops post-parturition so that lactation is an estrogen-deficient state [118]. Prolactin levels increase by more than 5-fold in pregnant rats compared to virgin control and then doubles again during lactation [38]. Both prolactin and estrogen levels are known to impact calcium regulation [39, 112, 149]. Other hormones such as progesterone, PTHrP, and serotonin also have been shown to impact calcium regulation and are at differing levels during pregnancy and lactation [33, 68, 217]. In total, all of these hormones drive maternal adaptations for pregnancy and lactation.

To quantify the impact of lactation-specific maternal adaptations on pregnancy calcium homeostasis, we set individual parameters in the pregnancy model to their corresponding values in the lactation model and solved for the resulting steady-state (Fig. 5.9). Given that the milk requirement (Γ_{Milk}) is 2.8 times more than the needs of the fetus (Γ_{Fetus}), setting Γ_x in the pregnancy model to Γ_{Milk} (see Eq. 5.17), led to extremely low $[\text{Ca}^{2+}]_p$, despite elevated levels of $[1,25(\text{OH})_2\text{D}_3]_p$ (Fig. 5.9). Other parameter changes to the lactation value results in minor fluctuations in $[\text{Ca}^{2+}]_p$.

Conversely, we set individual parameters in the lactation model with their corresponding values in the pregnancy model and solved for the resulting steady-state to investigate the impact of pregnancy-specific maternal adaptations on lactation calcium homeostasis (Fig. 5.9). The lactation model has higher intestinal absorption compared to the pregnancy model (Fig. 5.4a). This is primarily driven by increased calcium intake (I_{Ca}) and baseline fractional intestinal absorption (Γ_{abs0}). When these parameters are reduced to the pregnancy values, $[\text{Ca}^{2+}]_p$ reduces below normal range, despite compensatory increases in $[1,25(\text{OH})_2\text{D}_3]_p$ and $[\text{PTH}]_p$. Based on this analysis, we conclude that the maternal

adaptations during pregnancy are not sufficient to meet the calcium requirements of milk production.

Vitamin D deficiency. Vitamin D is important for overall health. We used our models to predict the impacts of vitamin D deficiency on calcium regulation (Fig. 5.10; Fig. 5.11; Fig. D.2). Simulation results for male and female rats showed no significant difference between the sexes on the impact of vitamin D deficiency (Fig. D.2). Note that the models provided in this study are for young rats. In experimental studies, no significant differences in the impacts of vitamin D deficiency between the sexes in young rats have been reported, although sex differences in serum calcium levels were observed in aged rats [219]. While important, the effect of aging is out of the scope of the current study.

Boass et al. [19] found that lactating rats on a vitamin D-deficient diet (serum inactive vitamin D levels were undetectable) developed severe hypocalcemia, but milk calcium levels were not significantly decreased. This suggests that the maternal body prioritizes the demands of milk calcium during lactation, likely making lactating rats more susceptible to hypocalcemia than non-pregnant rats under vitamin D deficiency. In our model predictions, we found the lactating rat model was most sensitive to vitamin D deficiency, resulting in the lowest levels of $[Ca^{2+}]_p$ during severe vitamin D deficiency (Fig. 5.10).

Notably, while vitamin D receptor-null rodents are able to get pregnant, their fertility is low: vitamin D receptor-null mothers conceive at 5-10% frequency of the control [119]. Additionally, both vitamin D receptor-null and vitamin D deficient mothers result in fewer viable fetuses as well as inhibited pup and maternal health [20, 117, 119]. In summary, our model results and experimental studies demonstrate how vitamin D plays a key role in maintaining $1,25(OH)_2D_3$ levels to support the extra calcium needs of the maternal body.

Implications of sex differences in calcitriol levels. Given the substantial increase in $[1,25(OH)_2D_3]_p$ levels observed during pregnancy and lactation, we hypothesize that female rats evolved to have a lower baseline synthesis of $1,25(OH)_2D_3$ to be able to increase $1,25(OH)_2D_3$ levels during pregnancy and lactation without depleting vitamin D levels. To explore the validity of that hypothesis, we conducted “what if” simulations to (1) predict the impact of maternal parameter changes on the male model (Fig. 5.12) and (2) investigate the implications of sex differences in $[1,25(OH)_2D_3]_p$ levels in a hypothetical pregnant or lactating male (Fig. 5.13).

Our steady-state predictions for “male-pregnancy” and “male-lactation” result in balanced changes in calcium fluxes (Fig. 5.12d-f) so that $[Ca^{2+}]_p$ is unchanged from the male baseline (Fig. 5.12b). In contrast, “male-pregnancy” and “male-lactation” $[1,25(OH)_2D_3]_p$

levels are double that of the corresponding female pregnancy and lactation model steady-state $[1,25(\text{OH})_2\text{D}_3]_p$. Notably, to support this extremely high $[1,25(\text{OH})_2\text{D}_3]_p$ level, “male-pregnancy” requires a 77% higher synthesis of $1,25(\text{OH})_2\text{D}_3$ than the female pregnancy model. It may be that the synthesis of $1,25(\text{OH})_2\text{D}_3$ at the rate required for the levels of $[1,25(\text{OH})_2\text{D}_3]_p$ (over 480 pmol/L; Fig. 5.12c) in “male-pregnancy” and “male-lactation” models is not sustainable, since $1,25(\text{OH})_2\text{D}_3$ synthesis requires both sufficiently high levels of its precursor vitamin D as well as 1α -hydroxylase activity. Therefore, having a lower baseline level of $[1,25(\text{OH})_2\text{D}_3]_p$ in female rats may prove more efficient to support maternal adaptations.

To investigate this hypothesis, we tested if female pregnancy and lactation $[1,25(\text{OH})_2\text{D}_3]_p$ levels were sufficient to maintain $[\text{Ca}^{2+}]_p$ within normal range for “male-pregnancy” and “male-lactation” (Fig. 5.13). Our analysis indicated that the compensatory increase in $[\text{PTH}]_p$ levels (Fig. 5.13a) is insufficient to offset the reduced intestinal absorption and bone resorption caused by lowered levels of $[1,25(\text{OH})_2\text{D}_3]_p$ (Fig. 5.13d; Fig. 5.13e). Consequently, these simulations result in severely hypocalcemic levels of $[\text{Ca}^{2+}]_p$ (Fig. 5.13b). In conclusion, these “what if” simulations support our hypothesis that the lower baseline $[1,25(\text{OH})_2\text{D}_3]_p$ in female rats may allow them to more readily adapt to pregnancy and subsequent lactation.

Despite the known impacts of sex, pregnancy, and lactation on various physiological systems, relatively few mathematical models specifically account for these factors [1, 122]. The study of maternal physiology and associated disorders has traditionally been challenging due to ethical concerns and potential risks to the fetus or neonate in clinical trials involving pregnant or lactating women. However, computational modeling provides a promising strategy to overcome these limitations by allowing the exploration of hypotheses and evaluation of potential therapies *in silico* using models specifically formulated to represent maternal physiology. In this study, we have introduced the first in a series of mathematical models focused on calcium homeostasis during pregnancy and lactation. We aim to contribute to the unravelling of the complexities of maternal physiologies as well as take a step towards having mathematical models that can be used to study underlying pathophysiological conditions and potential treatments for maternal populations.

5.4.1 Model limitations and future directions

The models presented in this study do not include the interactions between calcium and phosphate, which is especially relevant in bone remodeling. Indeed, more comprehensive

calcium-phosphate homeostasis models with detailed bone compartments exist [79, 165]. Mathematical models have long been used to study bone remodeling [45]. Indeed, a future work would be to develop lactation-specific bone remodeling models to understand the complexities of changes to bone remodeling during lactation and post-weaning [118, 189]. Note that pregnancy has more conflicting results about bone remodeling changes, but could be explored through modeling based on existing data [118].

In this study, we did not include pregnancy and lactation changes along the nephrons due to limited data. Future work may determine more detailed renal transport of calcium during pregnancy and lactation by using detailed epithelial transport models of kidney function in females [99, 100, 126, 143], models that simulate epithelial calcium transport [24, 54, 55, 221], and models of CKD [124, 125, 128]. Epithelial transport nephron models for pregnancy have been created (see Refs. [209, 215]), but do not include calcium at this time. Note that there are no known renal epithelial transport models that account for lactation adaptations since data on transporter changes during lactation is limited. As more data on renal transporter adaptations during pregnancy and lactation is published, the calcium nephron transport models (Refs. [24, 54, 55, 221]) may be adapted to understand the impacts of pregnancy and lactation on renal calcium handling in the same way as Refs. [209, 215]. (Update: Since the publication of this manuscript in Ref. [211], Hakimi et al. [86] included calcium in a pregnancy-specific epithelial transport model.) Additionally, these results could be coupled to the kidney compartment in the pregnancy and lactation models presented in this study.

It should also be noted that the models presented in this study do not explicitly account for the effects of specific hormonal changes that occur during pregnancy and lactation. For instance, estrogen levels significantly increase during pregnancy, but decrease post-parturition. In our current models, the impacts of hormones such as estrogen, progesterone, and prolactin are implicitly incorporated through the chosen parameters. However, future research could explore the inclusion of these individual hormones and their effects on calcium regulatory systems to create a more comprehensive model capable of predicting the impact of changes in maternal hormone levels.

Chapter 6

A physiology-based mathematical model of the renin-angiotensin system, bone remodeling, and calcium homeostasis: Effects of estrogen and renin-angiotensin system inhibitors

Abstract

During menopause, estrogen levels decline significantly, leading to substantial physiological changes due to estrogen's regulatory role in various systems. In particular, estrogen helps prevent excessive bone resorption by its impact on bone remodeling. When estrogen levels decrease, increased bone resorption occurs, often resulting in weakened bones and osteoporosis in post-menopausal women. Experimental studies have also shown that estrogen regulates the renin-angiotensin system (RAS), a hormone system involved in many physiological processes, including blood pressure. Additionally, the RAS has an interconnected relationship with calcium regulatory and bone remodeling systems. Given these interconnections, how would perturbations in one system affect the others? To answer that question, we developed a physiology-based

mathematical model that simulates the interactions of estrogen, key RAS components, calcium regulation, and bone remodeling. Through sensitivity analysis and model simulations, we investigated how declining estrogen levels affect the RAS and bone mineral density. Additionally, we quantified how RAS inhibitors, specifically angiotensin-converting enzyme inhibitors and angiotensin receptor blockers, may increase bone density during post-menopausal estrogen decline.

6.1 Introduction

Bone remodeling is a complex physiological process that maintains the balance between bone formation and resorption. This process is mediated by bone cells: osteoblasts, which are responsible for bone formation, and osteoclasts, which are responsible for bone resorption. The activity and proliferation of osteoblasts and osteoclasts is regulated by a system of various receptors, their corresponding ligands, and hormonal signals [7, 45]. Estrogen, in particular, impacts bone remodeling by inhibiting osteoclast activity, thereby protecting bones from excessive resorption. When estrogen levels in women decline post-menopause, osteoclast activity increases, resulting in increased resorption, and ultimately weakening bones. This often leads to post-menopausal osteoporosis. Post-menopausal osteoporosis is the most common form of osteoporosis, which is characterized by increased bone fragility, making fractures more likely from minor trauma or in severe cases, even spontaneous fracture. It is estimated that about 1 in 3 women over 50 years of age will suffer a fracture related to post-menopausal osteoporosis [94, 147].

The main component of bone structure is hydroxyapatite, a compound made up of phosphate and calcium. Given their role in developing the key structure of bones, maintaining balanced levels of calcium and phosphate is critical for bone health. The regulation of calcium and phosphate levels is driven by calciotropic hormones: calcitriol and PTH [184]. Researchers have reported a bidirectional relationship between the calciotropic hormones and the renin-angiotensin system (RAS) [222], a hormone system that plays a key role in many physiological processes, including blood pressure regulation, in addition to a direct impact of the RAS on bone remodeling [197]. The RAS is a hormone cascade that produces the bioactive peptide Ang II. Ang II binds to angiotensin type 1 receptor (AT1R) and angiotensin type 2 receptor (AT2R) to trigger various physiological effects. The RAS is also modulated by estrogen levels, and therefore is impacted by the rapidly declining estrogen levels in post-menopausal women [75]. This dysregulation contributes to the higher susceptibility of post-menopausal women to the development of hypertension, cardiovascular disease, and osteoporosis [75].

Inhibition of RAS components, particularly angiotensin-converting enzyme (ACE) and AT1R binding, has shown to be effective targets for antihypertensive treatment [102]. These drugs are classified as RAS inhibitors. Experimental data has shown that the use of RAS inhibitors improves bone quality independent of their effect on blood pressure [114, 190]. Given that RAS inhibitors are relatively well tolerated, safe, and are reasonably priced drugs, there has been interest in using RAS inhibitors to prevent osteoporosis in post-menopausal women [114, 190, 237]. Additionally, since hypertension is highly prevalent in post-menopausal women, understanding the impacts of antihypertensive treatments, such as RAS inhibitors, on bone remodeling is important for achieving blood pressure control and bone health in post-menopausal women [114, 190, 237].

Many mathematical models have been developed to study bone remodeling as recently reviewed in Ref. [45]. Peterson and Riggs [165] developed a mathematical model of calcium and phosphate regulation coupled to a detailed bone biology model. Their model consists of bone, renal, and blood compartments to predict levels of PTH, calcitriol, calcium, phosphate, and bone remodeling activity. This model has been applied to investigate applications related to bone remodeling and calcium-phosphate homeostasis, including the impact of estrogen [186], mineral bone disorder in patients with chronic kidney disease [187], and the impact of fibroblast growth factor 23 and vascular calcification [72]. Mathematical models have also been developed for the RAS to understand its role in blood pressure regulation. Specifically, Lo et al. [137] developed a model of the RAS in humans to investigate the differences between normo-tensive and hypertensive patients. This model was later included in a full body blood pressure model by Hallow et al. [90]. Leete and Layton [131] further developed this model to investigate the effects of sex-based differences in the RAS on blood pressure regulation. However, no known models have been developed that include the direct impact of estrogen on the RAS or have coupled the RAS impacts with calcium homeostasis and bone remodeling in humans.

In this study, we (i) investigate the impact of age-related estrogen decline by including the impact of estrogen on the RAS and (ii) couple the calcium homeostasis and bone remodeling model (Refs. [165, 186]) with the RAS model (Refs. [90, 131, 137]) to predict the impact of RAS inhibitors on this system.

6.2 Methods

In this study, we develop a coupled ODE model that represents (i) the impact of estrogen on the RAS (Section 6.2.1) and (ii) couple this estrogen-RAS model with a bone biology and calcium homeostasis model (Section 6.2.3). We then use this model to simulate the

effects of post-menopausal estrogen decline on the RAS, bone remodeling, and calcium homeostasis. Additionally, we simulate the impact of RAS inhibitors. To quantify the impact of parameter variation on model output, we conduct a global sensitivity analysis. A model schematic diagram is shown in Fig. 6.1. A list of the model equations is in Appendix E.4.

6.2.1 Renin-angiotensin system model

In this study, we use the RAS model with the female-specific parameter set from Ref. [131]. The model variables consist of the systemic concentrations of angiotensinogen (AGT), Ang I, Ang IV, Ang (1-7), AT1R-bound Ang II, and AT2R-bound Ang II. AT1R-bound Ang II inhibits renin activity through a feedback loop. For a detailed description of the underlying RAS model used in this study, see Refs. [90, 131, 137].

Impact of estrogen decline in aging women on the RAS

Estrogen levels impact the RAS by activating and inhibiting various key mechanisms [75]. Specifically, estrogen activates AGT production and AT2R binding, while inhibiting renin secretion, ACE activity, and AT1R binding [75]. These mechanisms are illustrated in Fig. 6.1. Model simulations of the estrogen-RAS model in isolation are shown in Appendix E.1.

After the onset of menopause, estrogen levels decrease dramatically. To model this decline in estrogen levels, we use the estrogen model for post-menopausal women from Ref. [105]. The normalized level of estrogen, denoted by E , is modeled through explicit time dependence so that

$$E(t) = \begin{cases} 1 & t < t_e \\ \frac{1}{1+(t-t_e)/\tau_e} & t \geq t_e \end{cases} \quad (6.1)$$

where t is the time in years (i.e., age), $t_e = 50$ years is the age of onset of estrogen decline, and $\tau_e = 2.6$ years is a parameter fit to normalized estrogen data from Ref. [206] that determines the slope of the decline in estrogen (see Ref. [105] for details). Note that before t_e , $E = 1$ so that estrogen is assumed to remain at baseline level pre-menopause. Indeed, estrogen levels vary during the menstrual cycle [173]. In this study, we do not consider variations in estrogen over the menstrual cycle in pre-menopausal women and consider baseline estrogen level pre-menopause as the average level through the full cycle.

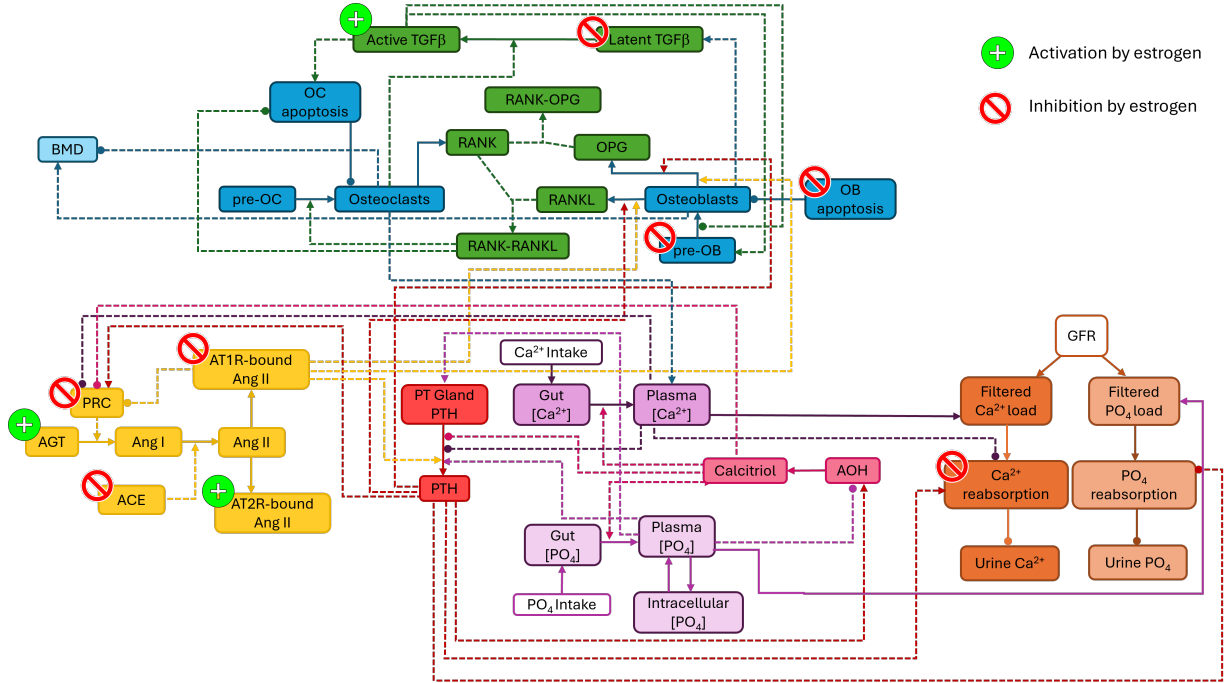


Figure 6.1: Schematic diagram of calcium regulation, bone remodeling, the renin-angiotensin system, and the impact of estrogen on these systems. Purple nodes represent phosphate and calcium levels, orange nodes represent phosphate and calcium renal handling, yellow nodes represent the RAS components, red nodes represent parathyroid hormone, pink nodes represent calcitriol, blue nodes represent bone cells and bone mineral density, green nodes represent signaling mechanisms in the bone. Nodes with a white background represent fixed values in the model. The impacts of estrogen are indicated by the red and green symbols for inhibition and activation, respectively. The solid arrows represent flux, secretion, synthesis, or expression, while the dashed lines represent impacts on other biological components. The pointed arrows represent activation and the rounded arrows represent inhibition. ACE: angiotensin-converting enzyme, AGT: angiotensinogen, Ang: angiotensin, AOH: 1 α -hydroxylase, AT1R: angiotensin type 1 receptor, AT2R: angiotensin type 2 receptor, BMD: bone mineral density, Ca²⁺: calcium, GFR: glomerular filtration rate, OB: osteoblast, OC: osteoclast, OPG: osteoprotegerin, PO₄: phosphate, PRC: plasma renin concentration, PT: parathyroid, PTH: parathyroid hormone, RANK: Receptor Activator of Nuclear Factor Kappa-B, RANKL: RANK ligand, RAS: renin-angiotensin system, TGF β : transforming growth factor β

To include the impact of estrogen on the RAS, we use functions of the form:

$$g_i^+(E) = V_{\max,i}^+ \times \frac{(E/e_i)}{1 + (E/e_i)} \quad (6.2)$$

$$g_i^-(E) = V_{\max,i}^- \times \frac{1}{1 + (E/e_i)} \quad (6.3)$$

to model activation (g^+) or inhibition (g^-) with

$$V_{\max,i}^+ = e_i + 1, \quad V_{\max,i}^- = \frac{e_i + 1}{e_i}$$

where i denotes the target mechanism, e_i is a parameter that is determined for each relevant target mechanism, and E is given by Eq. 6.1. This is a similar approach to the modeling of estrogen impacts in Ref. [105]. Note that when $E = 1$ we have that $g_i^+(E) = g_i^-(E) = 1$ for any value of e_i , so that at baseline estrogen levels, there are no changes.

The enzyme renin is secreted by the juxtaglomerular cells of the kidneys to convert AGT to angiotensin I (Ang I). Estrogen inhibits renin levels [63, 75, 194]. We let g_{renin}^- be the impact of estrogen on renin secretion so that renin secretion is modeled by

$$R_{\text{sec}} = N_{\text{rs}} \times \nu_{\text{AT1R}} \times g_{\text{renin}}^-(E) \quad (6.4)$$

where N_{rs} is baseline renin secretion and ν_{AT1R} is the feedback of [AT1R-bound Ang II] on renin secretion from Ref. [90]. Plasma renin concentration (PRC) is modeled by

$$\frac{d[\text{PRC}]}{dt} = R_{\text{sec}} - \frac{\ln(2)}{h_{\text{renin}}} \times [\text{PRC}] \quad (6.5)$$

where h_{renin} denotes the half life of renin as in Ref. [90].

AGT is synthesized in the liver and is stimulated by estrogen [63, 75, 76]. To model the impact of estrogen on AGT, we multiply the production of AGT, denoted by k_{AGT} , by $g_{\text{AGT}}^+(E)$ so that [AGT] is modeled by

$$\frac{d[\text{AGT}]}{dt} = g_{\text{AGT}}^+(E) \times k_{\text{AGT}} - \text{PRA} - \frac{\ln(2)}{h_{\text{AGT}}} \times [\text{AGT}] \quad (6.6)$$

where

$$\text{PRA} = X_{\text{PRCPRA}} \times [\text{PRC}] \times \frac{[\text{AGT}]}{K_{\text{AGT0}} + [\text{AGT}]} \quad (6.7)$$

is plasma renin activity (PRA) with parameter X_{PRCPRA} and h_{AGT} is the half-life for AGT as in Ref. [90]. The equation for PRA is a slight modification from the equation used in

Refs. [90, 131] to include the impact of AGT on PRA as well. The K_{AGT0} parameter was fit to predict the same model output in the original blood pressure model from Ref. [131]. In post-menopausal women, AGT levels decline from pre-menopause levels [63].

ACE converts Ang I into Ang II. Researchers have reported that both ACE expression and activity are inhibited by estrogen in rodent models [30, 31, 69, 101]. We let g_{ACE}^- represent the inhibition of estrogen levels on ACE activity so that the concentration of Ang I and Ang II are modeled by

$$\frac{d[\text{AngI}]}{dt} = \text{PRA} - (g_{\text{ACE}}^-(E) \times c_{\text{ACE}} + c_{\text{chym}} + c_{\text{nep}})[\text{AngI}] - \frac{\ln(2)}{h_{\text{AngI}}}[\text{AngI}] \quad (6.8)$$

$$\begin{aligned} \frac{d[\text{AngII}]}{dt} &= (g_{\text{ACE}}^-(E) \times c_{\text{ACE}} + c_{\text{chym}})[\text{AngI}] \\ &\quad - (c_{\text{ACE2}} + c_{\text{II,IV}} + g_{\text{AT1}}^-(E) \times c_{\text{AT1}} + g_{\text{AT2}}^+(E) \times c_{\text{AT2}})[\text{AngII}] \\ &\quad - \frac{\ln(2)}{h_{\text{AngII}}}[\text{AngII}] + k_{\text{inf}} \end{aligned} \quad (6.9)$$

where c_i denotes the corresponding enzyme activities and h_{AngI} and h_{AngII} are the half lives of Ang I and Ang II, respectively, as in Ref. [90]. k_{inf} is an input used for Ang II infusion (see Section 6.2.3) and is set to 0 in simulations with no Ang II infusion.

Ang II is reported to remain around the same in aging humans [106]. Ovariectomized rats showed a small decrease in plasma Ang II levels, which was reversed by estrogen replacement at pre-ovariectomy levels [218, 240]. We note that Ang II levels have been reported in post-menopausal women on estrogen replacement therapy, but researchers have reported significant differences in the impact of estrogen replacement therapy on the RAS when taking via oral or transdermal therapy [103]. This is likely due to the high exposure to estrogen in the liver when ingesting oral estrogen [103].

Ang II binds to AT1R and AT2R, triggering different physiological responses. Estrogen inhibits Ang II binding to AT1R, but activates Ang II binding to AT2R [75, 101, 192]. AT1R density is increased over 2-fold in ovariectomized rats [156]. We based our parameter value for e_{AT1} on these results. AT1R-bound Ang II ($[\text{AT1R-AngII}]$) and AT2R-bound Ang II ($[\text{AT2R-AngII}]$) concentrations are modeled by

$$\frac{d[\text{AT1R} - \text{AngII}]}{dt} = g_{\text{AT1}}^-(E) \times c_{\text{AT1}}[\text{AngII}] - \frac{\ln(2)}{h_{\text{AT1}}}[\text{AT1R} - \text{AngII}] \quad (6.10)$$

$$\frac{d[\text{AT2R} - \text{AngII}]}{dt} = g_{\text{AT2}}^+(E) \times c_{\text{AT2}}[\text{AngII}] - \frac{\ln(2)}{h_{\text{AT2}}}[\text{AT2R} - \text{AngII}] \quad (6.11)$$

with h_{AT1} and h_{AT2} denoting the half-lives of AT1R-bound Ang II and AT2R-bound Ang II from Ref. [90]. c_{AT1} and c_{AT2} denote the binding rates of Ang II to AT1R and AT2R, respectively, as in Ref. [90]. g_{AT1}^- and g_{AT2}^+ represent the impact of estrogen on the respective binding rate.

We chose parameter values for e_i based on reported changes in the respective RAS levels in the literature as described above. The values for e_i for each RAS component impacted by estrogen levels are listed in Table 6.1.

Name	Value	Equation(s)
e_{AGT}	0.15	g_{AGT}^+ , Eq. 6.6
e_{renin}	0.15	g_{renin}^- , Eq. 6.4
e_{ACE}	5.0	g_{ACE}^- , Eqs. 6.8,6.9
e_{AT1}	0.5	g_{AT1}^- , Eqs. 6.9,6.10
e_{AT2}	0.05	g_{AT2}^+ , Eqs. 6.9,6.11

Table 6.1: Values of the parameters e_i that determine the impact of estrogen on renin-angiotensin system components. The e_i parameters are unitless.

Renin-angiotensin system inhibitor simulations

To simulate the RAS inhibitors, we use the same approach as in Ref. [90]. Specifically, to simulate treatment with an angiotensin receptor blockers (ARB), we let

$$c_{\text{AT1}} = c_{\text{AT1},0} \times (1 - \sigma_{\text{ARB}}) \quad (6.12)$$

where $c_{\text{AT1},0}$ is the baseline value for c_{AT1} and σ_{ARB} is the percent inhibition of AT1R binding by the ARB. Similarly, to simulate treatment with an angiotensin-converting enzyme inhibitors (ACEi), we let

$$c_{\text{ACE}} = c_{\text{ACE},0} \times (1 - \sigma_{\text{ACEi}}) \quad (6.13)$$

where $c_{\text{ACE},0}$ is the baseline value for c_{ACE} and σ_{ACEi} is the percent inhibition of ACE by the ACEi.

6.2.2 Calcium homeostasis and bone biology model

The mathematical model developed by Peterson and Riggs [165] consists of detailed bone, renal, and blood compartments, and predicts levels of PTH, calcitriol, bone cell activity,

calcium, and phosphate. In this study, we primarily used the original model from Peterson and Riggs [165] for the calcium homeostasis and bone remodeling components with the following modifications from published model extensions:

- Gaweda et al. [72] extended this model to include other known regulatory mechanisms on calcium and phosphate homeostasis. In this study, we also include the impact of phosphate levels on parathyroid gland capacity as added by Gaweda et al. [72] (equation for $H_{5,11}$ in Ref. [72]). Note that Gaweda et al. [72] used the Peterson and Riggs [165] model as is with their extensions so all other parameters and biological components are the same as in Ref. [165].
- A compartment for bone mineral density was added by Riggs et al. [186] and is included in the model in this study.
- Riggs et al. [186] added the impact of estrogen on bone remodeling and calcium homeostasis to the model in Ref. [165]. The authors included the impact of estrogen on latent and active transforming growth factor- β (TGF- β), osteoblast signaling, and renal calcium handling. TGF- β is a key cytokine in bone remodeling that regulates osteoblast and osteoclast proliferation and activity. Estrogen inhibits latent TGF- β formation, while stimulating its activation. TGF- β impacts bone remodeling activity through stimulating the expression of receptor activator of nuclear factor kappa-B (RANK) on osteoclasts, stimulating osteoclast apoptosis, stimulating pre-osteoblast formation, and inhibiting osteoblast proliferation. Estrogen directly inhibits both the formation of pre-osteoblasts as well as the apoptosis rate of osteoblasts. Riggs et al. [186] also included the impact of estrogen on renal calcium reabsorption by decreasing renal calcium reabsorption with declining estrogen levels post-menopause. All of these estrogen impacts were included in the model use in this study.

A model schematic is shown in Fig. 6.1 and model equations are listed in Appendix E.4.

6.2.3 Composite calcium homeostasis, bone remodeling, and renin-angiotensin system model

To formulate a composite model, we connected the estrogen-RAS model (Section 6.2.1) with the calcium homeostasis and bone biology model (Section 6.2.2) through known regulatory mechanisms. Specifically, the composite model includes the impact of AT1R-bound

Ang II on PTH secretion, the impacts of extracellular calcium levels and the calciotropic hormones on renin secretion, and the impact of AT1R-bound Ang II on bone remodeling.

To represent those interactions, in the same way as Ref. [165], we characterize the changes in responses influenced by a given stimulant using hyperbolic functions:

$$H_x^- = \alpha - (\alpha - \rho) \times \frac{x^\gamma}{\delta^\gamma + x^\gamma} \quad (6.14)$$

$$H_x^+ = \rho + (\alpha - \rho) \times \frac{x^\gamma}{\delta^\gamma + x^\gamma} \quad (6.15)$$

where x denotes the stimulus variable, α is the maximum anticipated response, γ is the steepness of the response (note when $\gamma = 1$ the functions are sigmoidal), δ is the value of x that produces the half-maximal response, and ρ is the minimum anticipated response. The minus or plus superscript in H denotes whether the response is a decrease or increase from the baseline steady state, respectively. Note that when $\rho = 0$, the response is a classical $E_{\max}$ response often used in modeling in pharmacology (see Ref. [22]). Parameter values for the equations added in this study are listed in Table 6.2. Additional parameter values can be found in Refs. [72, 165, 186] or using our model code (see Section 6.2.5).

AT1R-bound Ang II impact on PTH secretion

AT1R-bound Ang II concentration, denoted by [AT1R-AngII], increases PTH secretion from the parathyroid gland [222]. To model this effect, we let $H_{\text{AT1R,PTH}}^+$ denote the impact of AT1R-bound Ang II on PTH secretion:

$$H_{\text{AT1R,PTH}}^+ = \rho_{\text{AT1R,PTH}} + (\alpha_{\text{AT1R,PTH}} - \rho_{\text{AT1R,PTH}}) \times \frac{[\text{AT1R} - \text{AngII}]^{\gamma_{\text{AT1R,PTH}}}}{\delta_{\text{AT1R,PTH}}^{\gamma_{\text{AT1R,PTH}}} + [\text{AT1R} - \text{AngII}]^{\gamma_{\text{AT1R,PTH}}}}. \quad (6.16)$$

where the parameters correspond to the function form of type Eq. 6.15. PTH concentration ([PTH]) in the blood is modeled by

$$\frac{d[\text{PTH}]}{dt} = S_{\text{PTH}} \times H_{\text{AT1R,PTH}}^+ - k_{\text{deg-PTH}} \times [\text{PTH}] \quad (6.17)$$

where S_{PTH} is the secretion of PTH from the parathyroid gland and $k_{\text{deg-PTH}}$ is degradation as in Ref. [165]. The parameters for Eq. 6.16 were fit using the Ang II infusion experiment data from Grant et al. [80] (Section 6.2.3). The parameter values are listed in Table 6.2.

Name	Units	Value	Equation
$\alpha_{\text{AT1R,PTH}}$	—	3.2	Eq. 6.16
$\rho_{\text{AT1R,PTH}}$	—	0.7	Eq. 6.16
$\delta_{\text{AT1R,PTH}}$	pmol/L	10.0	Eq. 6.16
$\gamma_{\text{AT1R,PTH}}$	—	1.5	Eq. 6.16
$\alpha_{\text{CaSR,renin}}$	—	1.2	Eq. 6.18
$\rho_{\text{CaSR,renin}}$	—	0.25	Eq. 6.18
$\delta_{\text{CaSR,renin}}$	mmol/L	2.6	Eq. 6.18
$\gamma_{\text{CaSR,renin}}$	—	5.0	Eq. 6.18
$\alpha_{\text{PTH,renin}}$	—	2.5	Eq. 6.19
$\rho_{\text{PTH,renin}}$	—	0.5	Eq. 6.19
$\delta_{\text{PTH,renin}}$	pmol/L	4.0	Eq. 6.19
$\gamma_{\text{PTH,renin}}$	—	3.0	Eq. 6.19
$\alpha_{\text{Ctriol,renin}}$	—	1.1	Eq. 6.20
$\rho_{\text{Ctriol,renin}}$	—	0.8	Eq. 6.20
$\delta_{\text{Ctriol,renin}}$	pmol/L	180	Eq. 6.20
$\gamma_{\text{Ctriol,renin}}$	—	1.0	Eq. 6.20
$\alpha_{\text{AT1R,RANKL}}$	—	5.0	Eq. 6.22
$\rho_{\text{AT1R,RANKL}}$	—	0.95	Eq. 6.22
$\delta_{\text{AT1R,RANKL}}$	pmol/L	16.0	Eq. 6.22
$\gamma_{\text{AT1R,RANKL}}$	—	3.0	Eq. 6.22
$\alpha_{\text{AT1R,OPG}}$	—	2.5	Eq. 6.23
$\rho_{\text{AT1R,OPG}}$	—	0.9	Eq. 6.23
$\delta_{\text{AT1R,OPG}}$	pmol/L	14	Eq. 6.23
$\gamma_{\text{AT1R,OPG}}$	—	2.0	Eq. 6.23

Table 6.2: Parameter values for coupling the renin-angiotensin system components to the calcium homeostasis and bone remodeling model. Additional parameters can be found in Refs. [72, 165].

Calcitropic hormones, calcium, and renin secretion

Renin is secreted from specialized cells in the kidneys called juxtaglomerular cells. Renin secretion, denoted by R_{sec} , is impacted by PTH, calcitriol (Ctriol), and extracellular calcium levels. Specifically, increased extracellular calcium and [Ctriol] inhibit renin secretion, while [PTH] stimulates renin secretion [222].

Extracellular calcium levels are detected by the CaSR in the juxtaglomerular cells [91]. To model the impact of CaSR on R_{sec} we let:

$$H_{\text{CaSR,renin}}^- = \alpha_{\text{CaSR,renin}} - (\alpha_{\text{CaSR,renin}} - \rho_{\text{CaSR,renin}}) \times \frac{[\text{Ca}]^{\gamma_{\text{CaSR,renin}}}}{\delta_{\text{CaSR,renin}}^{\gamma_{\text{CaSR,renin}}} + [\text{Ca}]^{\gamma_{\text{CaSR,renin}}}}. \quad (6.18)$$

Ortiz-Capisano et al. [160, 161] found that renin release was about 40% lower when using a calcimimetic or at elevated calcium levels in the juxtaglomerular cells of the kidneys. We used this result to estimate parameters for Eq. 6.18 so that at high calcium levels, renin release is decreased similarly.

To model stimulation of R_{sec} by [PTH] we let:

$$H_{\text{PTH,renin}}^+ = \rho_{\text{PTH,renin}} + (\alpha_{\text{PTH,renin}} - \rho_{\text{PTH,renin}}) \times \frac{[\text{PTH}]^{\gamma_{\text{PTH,renin}}}}{\delta_{\text{PTH,renin}}^{\gamma_{\text{PTH,renin}}} + [\text{PTH}]^{\gamma_{\text{PTH,renin}}}}. \quad (6.19)$$

The parameters (listed in Table 6.2) were fit using the PTH only infusion experiment from Grant et al. [80] (Section 6.2.3).

To model inhibition of R_{sec} by calcitriol we let:

$$H_{\text{Ctriol,renin}}^- = \alpha_{\text{Ctriol,renin}} - (\alpha_{\text{Ctriol,renin}} - \rho_{\text{Ctriol,renin}}) \times \frac{[\text{Ctriol}]^{\gamma_{\text{Ctriol,renin}}}}{\delta_{\text{Ctriol,renin}}^{\gamma_{\text{Ctriol,renin}}} + [\text{Ctriol}]^{\gamma_{\text{Ctriol,renin}}}}. \quad (6.20)$$

The parameters were chosen (listed in Table 6.2) based on the data from Tomaschitz et al. [220] that related calcitriol levels with renin concentration. Taken together, we incorporate the combined impact of calcium, PTH, and calcitriol on renin secretion by modifying Eq. 6.4 to

$$R_{\text{sec}} = N_{\text{rs}} \times \nu_{\text{AT1R}} \times g_{\text{renin}}^-(E) \times H_{\text{CaSR,renin}}^- \times H_{\text{PTH,renin}}^+ \times H_{\text{Ctriol,renin}}^-. \quad (6.21)$$

AT1R-bound Ang II activation of RANKL and OPG

Bone remodeling is primarily regulated by osteoblasts and osteoclasts. Osteoblasts develop the bone matrix, while osteoclasts break down bone tissue. Osteoblasts express receptor

activator of nuclear factor kappa-B ligand (RANKL), which binds to its receptor RANK on osteoclasts to promote osteoclast differentiation and activation. Osteoblasts also produce osteoprotegerin (OPG), a decoy receptor that binds to RANKL, preventing its binding with RANK and thereby inhibiting osteoclast activity. The balance between RANKL and OPG is essential for maintaining bone homeostasis as an increase in RANKL or decrease in OPG can lead to excessive or reduced bone resorption, respectively. This system is often referred to as the RANK-RANKL-OPG system and is illustrated in Fig. 6.1.

Shimizu et al. [197] reported that in ovariectomized rats, infusion of Ang II increased RANKL expression and led to a significant decrease in bone volume, but this impact was abolished by treatment with an AT1R blocker and not an AT2R blocker. Their results showed that RANKL production is activated by AT1R-bound Ang II levels. Additionally, Shimizu et al. [197] found that OPG expression is increased in osteoblast cells, but to a much lesser extent than RANKL.

The impact of AT1R-bound Ang II on RANKL and OPG expression are modeled using the form in Eq. 6.15 for activation so that:

$$H_{\text{AT1R,RANKL}}^+ = \rho_{\text{AT1R,RANKL}} + (\alpha_{\text{AT1R,RANKL}} - \rho_{\text{AT1R,RANKL}}) \times \frac{[\text{AT1R} - \text{AngII}]^{\gamma_{\text{AT1R,RANKL}}}}{\delta_{\text{AT1R,RANKL}}^{\gamma_{\text{AT1R,RANKL}}} + [\text{AT1R} - \text{AngII}]^{\gamma_{\text{AT1R,RANKL}}}}, \quad (6.22)$$

$$H_{\text{AT1R,OPG}}^+ = \rho_{\text{AT1R,OPG}} + (\alpha_{\text{AT1R,OPG}} - \rho_{\text{AT1R,OPG}}) \times \frac{[\text{AT1R} - \text{AngII}]^{\gamma_{\text{AT1R,OPG}}}}{\delta_{\text{AT1R,OPG}}^{\gamma_{\text{AT1R,OPG}}} + [\text{AT1R} - \text{AngII}]^{\gamma_{\text{AT1R,OPG}}}}. \quad (6.23)$$

These are included in the equations for the production of RANKL and OPG, respectively (Eqs. E.38 and E.40 in Appendix E.4). The values for $\alpha_{\text{AT1R,RANKL}}$ and $\alpha_{\text{AT1R,OPG}}$ represent the highest possible amount of activation of RANKL and OPG by AT1R binding, respectively. These values were set to the fold-change in RANKL and OPG expression in Shimizu et al. [197]. The parameter values are listed in Table 6.2.

Parameter fitting to PTH and Ang II infusion experiments

Grant et al. [80] conducted infusion experiments in healthy volunteers to determine the relationship between the RAS and PTH in humans. To test this, the authors gave healthy volunteers 1 hour infusions on different days of (i) Ang II only, (ii) PTH (i.e., teriparatide) only, and (iii) Ang II and PTH together. We simulated these experiments and used the

data from this study to determine parameters values (listed in Table 6.2) for Eqs. 6.16, 6.18, and 6.19.

In their experiments, Ang II infusions were given in graded doses of 1, 3, and 10 ng/kg·min for infusions of 20 minutes each. We simulated Ang II infusion by letting $k_{\text{inf}} = 1$ ng/kg·min (Eq. 6.9) for the first 20 minutes, 3 ng/kg·min for the next 20 minutes, and 10 ng/kg·min for the final 20 minutes of the 1 hour infusion to simulate this infusion type. For PTH infusions we used the pharmacokinetic parameters from Peterson and Riggs [165] with a dose of 200 U of teriparatide as reported by Grant et al. [80]. To fit the parameters, we used the `fmincon` function from MATLAB using the `interior-point` algorithm to minimize the difference between the predicted values and the data for PRA, endogenous PTH, and [Ang II] reported in Grant et al. [80]. In all of these simulations we let $E=1$ so that there are no estrogen effects.

6.2.4 Sobol sensitivity analysis

Mathematical models of complex biological systems, like the one used in this study, involve a large number of parameters with unknown or uncertain values. To investigate the impact of variations in parameter values on model output, we can use methods such as sensitivity analysis [176, 191]. Global sensitivity analysis methods evaluate the model at many points in the parameter space, providing an assessment of how individual parameters contribute to variations in model output across the parameter space, including interactions with other parameter changes [176, 182, 191].

The Sobol method is a variance-based global sensitivity analysis technique that decomposes the variance in the model output into contributions from each individual parameter as well as its interactions with other parameters [176, 200]. The results of a Sobol sensitivity analysis are called “Sobol sensitivity indices” (or “Sobol indices” for short) and are computed for each parameter included in the analysis. First-order Sobol indices (denoted by S_{first}) are the fraction of the variance in the model output the individual parameter contributes to. Total order Sobol indices (denoted by S_{total}) quantify the fraction of output variance the parameter contributes to, including interactions with other parameter changes. By evaluating Sobol indices, we can identify which parameters are the most influential on model output. For more information about Sobol sensitivity analysis and its use in applications in biology, we refer readers to Refs. [176, 182, 191, 200].

We conducted a Sobol sensitivity analysis on the full composite model (described in Section 6.2.3). The model outputs we considered are bone mineral density, AT1R-bound Ang II, PTH, calcitriol, extracellular calcium, and renin concentration over a simulation

time from 20 to 80 years with age-related estrogen decline (i.e., same as the “no RASi” simulations in Figs. 6.3, 6.4). We computed Sobol indices at every 10 years of simulation time. The full composite model involves 181 parameters, so running a Sobol sensitivity analysis over all the parameters can lead to large numerical errors and computation time. To reduce the number of parameters used in the full Sobol sensitivity analysis, we applied a two-step approach:

1. We first conducted a Sobol sensitivity analysis varying all parameters only in the calcium homeostasis and bone biology model described in Section 6.2.2 by $\pm 10\%$. This includes the baseline biological components of bone remodeling (including the detailed impacts of RANKL, RANK, OPG, and TGF- β on osteoblast and osteoclast activity), PTH secretion and degradation, calcitriol synthesis and degradation, renal calcium and phosphate reabsorption, intestinal calcium and phosphate absorption, and extracellular calcium and phosphate levels. We screened the 121 parameters included in this analysis for parameters that had an S_{first} value of greater than 1% in at least one of the outputs of interest (bone mineral density, AT1R-bound Ang II, PTH, calcitriol, or calcium) at some point in the simulation. This resulted in 30 sensitive parameters.
2. We then included all the new parameters from this study (listed in Tables 6.1 and 6.2) with the 30 parameters selected from the initial screening in Step 1, resulting in 59 total parameters. We conducted a Sobol sensitivity analysis for the full composite model with parameters varied by $\pm 25\%$ of their baseline value.

The results of the Sobol sensitivity analysis are described in Section 6.3.3.

6.2.5 Code availability

All model code used in this study is available at <https://github.com/Layton-Lab/est-ras-bone>. Model code and model simulations were conducted using MATLAB R2022a. The Sobol sensitivity analysis was conducted using Simbiology. Data originally presented in graphs from published papers (e.g., the data from Grant et al. [80] shown in Fig. 6.2) were extracted using Web Plot Digitizer (version 4.7).

6.3 Results

6.3.1 PTH and Ang II infusion experiments simulations

We conducted simulations of infusion of (i) Ang II, (ii) PTH, and (iii) PTH and Ang II together (described in Section 6.2.3). Model results, together with the experimental data reported for the clinical studies done by Grant et al. [80], are shown in Fig. 6.2.

When Ang II is infused in isolation, Ang II levels rise resulting in an increase in AT1R-bound Ang II levels (Fig. 6.2B, D), which stimulates PTH secretion so that endogenous PTH (intact PTH, denoted by [iPTH]) increases (Fig. 6.2C). Grant et al. [80] reported that during an Ang II infusion, [iPTH] increased, while PRA decreased slightly likely due to the negative feedback on renin secretion by AT1R-bound Ang II (Fig. 6.2 “Ang II only” data). Model simulations capture both the rise in [iPTH] (Fig. 6.2C “Ang II only”) and slight decrease in PRA (Fig. 6.2A “Ang II only”) during infusion.

When infusing PTH in healthy volunteers, PRA nearly doubled on average (Fig. 6.2A “PTH only” data) [80]. Model simulations predicted a 69% increase in PRA (Fig. 6.2A “PTH only”). While total PTH levels increase substantially during the PTH infusion experiment, endogenous PTH production steadily decreased (Fig. 6.2C “PTH only” data). Model simulations did not result in a significant decrease [iPTH]. That discrepancy may be attributed to a more detailed impact of PTH on renal calcium reabsorption that is not represented in the model, which would increase calcium levels and thus decrease PTH secretion via the CaSR.

When infusing both Ang II and PTH together in healthy volunteers, the authors reported that Ang II levels were similar to those reported in the Ang II only experiment, likely due to PTH having only a small impact on Ang II levels [80]. In our model simulations, Ang II levels were slightly higher in the Ang II and PTH infusion experiment than the Ang II only experiment, but were still within the range reported by Grant et al. [80] at the end of the 1 hour infusion time (Fig. 6.2B “Ang II + PTH”). Grant et al. [80] reported that PRA levels remained near baseline for the Ang II and PTH infusion experiment. In our model simulations, the “Ang II + PTH” simulations resulted in a transient increase in PRA, but declines to near the levels prior to infusion by the end of the simulation time (Fig. 6.2A).

To summarize, model parameters were chosen to fit the observed responses of key RAS components and PTH following Ang II and PTH infusions with baseline estrogen levels.

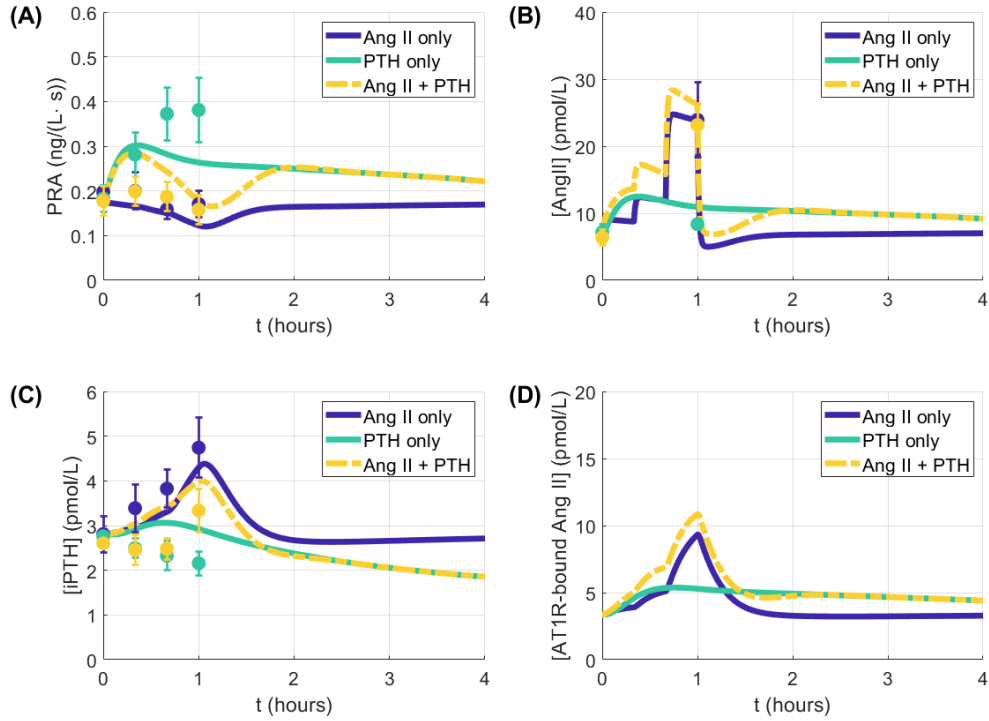


Figure 6.2: Simulation results for infusion of Ang II (“Ang II only”), PTH (“PTH only”), and Ang II and PTH together (“Ang II + PTH”). Results for (A) plasma renin activity, (B) angiotensin II plasma concentration, (C), intact parathyroid hormone, and (D) angiotensin receptor type 1-bound Ang II are shown for during and after 1 hour infusions. Lines indicate simulations, while the dots and error bars in panels A–C are from the relevant experimental data presented in Grant et al. [80]. Replication of these simulations for a longer simulation time is shown in Fig. E.3. Ang II: angiotensin II, AT1R: angiotensin receptor type 1, iPTH: intact parathyroid hormone, PRA: plasma renin activity

6.3.2 Impact of estrogen decline and RAS inhibitors

How do declining estrogen levels in post-menopausal women impact the RAS? How do changes in the RAS and declining estrogen levels contribute to altered bone remodeling? Does bone mineral density improve with RAS inhibition? To investigate these questions, we conducted simulations of the composite calcium homeostasis, bone remodeling, and estrogen-RAS model (Section 6.2.3) with estrogen decline observed in post-menopausal women with and without drug interventions.

In each simulation, estrogen decline starts at 50 years, which is about the average age of menopause onset in women. We conducted simulations with key RAS inhibitors (ACEi and ARB) starting at age 60 years, unless otherwise indicated. For ARB simulations, we let σ_{ARB} (Eq. 6.12) vary from 88–98.3% as reported for different types and dose levels of common types of ARB by Hallow et al. [90]. For ACEi, we conducted simulations with σ_{ACEi} (Eq. 6.13) varying from 94–97.2% as reported for common types of ACEi and doses by Hallow et al. [90]. The results are shown in Figs. 6.3 and 6.4.

ACEi inhibit ACE activity and ARB inhibit AT1R binding. While these are different components of the RAS, both drugs result in reduced levels of AT1R-bound Ang II (Fig. 6.3E). The impact of these drugs on the various components of the RAS are drug and dose-dependent. The varied range in values of σ_{ACEi} had a negligible effect on our model simulation results (Fig. 6.3, “ACEi”). In the ARB simulations, the impact on the RAS has a wider variation based on the value of σ_{ARB} (Fig. 6.3, “ARB”). Note that we simulated a wider range for σ_{ARB} than σ_{ACEi} .

We first consider the impact of ACEi and ARB on the RAS components. ARB directly inhibit the binding of Ang II to AT1R. As a result, AT1R-bound Ang II levels decreased by about 66–89%, depending on efficacy (Fig. 6.3E, “ARB” vs. “no RASi”). Recall that AT1R-bound Ang II inhibits renin secretion (Fig. 6.1). Hence, decreased AT1R-bound Ang II levels result in increased renin levels (Fig. 6.3B) as well as increased downstream Ang I (Fig. 6.3C) and Ang II levels (Fig. 6.3D). The lower AT1R binding along with increased Ang II levels results in more Ang II binding to AT2R, so that AT2R-bound Ang II levels increase by about 4-fold on average (Fig. 6.3F “ARB” vs. “no RASi”). ACEi impact the RAS by inhibiting the activity of ACE, which is the enzyme that converts Ang I into Ang II (Fig. 6.1). Ang II levels decreased by an average of 52% in the ACEi simulations despite increased Ang I levels (Fig. 6.3D, C “ACEi” vs. “no RASi”). As a result, both AT2R-bound Ang II and AT1R-bound Ang II decreased by about 52% from the no RAS inhibitor case (Fig. 6.3E–F, “ACEi” vs. “no RASi”).

Next, we analyze the age-related estrogen decline effect on bone mineral density and its response to ACEi and ARB. Estrogen maintains bone density by regulating factors

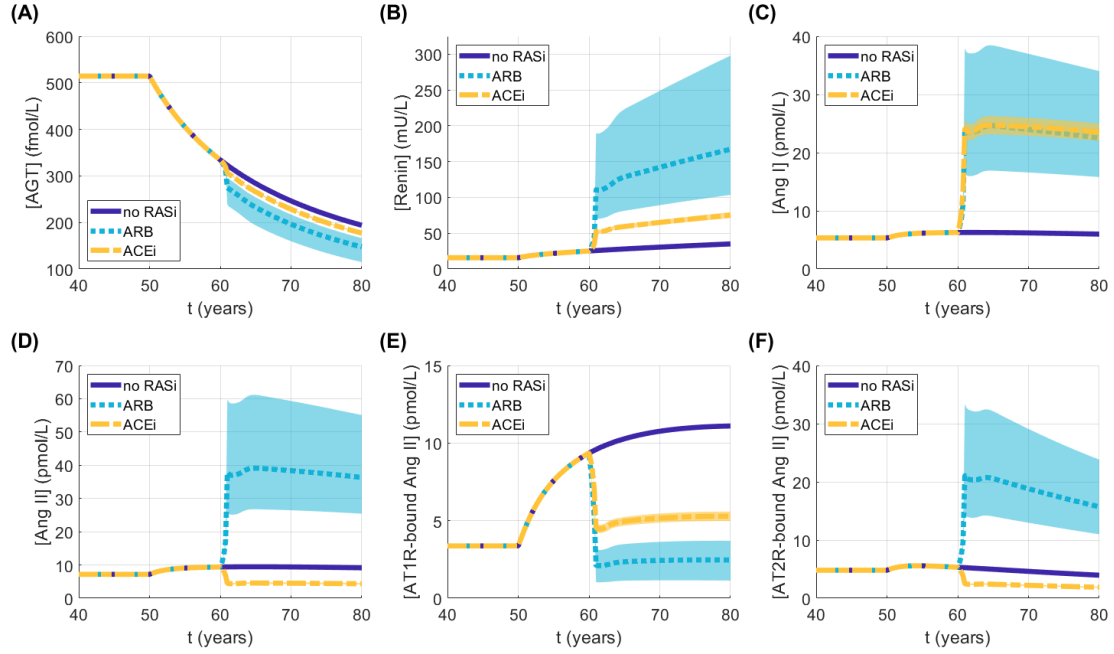


Figure 6.3: Impact of post-menopausal estrogen decline and renin-angiotensin system inhibitors on the renin-angiotensin system. For the angiotensin receptor blocker simulations (labeled “ARB” in the legend), σ_{ARB} (Eq. 6.12) was varied between 88–98.3% based on the ranges reported in Ref. [90]. For the angiotensin-converting enzyme inhibitor simulations (labeled “ACEi” in the legend), σ_{ACEi} (Eq. 6.13) was varied between 94–97.2% as reported in Ref. [90]. Lines represent the mean values over the varied σ_i values for the respective treatment with shaded area representing the range. AGT: angiotensinogen, Ang I: angiotensin I, Ang II: angiotensin II, AT1R: angiotensin type 1 receptor, AT2R: angiotensin type 2 receptor, RAS: renin-angiotensin system, RASi: RAS inhibitor, ARB: angiotensin receptor blocker, ACEi: angiotensin-converting enzyme inhibitor

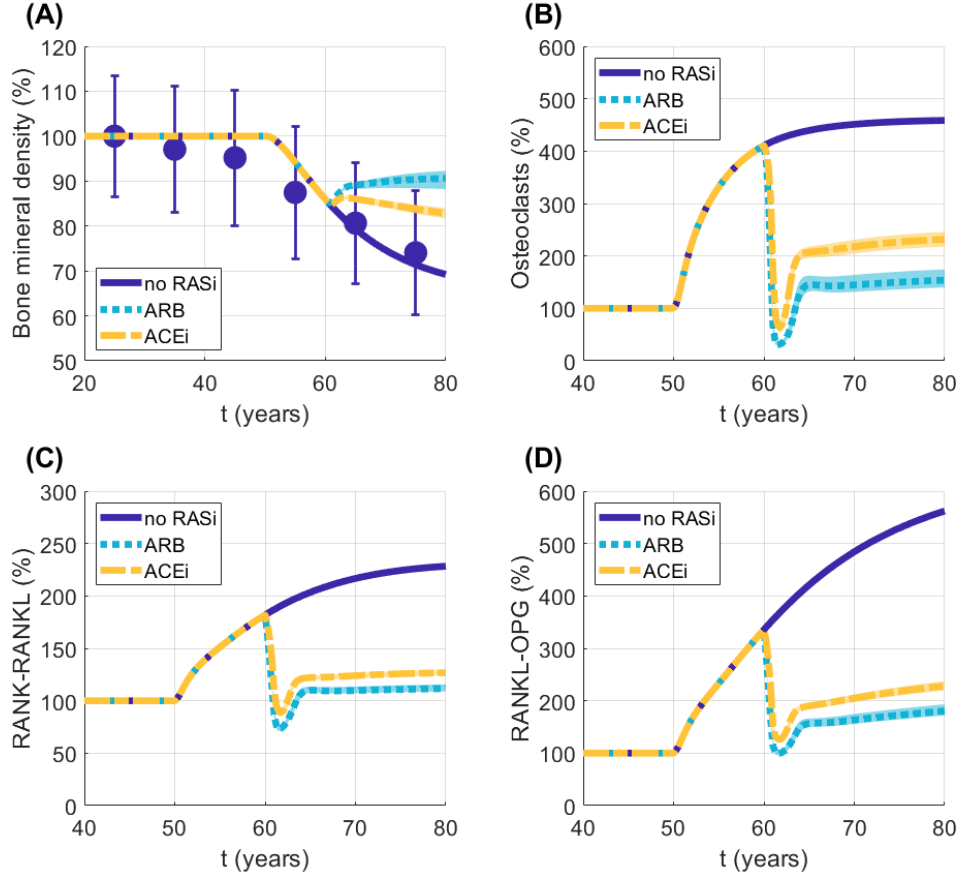


Figure 6.4: Impact of post-menopausal estrogen decline and renin-angiotensin system inhibitors on (A) bone mineral density, (B) osteoclast levels, (C) RANK-RANKL levels, and (D) RANK-OPG levels. The values are normalized to their baseline levels and shown as a percent of the baseline value. For the angiotensin receptor blocker simulations (labeled “ARB” in the legend), σ_{ARB} (Eq. 6.12) was varied between 88–98.3% based on the ranges reported in Ref. [90]. For the angiotensin-converting enzyme inhibitor simulations (labeled “ACEi” in the legend), σ_{ACEi} (Eq. 6.13) was varied between 94–97.2% as reported in Ref. [90]. Lines represent the mean values over the varied σ_i values for the respective treatment with shaded area representing the range. Data in panel (A) are used from the female data for femoral neck bone mineral density in Looker et al. [138]. The simulations and data are for femoral neck bone mineral density. BMD: bone mineral density, RANK: receptor activator of NF-kappaB, RANKL: RANK ligand, OPG: osteoprotegerin, RASi: RAS inhibitor, ARB: angiotensin receptor blocker, ACEi: angiotensin-converting enzyme inhibitor

involved in bone remodeling that promote the differentiation and activation of osteoblasts, while inhibiting osteoclast activity (Fig. 6.1). When estrogen levels decline, osteoclast activity increases, which increases bone resorption, ultimately resulting in a decrease in bone mineral density (Fig. 6.4A–B “no RASi”). The bone mineral density simulations results with the decline in estrogen levels is consistent with the data reported for aging women in Looker et al. [138] (Fig. 6.4A “no RASi” data). This decrease is a result of a combination of the direct impacts of estrogen on bone remodeling and increased AT1R-bound Ang II levels, which stimulates RANKL production to bind with RANK, forming RANK-RANKL (Fig. 6.4C; Fig. 6.3E “no RASi”). RANK-RANKL stimulates osteoclast differentiation and activation. Through estrogen decline simulations, the calciotropic hormones, PTH and calcitriol, as well as key electrolytes, calcium and phosphate, remained within normal range (Fig. E.4).

Age-related decreases in estrogen levels result in a large increase in osteoclast activity (Fig. 6.4B “no RASi”). However, with ARB treatment, AT1R-bound Ang II levels decrease (Fig. 6.3E), resulting in reduced RANKL production, so that osteoclast levels return to about 40% above baseline levels (Fig. 6.4B,C). As a result, by the end of the simulation time (i.e., $t = 80$ years), bone mineral density is at about 90% of baseline for ARB users versus 75% before estrogen decline with no treatment (Fig. 6.4A “ARB” vs. “no RASi”). ACEi also decreased AT1R-bound Ang II levels by a lesser amount (Fig. 6.3E) so that osteoclast levels are lowered to about 2-fold baseline levels. This results in an improvement in bone mineral density to about 85% of the level before estrogen decline (Fig. 6.4A “ACEi”). We observed that the range in the amount of AT1R-bound Ang II inhibition due to varied ARB efficacy did not have a large impact the change in bone levels on these drugs (Fig. 6.4; Fig. 6.3). It seems that the improvement to bone mineral density is not significantly dependent on the dose of the RAS inhibitors.

We conducted simulations of RAS inhibitor treatment starting at different ages with age-related estrogen decline as in Eq. 6.1. In these simulations, we let $\sigma_{\text{ARB}} = 93\%$ and $\sigma_{\text{ACEi}} = 95\%$ based on the average value for the respective drugs from Ref. [90]. We did this because varying σ_i did not have much impact on bone mineral density (Fig. 6.4A). The results are shown in Fig. 6.5. In all simulations, bone mineral density improved on both the ARB and ACEi drugs with a greater improvement with ARB treatment. When the administration of ARB or ACEi was started before the onset of estrogen decline (i.e., starting a RAS inhibitor before 50 years old), bone mineral density increased from baseline by about 5% (Fig. 6.5A1–A2). When estrogen decline started at 50 years, the bone mineral density decreased, but stays above 95% for patients on ARB (Fig. 6.5A1). When the administration of ACEi began before estrogen decline, bone mineral density declined below 90% of baseline during age-related estrogen decline (Fig. 6.5A2). Our model simulations

suggest that early RAS inhibition prevents bone loss driven by age-related estrogen decline.

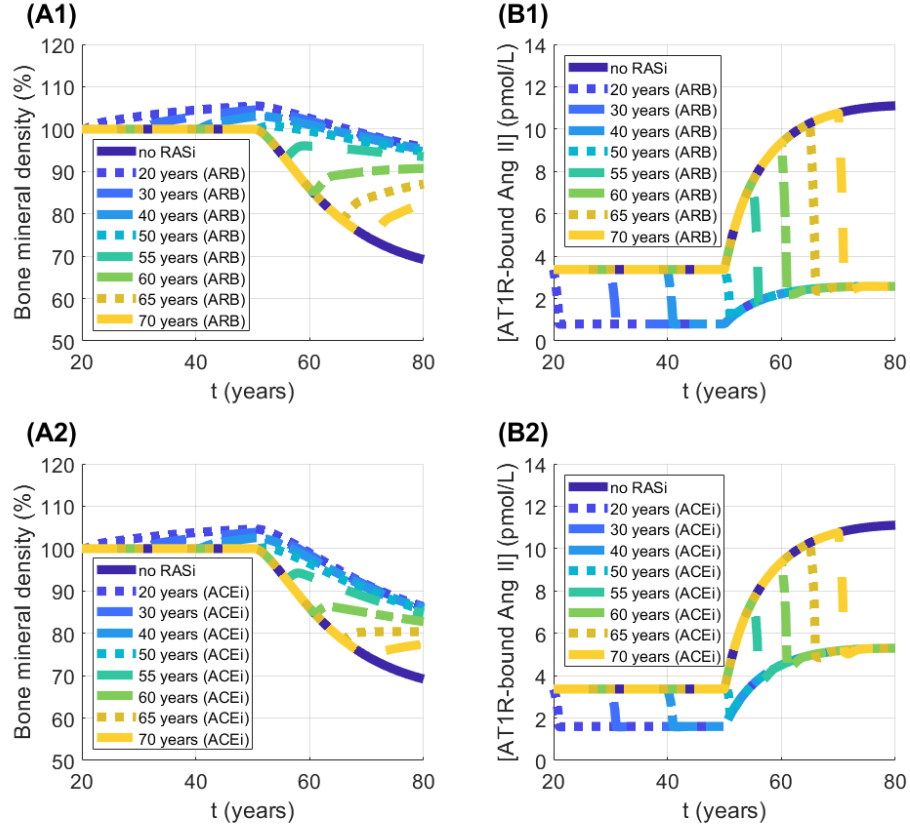


Figure 6.5: Model simulation results for varied starting age of ARB (A1–B1) and ACEi (A2–B2). $\sigma_{\text{ARB}} = 93\%$ in all simulations for ARB and $\sigma_{\text{ACEi}} = 95.6\%$ in all ACEi simulations. The bone mineral density is femoral neck bone mineral density. AT1R: angiotensin type 1 receptor, ARB: angiotensin receptor blocker, ACEi: angiotensin-converting enzyme inhibitor, RASi: RAS inhibitor

In summary, the RAS, calcium, and bone remodeling model developed in this study represents the expected decrease in bone mineral density due to age-related estrogen decline. This is driven by both the direct impacts of estrogen on bone remodeling and calcium regulation, as well as RAS activation by estrogen decline. Model simulations suggest that ARB offer stronger protection for bone health than ACEi, and that result is consistent when starting treatment at different ages.

6.3.3 Sensitivity analysis

We conducted a Sobol sensitivity analysis for the full composite model during age-related estrogen decline (see Section 6.2.4 for details). The parameters with the largest average S_{total} values in Fig. 6.6A are the parameters that have the largest impact on variation in bone mineral density. The top five parameters, based on the average S_{total} value, are all related to the regulation of RANK and RANKL (Fig. 6.6A). The RANK-RANKL complex directly impacts bone remodeling the stimulating osteoclasts (Fig. 6.1). Specifically, the parameters $k_{\text{in,L}}$ and $k_{\text{in,RNK}}$ are baseline production rates of RANKL and RANK, respectively. The parameter $E_{\text{max,L-PTH}}$ relates to the impact of PTH on RANKL production. The parameters k_3 and k_4 are the binding and unbinding rate of the RANK-RANKL complex, respectively. The next parameter with the largest impact on bone mineral density is $\rho_{\text{AT1R,L}}$, which represents the minimum impact of AT1R on RANKL production (Eq. 6.22). In summary, the top parameters that impact bone mineral density all primarily relate to RANK, RANKL and RANK-RANKL binding.

AT1R-bound Ang II is the key biological component that connects the RAS with the calcium homeostasis and bone remodeling model (Fig. 6.1). The parameters with the 7 largest average S_{first} and S_{total} values for the model output of [AT1R-bound Ang II] are $\delta_{\text{CaSR,renin}}$, $\Phi_{\text{Plas2BoneCa0}}$, $K_{\text{Ca,PTH}}$, $\Phi_{\text{Bone2PlasCa0}}$, N_{rs} , c_{AT1R} , and X_{PRCPRA} (Fig. 6.6B). $\delta_{\text{CaSR,renin}}$ represents half-maximal response of renin secretion to calcium levels (Eq. 6.18). $\Phi_{\text{Plas2BoneCa0}}$ and $\Phi_{\text{Bone2PlasCa0}}$ represent the baseline fluxes of calcium between the plasma and bone and both impact variation in calcium and renin levels (Fig. 6.7). Calcium level changes affect the RAS via the CaSR in the renin-secreting juxtaglomerular cells of the kidneys. $K_{\text{Ca,PTH}}$ is the half-maximal response of PTH secretion to calcium levels as mediated by the CaSR. This parameter plays a key role in variations in model output for [PTH], [Calcitriol], [Calcium], and [Renin] (Fig. 6.7 and 6.8), which all regulate the RAS. N_{rs} , c_{AT1R} , and X_{PRCPRA} are all directly related to the RAS (Section 6.2.1). Specifically, N_{rs} determines baseline renin secretion, c_{AT1R} is the binding rate for AT1R, and X_{PRCPRA} is baseline conversion rate from renin concentration to activity. Our sensitivity analysis shows that variations in [AT1R-bound Ang II] are driven by the CaSR impact on renin secretion and direct changes to the RAS components.

The calciotropic hormones are the primary regulators of calcium and bone remodeling. Additionally, these hormones have a bidirectional relationship with the RAS (Fig. 6.1). In our Sobol sensitivity analysis, the parameters with the largest impact on PTH had high S_{total} values relative to the respective S_{first} values. This is likely due to the large number of regulators on PTH. The parameters with the largest impact are $V_{\text{max,PTH-reabs}}$, $K_{\text{Ca,PTH}}$, and $V_{\text{PTH-AOH}}$ (Fig. 6.8A). $K_{\text{Ca,PTH}}$ denotes the half-maximal response of PTH secretion

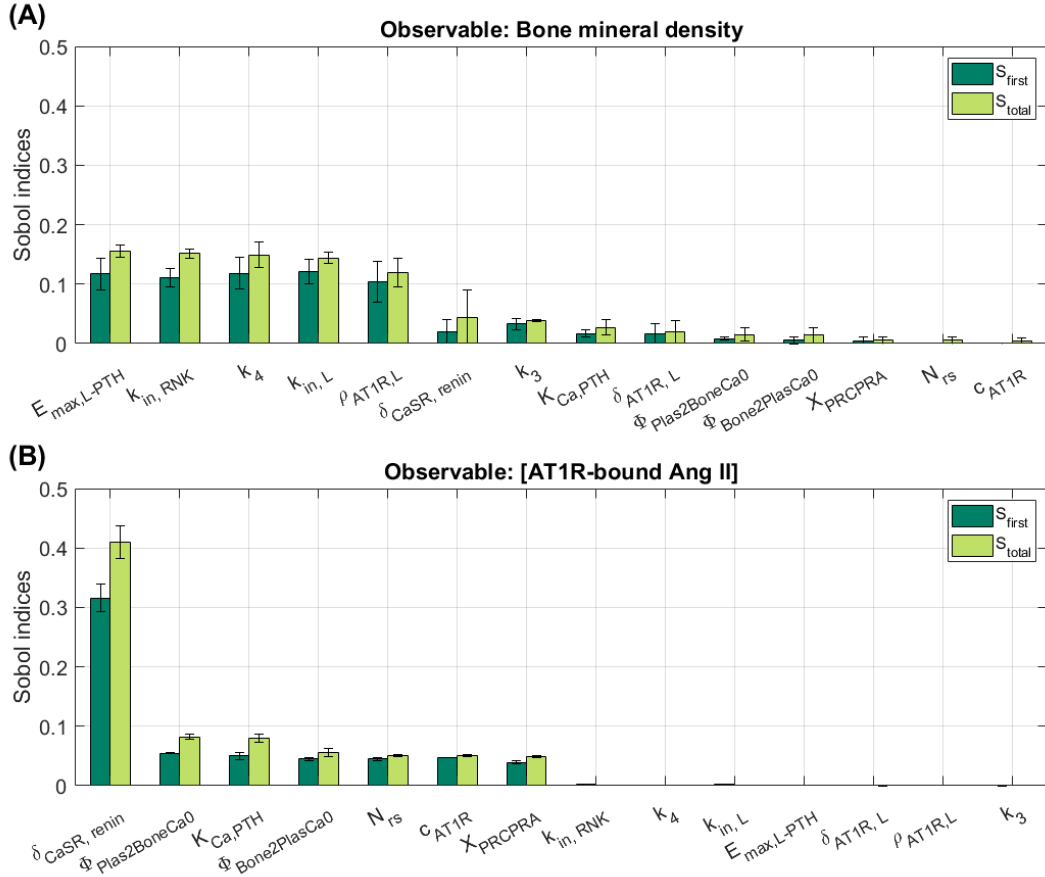


Figure 6.6: Mean (bar) and standard deviation (error bar) of first and total order Sobol indices (denoted by S_{first} and S_{total} in legend, respectively) of full model ran from age 20 to 80 years with estrogen decline for output variables (A) femoral neck bone mineral density and (B) AT1R-bound Ang II. Parameters shown had a maximal value of $S_{\text{total}} > 0.05$ for at least one output variable. A description of the parameters is given in Table E.1. AT1R: angiotensin type 1 receptor, Ang II: angiotensin II

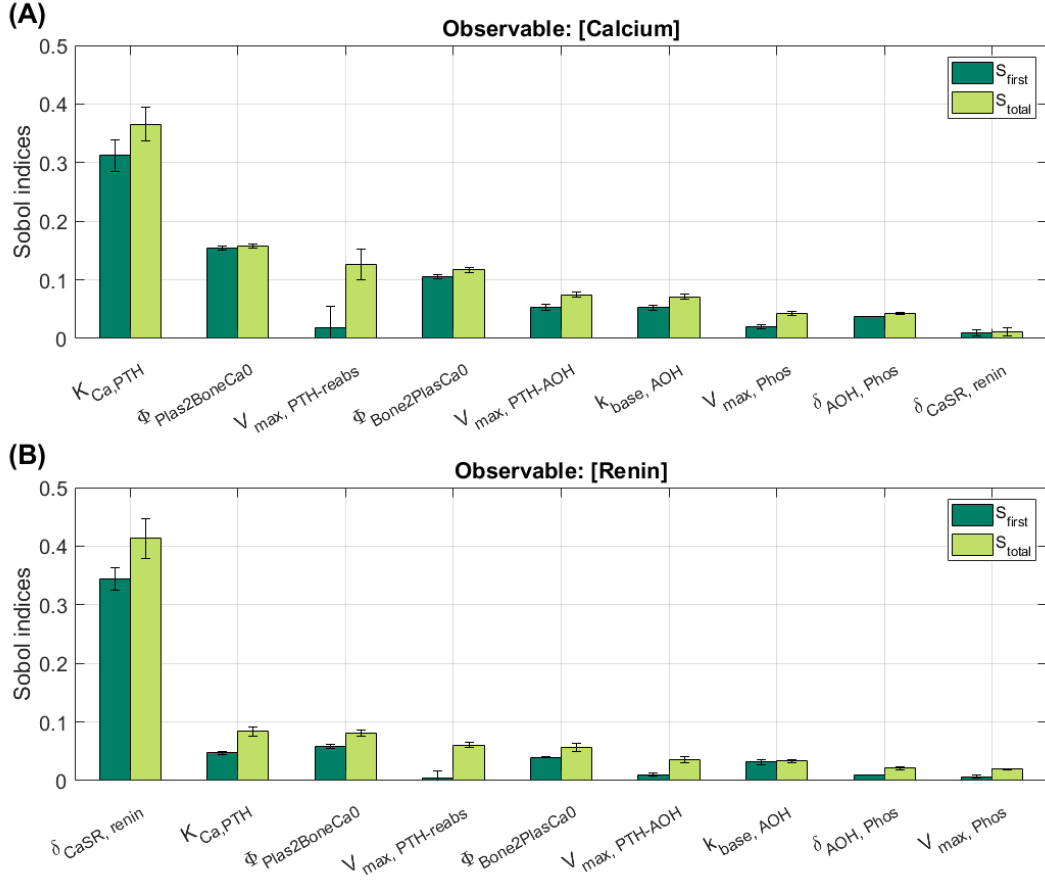


Figure 6.7: Mean (bar) and standard deviation (error bar) of first and total order Sobol indices (denoted by S_{first} and S_{total} in legend, respectively) of full model simulation from age 20 to 80 years with estrogen decline. Parameters shown had a maximal value of $S_{\text{total}} > 0.05$ for at least one output variable. A description of the parameters is given in Table E.1.

driven by calcium sensing via the CaSR in the parathyroid glands. $V_{\max, \text{PTH-reabs}}$ relates to the impact of PTH on calcium reabsorption in the kidneys. This parameter has a high S_{total} value for [PTH] as well as [Calcium] (Fig. 6.7 and 6.8), likely due to the interactions between renal calcium reabsorption, calcium levels, and PTH secretion. $V_{\text{PTH-AOH}}$ represents the maximal impact of PTH on 1α -hydroxylase, the enzyme that synthesizes calcitriol. PTH and calcitriol regulate one another, as calcitriol inhibits PTH secretion and PTH stimulates calcitriol production (Fig. 6.1). Both PTH and calcitriol are sensitive to the parameter $k_{\text{base, AOH}}$ which is the baseline production rate of 1α -hydroxylase, and to the parameters $\Phi_{\text{Bone2PlasCa0}}$ and $\Phi_{\text{Plas2BoneCa0}}$, which denote the baseline rate of calcium exchange between the bone and plasma and vice versa (Fig. 6.8). The latter is likely due to the sensitivity of PTH to calcium levels, which are impacted by these two parameters as well (Fig. 6.7A).

The Sobol sensitivity analysis results shown here are the average (bar) and standard deviation (error bar) of S_{first} and S_{total} values over the full simulation time from age 20 to 80 years. We also analyzed the results over the simulation time from age 20 to 50, i.e., before estrogen decline. The parameters with the largest impact on model output were the same (results not shown) as those for the full simulation time. This suggests that the model output is most sensitive to parameters that represent the overall biological components of the composite model rather than parameters that represent the impact of estrogen on calcium regulation and bone remodeling.

In summary, our global sensitivity analysis shows that, in our model, bone remodeling is most sensitive to RANK-RANKL binding and that the RAS is sensitive to both calcium levels and the calciotropic hormones. These are the key biological components that impact RAS levels and bone remodeling.

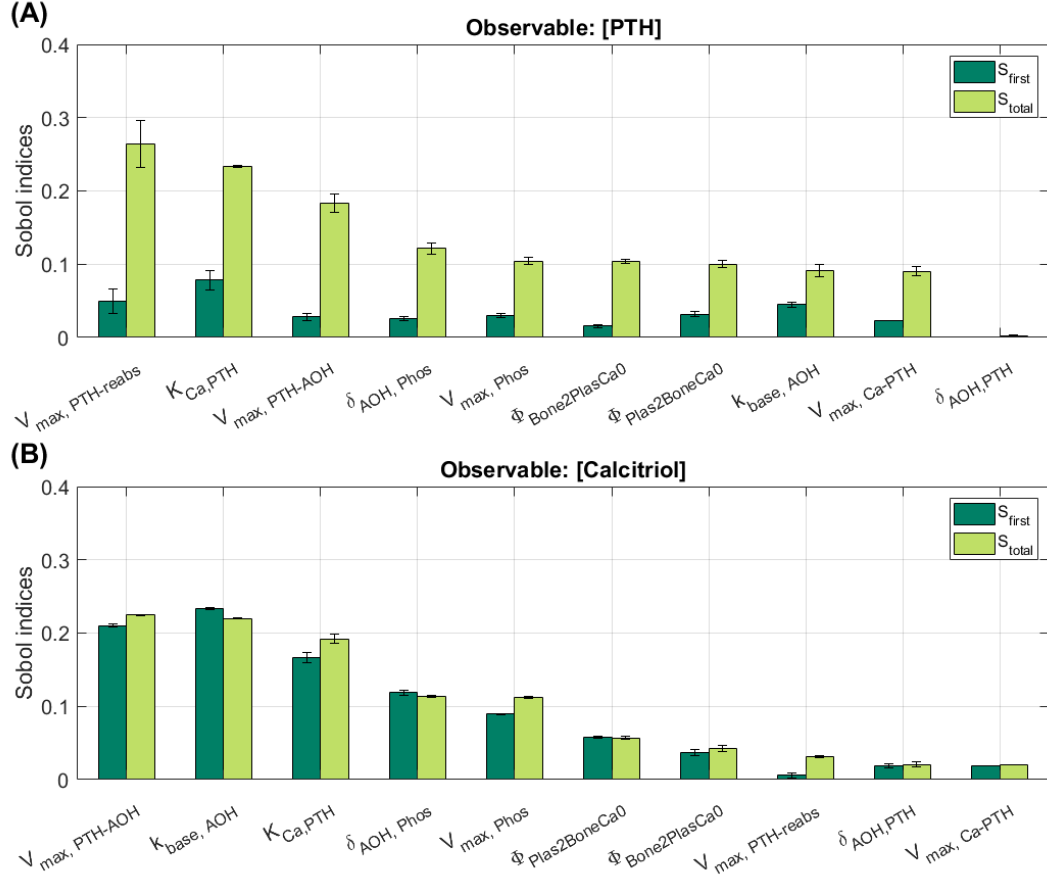


Figure 6.8: Mean (bar) and standard deviation (error bar) of first and total order Sobol indices (denoted by S_{first} and S_{total} in legend, respectively) of full model ran from age 20 to 80 years with estrogen decline for output variables (A) parathyroid hormone and (B) calcitriol. Parameters shown had a maximal value of $S_{\text{total}} > 0.05$ for at least one output variable. A description of the parameters is given in Table E.1. PTH: parathyroid hormone

6.4 Discussion

In this study we developed a mathematical model that includes the interconnected systems of estrogen, the RAS, PTH, calcitriol, calcium regulation, and bone remodeling. We used this model to:

- Investigate the impact of estrogen decline, as experienced in post-menopausal women, on the RAS, bone remodeling, and calcium homeostasis.
- Simulate potential therapeutic effects of ACEi and ARB on bone remodeling and calcium regulation.
- Assess the sensitivity of key model outputs to variations in the model’s many unknown parameters using a Sobol sensitivity analysis.

To the best of our knowledge, this is the first model to incorporate the direct effects of estrogen on the RAS, as well as the integrated effects of the RAS on calcium regulatory and bone remodeling systems. As such, our model development and analysis have brought major advances to the active area of using mathematical modeling to investigate bone remodeling and calcium regulation.

We note that the parameter fit in Fig. 6.2, especially for “PTH only” may be improved by adjusting parameters in the baseline calcium homeostasis and bone remodeling model. It seems that the parameter labeled “T16” in the code, which relates to PTH impact on renal function can be adjusted to get a better fit to the data in Fig. 6.2C. In this study, we chose not to adjust this parameter, but note that adjusting the renal impact of PTH may improve this fit in future work.

Given that the prevalence of hypertension increases markedly with aging, hypertension and osteoporosis often coexist. RAS inhibitors are widely used, safe, reasonably priced long term treatments for hypertension. RAS inhibitors have also been proposed to be a beneficial treatment to improve bone density due to the relationship between the RAS and bone remodeling [114, 190]. Since RAS inhibitors are already widely used for the treatment of hypertension in aging populations, including post-menopausal women, it may be beneficial to consider which RAS inhibitor may prevent or improve bone loss.

We conducted model simulations to investigate the impacts of RAS inhibitors, specifically ACEi and ARB, on bone remodeling for various stages of age-related estrogen decline. Since treatment with an ARB results in a larger reduction in AT1R-bound Ang II for all dose levels, the improvement in bone mineral density was greater in simulations of ARB

than ACEi (Fig. 6.3E, Fig. 6.4A). It seems that the targeted impact of ARB on AT1R binding results in a better improvement in bone mineral density during post-menopausal estrogen decline than ACEi, which inhibit Ang II production via ACE. These results were consistent when we varied the starting age for ACEi and ARB before and after estrogen decline (Fig. 6.5). In summary, our model simulations showed that reductions in AT1R-bound Ang II abundance results in reduced osteoclast activity, which prevents and improves declines in bone mineral density driven by low estrogen levels.

In a clinical study, Kim et al. [114] reported that patients that have used an ARB experienced fewer fractures than patients who have never used a RAS inhibitor. Similarly, Solomon et al. [203] reported reduced fracture risk in users of ARB, while ACEi users had no change in fracture risk. The systemic review by Wu et al. [237] reported that lower fracture risk is consistently reported male users of ARB, but not ACEi. Our mathematical model predicted that bone mineral density is increased not only by ARB treatment, but ACEi as well (Fig 6.4A). That discrepancy may be due to mechanisms not captured by our model. It is possible that the improvement in bone mineral density predicted by our model for ACEi is not enough to have a notable impact on fracture risk. We also note that bone mineral density is not the only factor that impacts fracture risk; the architecture of the skeleton, alcohol usage, smoking, physical activity, and previous fractures are also major factors in fracture risk that are not represented in the model in this study [141].

6.4.1 Clinical perspectives

Although estrogen replacement therapy (or hormone replacement therapy) can effectively manage menopause symptoms and post-menopausal osteoporosis, its benefits must be weighed against the heightened risks of blood clots, stroke, and breast cancer, especially with long-term use [65, 226]. Furthermore, side effects such as weight gain, diarrhea, and mood changes can affect patient well-being and adherence [226]. The balance of risks and benefits associated with hormone replacement therapy varies considerably based on individual physiology, time since menopause onset, and the specific type of hormone replacement therapy [65]. Other current osteoporosis treatments, including antiresorptives and osteoanabolic therapies, have limitations on their duration of use, typically ranging from one to five years [66]. Therefore, the development of alternative long-term osteoporosis treatments are needed to broaden the range of effective options for patients. Our mathematical modeling analysis presented in this study supports clinical evidence suggesting that ARB may contribute to maintaining bone health during post-menopausal estrogen decline by reducing AT1R-bound Ang II levels.

Indeed, there are a number of clinical applications that this model could be extended to study. The RAS is a key hormone system in blood pressure regulation. The RAS model used in this study is part of a larger model that involves whole-body blood pressure regulation [90, 131]. Using a similar approach to Ref. [207], the model presented in this study may be incorporated into the blood pressure model (Ref. [131]) through the RAS to investigate the interactions of hypertension and bone remodeling. CKD is a disorder of the loss of kidney function that affects over 10% of the global population [67]. Many patients with CKD experience CKD-metabolic bone disorder, where their impaired renal function results in loss of bone mineral density. Previous mathematical modeling studies have used the underlying calcium homeostasis and bone remodeling model to study this disorder [71, 72, 187]. However, none have considered the RAS. Many CKD patients take RAS inhibitors to protect their kidney function, prevent hypertension, and have been shown to improve bone health [73]. The model presented in this study may be used to investigate how RAS inhibitors may impact bone density and electrolyte balance in patients with CKD.

6.4.2 Limitations and future directions

In this study, we developed a mathematical model that describes the interactions between the RAS, calciotropic hormones, calcium regulation, and bone remodeling. One major limitation of this model is the large number of unknown parameters, resulting in uncertainty in the model outcomes. In this study, we analyzed the impacts of variations in parameter values on model output by conducting a global sensitivity analysis using the Sobol method. However, we do recognize that there may be parameters and mechanisms that are not included or could be changed in this model.

A worthwhile future direction is to accurately model an aging population. In this study, we conducted simulations that represent the estrogen decline that is seen in post-menopausal women. However, aging populations, including post-menopausal women, have shown other physiological changes related to the systems in the model presented in this study. For example, aging populations have shown a blunted response to ACEi, have a lower GFR, decreased intestinal calcium absorption, lower vitamin D levels, and higher PTH levels [15, 37, 243]. These effects are not included in the model developed in this study at this time. Indeed, future work may consider the specific physiological impacts of aging for a more comprehensive description of post-menopausal physiology.

Given the importance of estrogen and using a female-specific RAS, the model presented in this study only represents a female population. However, sex differences have been found

in the RAS [93] and renal handling of calcium [85, 112]. Men also can form osteoporosis as they age, which is not driven by the declining estrogen levels found in post-menopausal women. Rianon et al. [185] reported sex- and race-specific effects of ACEi on the prevention of age-related bone loss. Specifically, the authors reported that ACEi were effective at protecting against bone loss in African-American elderly men [185]. This is different than reports that did not consider sex and race differences and only reported significant changes in fracture risk in patients using ARB [114, 203, 237]. The model presented in this study may be extended to consider how underlying sex and racial differences may explain altered treatment outcomes [123].

In this study, we do not include the hormone aldosterone, which is connected with the RAS as a part of the renin-angiotensin-aldosterone system. Researchers have shown that aldosterone also has a bidirectional relationship with the calciotropic hormones [222]. Clinical reports have shown that the drug spironolactone (an aldosterone inhibitor), has a significant impact on the bone health of hypertensive patients [204]. Indeed, this hormone may be added to this model to study the impacts of hypertension, spironolactone, hyperaldosteronism, or other relevant conditions and medications on bone density and calcium regulation. Additionally, in this study, we assume that the RAS activity in the bone is the same as the systemic RAS, though this may not always be the case. Experimental evidence has shown that there is a bone-specific RAS [196]. Future work may involve modeling a bone-specific RAS to consider the impacts on bone remodeling similar to the renal-specific RAS models in Refs. [137, 199].

Estrogen levels vary during the different phases of the menstrual cycle in menstruating women [173]. Women who take oral contraceptives also have different estrogen levels [198]. The mathematical model presented here may be adapted to consider how changes in estrogen impacts the RAS, calcium homeostasis, and bone remodeling during the different phases of the menstrual cycle or on oral contraceptives [1]. During pregnancy, estrogen levels rise dramatically, while post-parturition, the estrogen levels drop below pre-pregnancy levels to support lactation [155]. Each of these changes results in major impacts on physiology, including the RAS, calcium homeostasis, and bone remodeling [118, 155]. Previously, we developed mathematical models that represent calcium homeostasis in rats that consider sex differences and maternal adaptations of pregnancy and lactation in Ref. [211] (Chap. 5), but these models do not include a detailed bone compartment or the impact of the RAS. The model presented in this study may be similarly adapted to consider the major changes that women undergo during pregnancy and lactation.

Chapter 7

Conclusions and Future Directions

Mammals have a system of regulatory processes to ensure that electrolyte levels stay within normal range during healthy conditions. When these regulatory processes are altered from factors such as a drug side effect, disease, or diet, this can lead to too high or too low electrolyte levels. When electrolyte levels are out of balance, symptoms such as muscle weakness, cardiac arrhythmia, and fatal cardiovascular events may occur. In the studies presented in this thesis, we developed and analyzed mathematical models of electrolyte homeostasis in rats and humans. Specifically, the focus of the studies presented in this thesis was the electrolytes potassium and calcium. Indeed, similar approaches may be used to study other electrolytes.

7.1 Summary of contributions

We developed mathematical models as well as conducted model simulations and analysis to study the impact of individual regulatory mechanisms on potassium regulation (Chap. 2), parameter variation on model output (Chap. 3), renal adaptations of high potassium intake (Chap. 4), sex-differences and maternal adaptations on calcium homeostasis (Chap. 5), and the impact RAS inhibitors on bone remodeling and calcium regulation (Chap. 6).

In Chap. 2, we presented a mathematical model representing the known regulatory mechanisms of potassium homeostasis. This model served as a base for the work in Chap. 3 and Chap. 4 as well. The novelty of this model, in contrast to other published potassium homeostasis models, lies in the inclusion of the impacts of insulin and ALD on $\text{Na}^+\text{-K}^+\text{-ATPase}$ intracellular potassium uptake, a detailed renal component representing potassium

reabsorption and secretion along different nephron segments, and the gastrointestinal feed-forward effect. We used this model to quantify the individual contribution of each regulatory mechanism in maintaining potassium levels during typical dietary potassium intake on a Western diet. Furthermore, we conducted *in silico* experiments to test a hypothesized regulatory mechanism: “muscle-kidney cross talk”.

In Chap. 3, we presented the results of global sensitivity analyses of our potassium regulation model (Chap. 2, Ref. [214]). The analyses in Chap. 3 aimed to evaluate the model’s underlying structure and quantify the impact of parameter variations on key model outputs. Specifically, we evaluated the sensitivity of steady-state intracellular and plasma potassium concentrations, representing long-term potassium regulation, as well as the dynamic responses of intracellular and plasma potassium concentrations following single meals of varying compositions, representing short-term potassium regulation. Our analyses showed that different simulation types are sensitive to different parameters. Steady-state results were primarily dependent on parameters related to kidney function, whereas single-meal simulations were predominantly influenced by hormonal signaling. This result aligns well with physiological understanding, where long-term potassium homeostasis is maintained by the kidneys, while rapid fluctuations, such as those occurring during meals, are modulated by hormonal signaling for increased intracellular potassium uptake. The sensitivity analyses in Chap. 3, demonstrate that our model effectively captures key regulatory mechanisms of potassium balance across a range of parameter values. Furthermore, the detailed sensitivity results offer valuable insights for future model development, particularly in guiding parameter reduction by identifying and potentially eliminating parameters with negligible impact on model output.

Following the publication of our initial potassium homeostasis model (Chap. 2, Ref. [214]), Wang et al. [230] published new experimental results regarding the impact of a high potassium diet on renal function. Specifically, the authors observed a significant reduction in PT potassium and sodium reabsorption, as well as GFR, in rats consuming a high potassium diet compared to rats on a control diet [230]. In Chap. 4, we incorporated these experimental observations into our mathematical model to simulate the effects of these renal adaptations on whole-body potassium regulation under a high potassium diet. Using our model, we conducted simulations to unravel the individual and combined impacts of decreased PT reabsorption and GFR on a high potassium diet. Our simulations revealed that, in isolation, reduced fractional PT potassium reabsorption leads to stable potassium levels, but the lowered GFR due to the TGF signal results in less efficient potassium excretion. We conducted further analysis by varying parameter values to understand the impact of reduced PT and GFR impacts on a high potassium diet. Additionally, we conducted local and global sensitivity analyses to assess the impact of all model parameters on plasma

and intracellular potassium concentrations under a high potassium diet.

The other major electrolyte studied in this thesis is calcium. Calcium plays a key role in many physiological processes, including muscle contractions as well as bone structure. In Chap. 5, we developed mathematical models representing calcium regulation in male, female, late pregnant, and lactating rats. We conducted model simulations and sensitivity analysis to quantify how sex-specific differences in calcitriol result in different set points for the stimulation of intestinal calcium absorption during pregnancy in female versus male rats. We hypothesized that the altered calcitriol set point in female rats allows for the substantial increase in intestinal calcium absorption that primarily supports the calcium demands of the developing fetus. During lactation, much of the calcium needs of milk are met by increased bone resorption, intestinal absorption, and renal calcium reabsorption. We conducted model simulations to unravel how these different maternal adaptations contribute to maternal calcium homeostasis. Furthermore, we conducted simulations to predict the impacts of vitamin D deficiency on male, female, pregnant, and lactating rats.

In Chap. 6, we integrated a model of the RAS with a model of calcium homeostasis and bone remodeling to study the interplay between these physiological systems. Additionally, we incorporated the impact of estrogen on these systems. Using this model, we investigated the impact of declining estrogen levels in post-menopausal women on the RAS, bone remodeling, and calcium homeostasis. We used this model to investigate the impact of antihypertensive therapies, ARB and ACEi, on bone remodeling, the RAS, and calcium balance. We also conducted global sensitivity analysis to determine which parameters and their related biological components have the largest impact on key model outputs using the Sobol method. Given the high rates of both hypertension and osteoporosis in post-menopausal women, these model results have clinical significance by supporting the use of ARB to mitigate bone loss. This model offers a platform for future hypothesis testing and for simulating the potential effects of various treatments on bone remodeling, calcium homeostasis, and the RAS.

7.2 Future directions

The mathematical models developed in this thesis offer numerous directions for future research into electrolyte homeostasis. Some potential future directions are highlighted below. We note that each chapter also has relevant future directions suggested in the discussion sections. To facilitate the application and extension of the work presented in this thesis, and in the spirit of Open Science, all code associated with the chapters of this thesis is publicly available at

<https://github.com/Layton-Lab>. These repositories are intended to enable the reproducibility of our findings and serve as a starting point for model updates and further studies on potassium and calcium homeostasis via mathematical modeling.

Indeed, the models in this study may be analyzed in a different way, or equations changed based on improved information or the addition of more detailed mechanisms. For example, in this thesis, we present a new mathematical in Chap. 2 and then conducted a global sensitivity analysis using the Morris method in Chap. 3 to conduct parameter ranking. Global sensitivity analysis with the Sobol method may be done to quantify how the model parameters contribute to the variation in model output. Additionally, with these analyses we identified parameters that may contribute a negligible amount to the model output. This may be adjusted to reduce the number of parameters in the model for a model reduction.

Circadian rhythms have been reported to impact bone remodeling [92], parathyroid hormone [34], renal function [34, 62, 82], and ALD secretion [83]. We have previously studied the impact of circadian rhythms on renal electrolyte handling using a cell-based epithelial nephron model [52, 208], but have not included circadian rhythms in the whole body electrolyte homeostasis models presented in this thesis. Clegg et al. [42] added a circadian rhythm impact on GFR in their potassium homeostasis model, but did not include other circadian rhythm impacts. Indeed, the models presented in this thesis may be extended to include circadian rhythms to study the impact on electrolyte homeostasis.

Sex differences have been reported in renal calcium and potassium handling [85, 99, 112, 225], ALD concentration [148], and CKD [238]. With the exception of the sex-specific models in Chap. 5, the models in this thesis are not sex-specific. To study the physiological implications of sex differences and unravel how underlying sex differences may contribute to different outcomes in disorders such as CKD, sex-specific calcium and potassium models can be developed.

CKD is estimated to impact over 10% of the global population [67]. Given the importance of kidneys in regulating electrolyte balance, a major concern for CKD patients is maintaining normal electrolyte levels. In CKD patients, potassium levels must be monitored tightly because hyperkalemia and hypokalemia are strongly associated with adverse health outcomes [120]. The model used in Chaps. 2, 3, and 4 could be used to consider the impacts of CKD to predict patient risk for hyperkalemia or hypokalemia potassium levels and predict the impact of potential treatments, such a dialysis or other drug interventions. Dysregulated calcium due to renal insufficiency often impacts the bones, leading to mineral bone disorder (i.e., weakened bones) in patients with CKD [77]. Several modeling studies have used the calcium homeostasis and bone remodeling model from Peterson

and Riggs [165] to study CKD mineral bone disorder [71, 72, 187], but none have included the RAS. RAS inhibitors are a common treatment method for CKD patients due to their protective benefits on further renal impairment as well as prevention of hypertension [73]. The model in Chap. 6 may be used to understand the impact of RAS inhibitors on the bones of patients with CKD.

Another major disorder related to the studies presented in this thesis is hypertension, the leading cause of cardiovascular disease and premature death worldwide [110]. Recently, we coupled the potassium homeostasis model (Chap. 2, Ref. [214]) to the whole-body blood pressure model (Ref. [131]) to study the impact of the ratio of potassium and sodium intake on blood pressure regulation by describing the interactions between sodium and potassium with the blood pressure system [207]. This model extension brings in the interactions of sodium and potassium rather than just studying sodium impact on blood pressure in isolation. The study in Chap. 6 involves including the RAS with the calcium homeostasis and bone remodeling model and studying the impact of RAS inhibitors, which are anti-hypertensive drugs. This model is not coupled to the whole-body blood pressure model but could be through the impact of the RAS. This model extension may be useful for understanding how hypertension impacts bone health [171, 242].

Indeed, there are other future directions that can be studied based on the work presented in this thesis including studying the unique physiological states of pregnancy and lactation, predicting the impact of hypo- or hyperparathyroidism or hyperaldosteronism, vitamin D deficiency, exercise, aging and more. Some of these are detailed with relevant references in the chapters presented in this thesis.

7.3 Concluding remarks

The work in this thesis demonstrates the value of mathematical modeling as a tool to unravel, understand, analyze, and predict the intricate dynamics of electrolyte homeostasis. Through the presented model simulations and analyses, we investigated many questions related to calcium and potassium regulation. Recognizing the inherent uncertainty in parameters values within all of the models presented in this thesis, we used global and local sensitivity analyses to quantify the impact of parameter variation on model outputs. Ultimately, this research enhances our understanding of the complexities underlying potassium and calcium homeostasis, providing a foundation for informing future biological studies and generating predictions for the impacts of diseases or potential drug targets.

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APPENDICES

Appendix A

Supplementary materials for Chap. 2

A.1 Effect of feedback and feedforward control mechanisms

We conducted additional simulations to investigate the effects of the individual control mechanisms. This study is analogous to those shown in Fig. 2.6 of the manuscript, however instead of showing the impact of showing only one feedforward or feedforward control mechanism, we started by turning all the mechanisms off and then include one control mechanism on at a time to see the impact on plasma $[K^+]$ and intracellular $[K^+]$. The results are shown in Fig. A.1.

A.2 Response to repeated K^+ loading

The simulation results for Fig. 2.7 on the model's response to repeated K^+ loading for various muscle-kidney cross talk mechanisms is shown in Fig. A.2 for days 2-6 where K^+ loading occurs. This shows more clearly the impact on renal K^+ handling during this period.

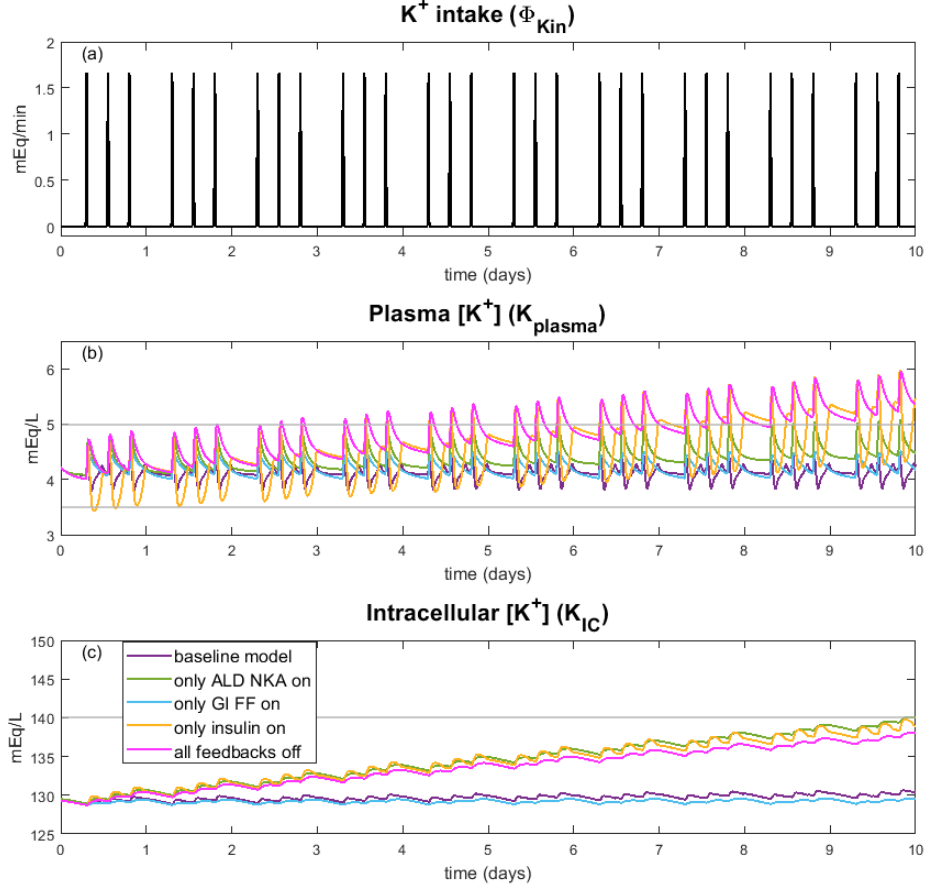


Figure A.1: Simulation results for plasma K^+ concentration (b) and intracellular K^+ concentration (c) for 10 days of normal K^+ intake ((a); 100 mEq/day, 3 typical meals) for the baseline model, all control mechanisms off except the aldosterone effect on $Na^+-K^+-ATPase$ uptake (ρ_{al}), all control mechanisms off except the gastrointestinal feedforward mechanism, all control mechanisms off except insulin ($\rho_{insulin}$), and baseline model with all feedback effects off. Grey lines indicate normal range for K_{plasma} and K_{IC} . ALD: aldosterone, NKA: $Na^+-K^+-ATPase$, GI: gastrointestinal, FF: feedforward

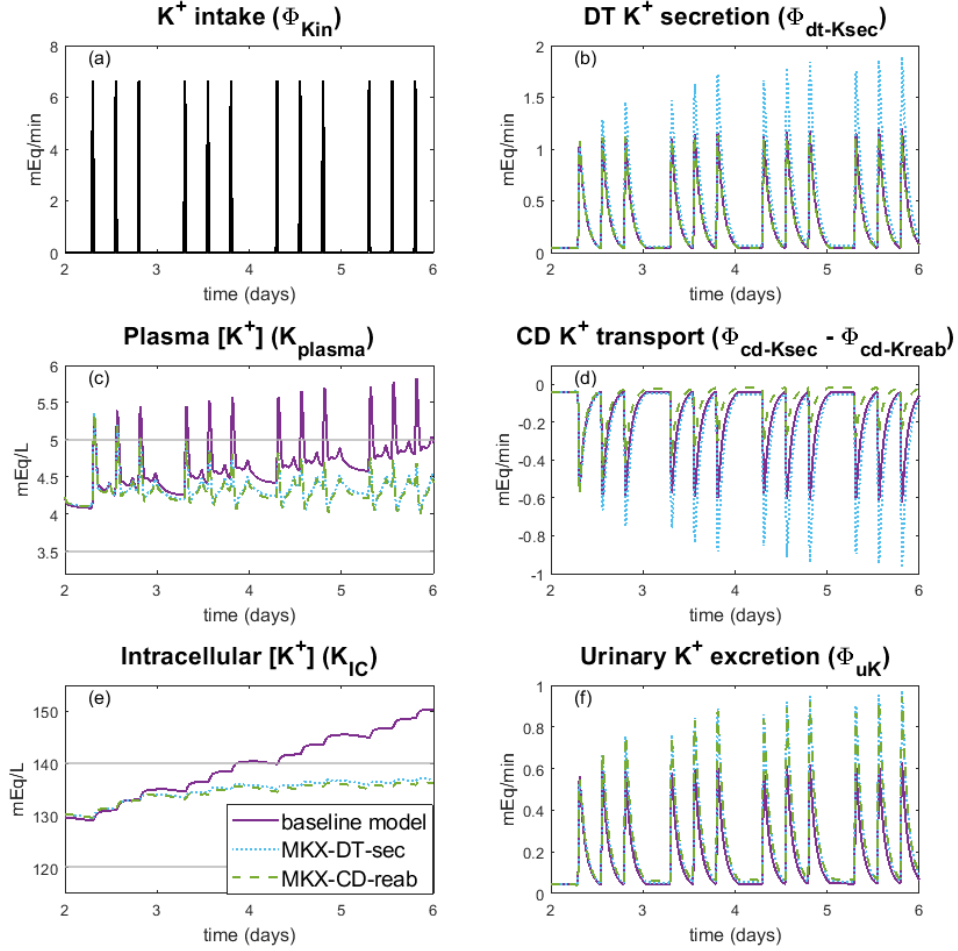


Figure A.2: Same simulation results as shown in Fig. 2.7 of manuscript. Simulation results for K^+ loading experiments for the baseline model and muscle-kidney cross talk simulations (Case MKX-DT-sec and Case MKX-CD-reab). Potassium intake (a) is the same for all three simulation types. Note that Case MKX-CD-sec is not plotted since it had little impact from the baseline model results. Horizontal grey line shows normal range for plasma K^+ concentration (c) and intracellular K^+ concentration (e). CD: collecting duct, DT: distal tubule

A.3 Impact of parameters on muscle-kidney cross talk during K^+ loading and depletion

To investigate the impact of the parameter m_{Kic} in Eq. 2.28 from the manuscript on muscle-kidney cross talk simulations, we conducted simulations analogous to the K^+ loading and depletion experiments shown in manuscript Figs. 2.7 and 2.8, respectively. We conducted simulations for varied values of m_{Kic} using the muscle-kidney cross talk mechanism that targets distal tubule K^+ secretion (i.e., Case MKX-DT-sec). The results for loading and depletion are shown in Fig. A.3 and A.4, respectively. Notably, when we decreased m_{Kic} by 50%, we can see that indeed muscle-kidney cross talk has a large impact on the predicted intracellular and plasma K^+ concentrations in both the loading and depletion experiments. This shows how muscle-kidney cross talk likely plays a key role in ensuring that intracellular K^+ concentration returns to baseline after a period of K^+ loading or depletion.

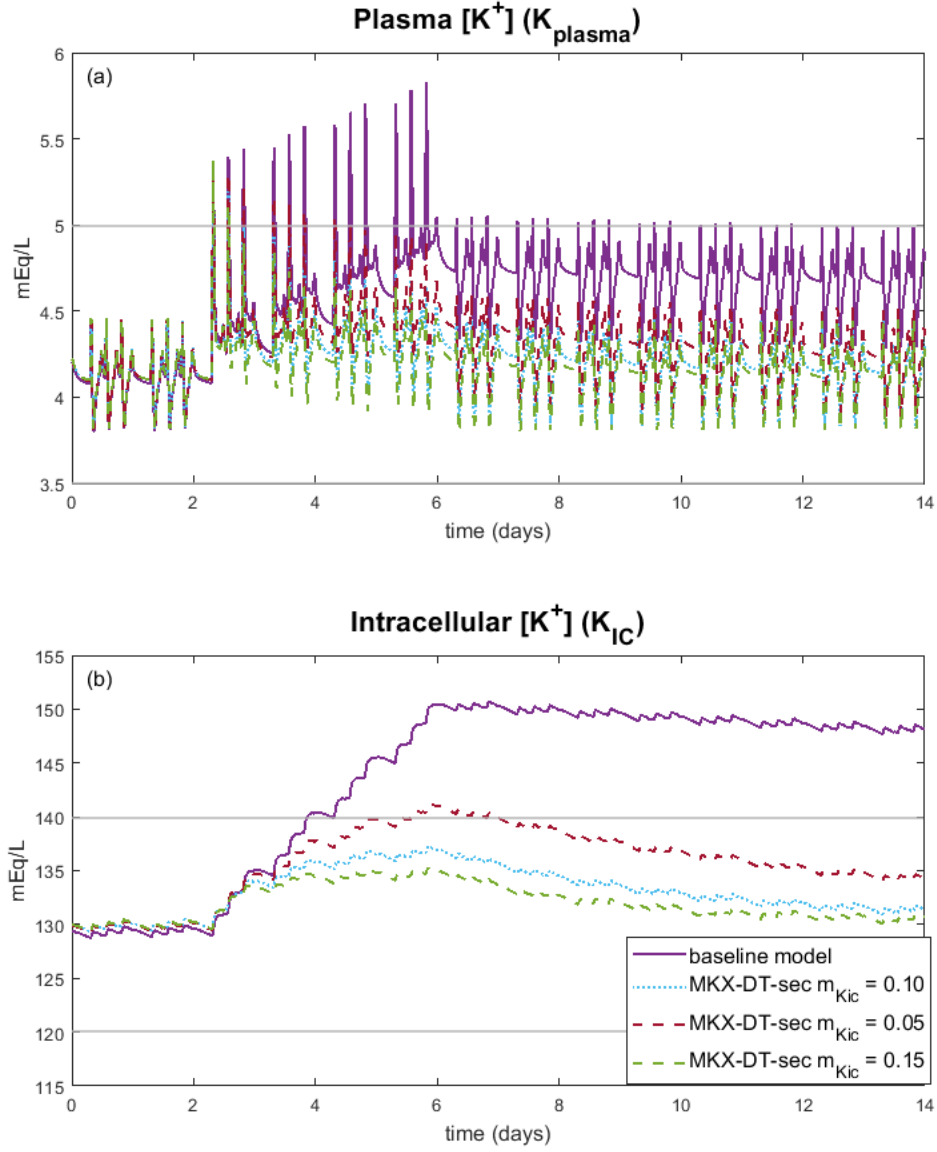


Figure A.3: Simulation results for K^+ loading experiments for the baseline model and distal tubule K^+ secretion muscle-kidney cross talk simulations (Case MKX-DT-sec) for varied values of the parameter $m_{K_{ic}}$. Potassium intake is the same for all simulation types. Horizontal grey line shows normal range for plasma K^+ concentration (a) and intracellular K^+ concentration (b).

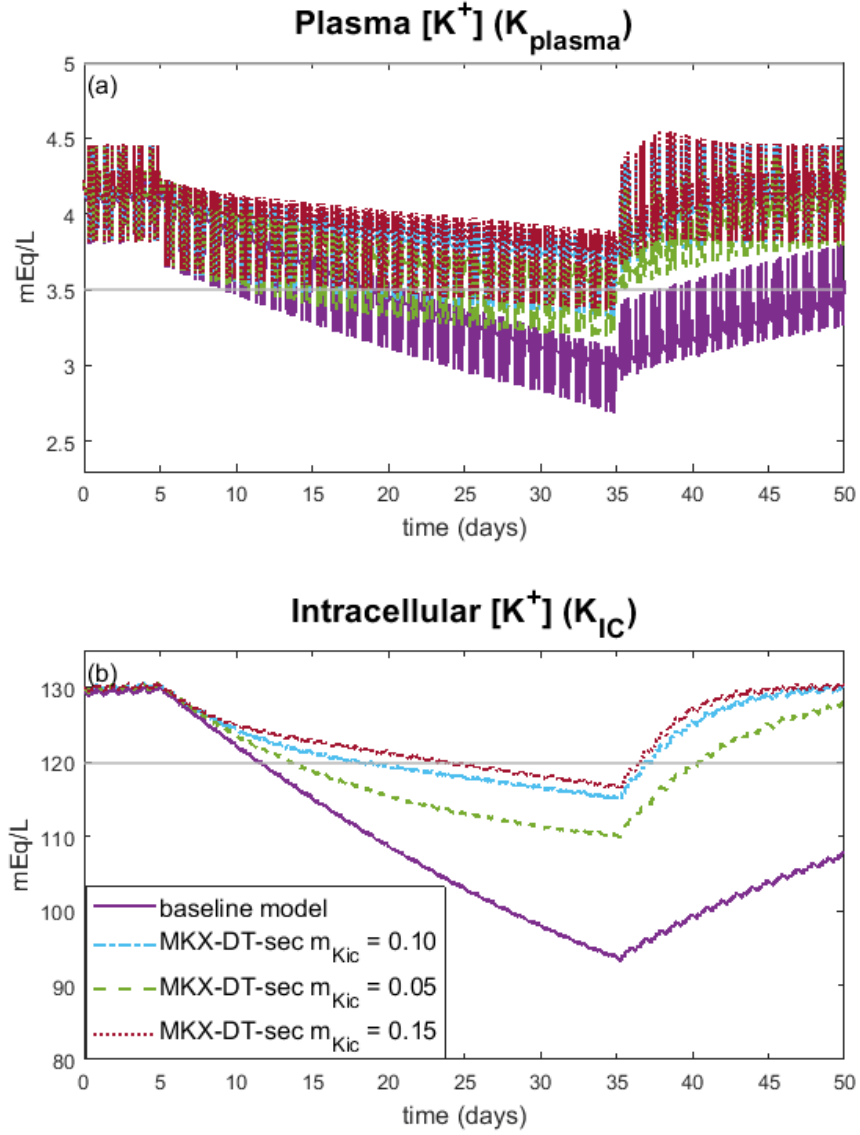


Figure A.4: Simulation results for K^+ depletion experiments for the baseline model and distal tubule K^+ secretion muscle-kidney cross talk simulations (Case MKX-DT-sec) for varied values of the parameter $m_{K_{\text{ic}}}$. Potassium intake is the same for all simulation types. Horizontal grey line shows normal range for plasma K^+ concentration (a) and intracellular K^+ concentration (b).

A.4 Local sensitivity analysis

We conducted a local sensitivity analysis by increasing selected parameters by 10% and computing the resulting changes in steady-state plasma and intracellular K^+ concentration. Results are shown in Fig A.5.

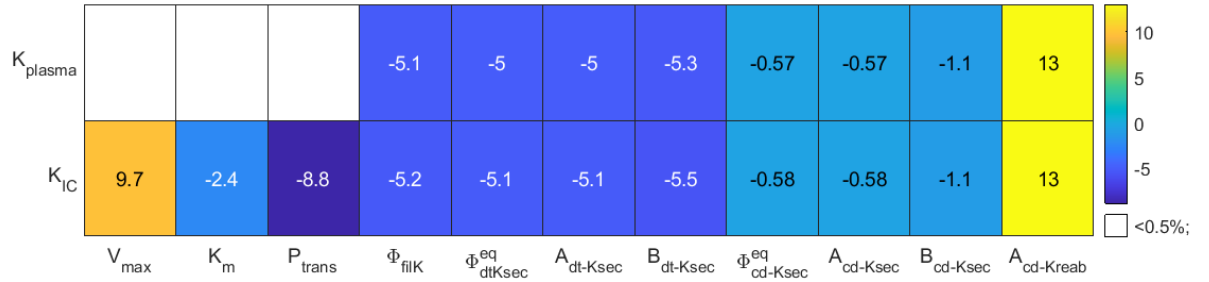


Figure A.5: Percent changes in baseline model steady-state solution for plasma K^+ concentration (K_{plasma}) and intracellular K^+ concentration (K_{IC}) in response to a perturbation of 10% increase of the given parameter value.

Among the parameters that affect renal handling of K^+ (i.e., filtration, distal tubule K^+ secretion, and collecting duct K^+ secretion and reabsorption), plasma and intracellular K^+ concentration are most sensitive to $A_{\text{cd-Kreab}}$ (Fig A.5). This sensitivity may, in large part, attributed to the lack of downstream segments that can compensate changes in collecting duct K^+ reabsorption. As such increasing $A_{\text{cd-Kreab}}$ by 10% increases collecting duct K^+ reabsorption proportionally (see Eq. 2.17), significantly lowering K^+ excretion, which leads to a 13% increase in plasma and intracellular K^+ concentrations (Fig A.5).

Intracellular K^+ concentration is also highly sensitive to parameters that impact the K^+ fluxes in and out of the intracellular compartment (Fig A.5): the Michaelis-Menten parameter $V_{\max}$ (Eq. 2.8) and transmembrane permeability P_{trans} (Eq. 2.9). In contrast, plasma K^+ concentration is insensitive to changes in these parameters, as explained below. Recall that in this analysis K^+ intake is fixed, so then an increase in $V_{\max}$ will cause an immediate influx of K^+ into the intracellular compartment, which decreases plasma K^+ . This causes a proportional decrease in K^+ nephron filtration, which in turn decreases urinary K^+ excretion. Together, the fixed K^+ intake and (transiently) lower K^+ excretion yield net K^+ retention, which accumulates in the extracellular K^+ compartments until plasma K^+ concentration is back to baseline, with urinary K^+ matching K^+ intake, and a new steady-state is obtained.

Appendix B

Supplementary for Chap. 3

B.1 Description of mathematical model of K^+ homeostasis

K^+ primarily enters the body through oral K^+ intake. The amount of K^+ in the gastrointestinal and hepatoportal circulation (denoted by $M_{K_{\text{gut}}}$) is determined by the difference between 90% of oral K^+ intake (denoted by $\Phi_{K_{\text{in}}}$) and K^+ entering the blood stream:

$$\frac{dM_{K_{\text{gut}}}}{dt} = 0.9 \times \Phi_{K_{\text{in}}} - k_{\text{gut}} \times M_{K_{\text{gut}}} \quad (\text{B.1})$$

where k_{gut} is a parameter that determines the rate of K^+ delivery to the plasma. 10% of the K^+ intake is assumed to be excreted via feces.

Internal K^+ balance

Internal K^+ balance refers to the distribution of K^+ between the intracellular and extracellular fluids. In the mathematical model, the extracellular K^+ is divided into two compartments: plasma and interstitial fluid K^+ . Plasma $[K^+]$, denoted by K_{plasma} , is modeled by the amount of K^+ coming into the plasma from $M_{K_{\text{gut}}}$ minus the amount leaving the plasma via urinary excretion and diffusion to the interstitial fluid:

$$V_{\text{plasma}} \frac{dK_{\text{plasma}}}{dt} = k_{\text{gut}} \times M_{K_{\text{gut}}} - \Phi_{\text{ECF}} - \Phi_{\text{uK}} \quad (\text{B.2})$$

where V_{plasma} is plasma volume, Φ_{uK} denotes urine K^+ excretion (see Eq. B.13), Φ_{ECF} denotes the diffusion of K^+ from the plasma to the interstitial fluid and is modeled by simple diffusion:

$$\Phi_{\text{ECF}} = P_{\text{ECF}} \times (K_{\text{plasma}} - K_{\text{inter}}) \quad (\text{B.3})$$

where P_{ECF} is a permeability parameter and K_{inter} is the interstitial $[\text{K}^+]$. K_{inter} is modeled as

$$V_{\text{inter}} \frac{dK_{\text{inter}}}{dt} = \Phi_{\text{ECF}} - \Phi_{\text{ECtoIC}} + \Phi_{\text{ICtoEC}} \quad (\text{B.4})$$

where V_{inter} is the interstitial fluid volume, Φ_{ECtoIC} is intracellular K^+ uptake (see Eq. B.6), and Φ_{ICtoEC} is the flux of K^+ from the intracellular fluid to the extracellular fluid (Eq. B.7). Intracellular $[\text{K}^+]$, denoted by K_{IC} , is determined by the flux of K^+ into and out of the cells

$$V_{\text{IC}} \frac{dK_{\text{IC}}}{dt} = \Phi_{\text{ECtoIC}} - \Phi_{\text{ICtoEC}}, \quad (\text{B.5})$$

where V_{IC} denotes the intracellular fluid volume. Flow of K^+ into the intracellular fluid is driven by Na^+ - K^+ -ATPase uptake and is modeled by

$$\Phi_{\text{ECtoIC}} = \rho_{\text{al}} \times \rho_{\text{insulin}} \times \frac{V_{\text{max}} \times K_{\text{inter}}}{K_{\text{m}} + K_{\text{inter}}} \quad (\text{B.6})$$

where V_{max} is the maximum rate, K_{m} denotes the half maximal activation level, and ρ_{al} and ρ_{insulin} are the effects of ALD and insulin on Na^+ - K^+ -ATPase, respectively (see Eq. B.15 and Eq. B.19). K^+ returns to the interstitial fluid via diffusion through a permeable membrane:

$$\Phi_{\text{ICtoEC}} = P_{\text{trans}} \times (K_{\text{IC}} - K_{\text{inter}}) \quad (\text{B.7})$$

where P_{trans} is a parameter that denotes the transmembrane permeability.

Renal K^+ regulation

To determine urinary K^+ excretion (Φ_{uK}), we consider K^+ reabsorption and secretion along the nephrons of the kidney following glomerular filtration. The K^+ that reaches the end of the nephrons is excreted in urine. A schematic of the key segments for renal K^+ transport is given in Fig. B.1. In the kidney, plasma is filtered into the nephrons through the glomerulus. Since K^+ is a freely filtered electrolyte, the filtered K^+ load (Φ_{filK}) is proportional to the GFR, denoted by Φ_{GFR} , and K_{plasma} so that

$$\Phi_{\text{filK}} = \Phi_{\text{GFR}} \times K_{\text{plasma}}. \quad (\text{B.8})$$

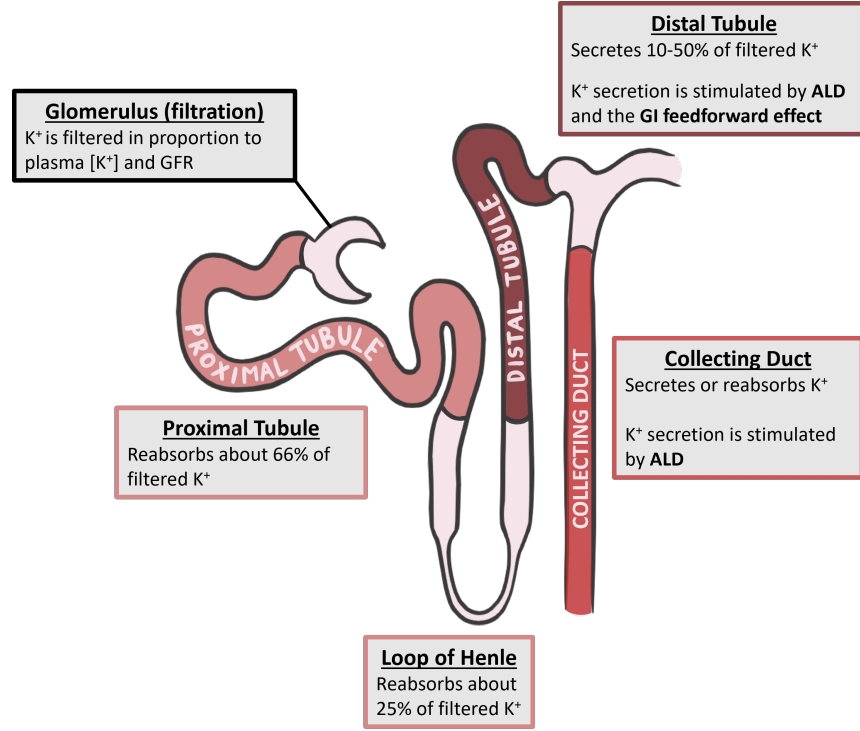


Figure B.1: Filtration, reabsorption, and secretion of K⁺ in key nephron segments. Note that the proximal segment consists of the proximal tubule and the loop of Henle. Figure modified from Stadt et al. [214]. GFR: glomerular filtration rate; ALD: aldosterone; GI: gastrointestinal

The first segment is the “proximal segment (ps)”, which includes the proximal tubule and loop of Henle. This is where most of the filtrate K⁺ is reabsorbed. Let $\eta_{ps-Kreab}$ denote the fractional K⁺ reabsorption along the proximal segment. Then net proximal segment K⁺ reabsorption is given by

$$\Phi_{ps-Kreab} = \eta_{ps-Kreab} \times \Phi_{filK}. \quad (B.9)$$

After the proximal segment, the model includes the “distal tubule (dt)”, which includes the distal convoluted tubule and the connecting tubule. Along this segment, there is net K⁺ secretion. Distal tubule K⁺ secretion is modeled using a baseline distal tubule secretion value of $\Phi_{dt-Ksec}^{eq}$ and is regulated by ALD and the gastrointestinal feedforward effect so that

$$\Phi_{dt-Ksec} = \Phi_{dt-Ksec}^{eq} \times \gamma_{al} \times \gamma_{Kin} \quad (B.10)$$

where γ_{al} denotes the regulatory effect of ALD (Eq. B.16) and γ_{Kin} represents the gastrointestinal feedforward effect (Eq. B.20).

The last segment before urinary excretion is the collecting duct (cd), which both reabsorbs and secretes K^+ . Collecting duct K^+ secretion ($\Phi_{\text{cd-Ksec}}$) is modeled using a baseline value of $\Phi_{\text{cd-Ksec}}^{\text{eq}}$ and is regulated by ALD so that:

$$\Phi_{\text{cd-Ksec}} = \Phi_{\text{cd-Ksec}}^{\text{eq}} \times \lambda_{\text{al}} \quad (\text{B.11})$$

where λ_{al} denotes the regulatory effect of ALD (Eq. B.17). Collecting duct K^+ reabsorption ($\Phi_{\text{cd-Kreab}}$) is modeled as a linear function depending on the filtrate entering the collecting duct based on transport from the previous segments:

$$\Phi_{\text{cd-Kreab}} = (\Phi_{\text{filK}} - \Phi_{\text{ps-Kreab}} + \Phi_{\text{dt-Ksec}}) \times A_{\text{cd-Kreab}}. \quad (\text{B.12})$$

where $A_{\text{cd-Kreab}}$ denotes the fraction of K^+ that is reabsorbed along the collecting duct. Finally, urine K^+ excretion (Φ_{uK}) is given by the filtration and the net transport along the various segments:

$$\Phi_{\text{uK}} = \Phi_{\text{filK}} - \Phi_{\text{ps-Kreab}} + \Phi_{\text{dt-Ksec}} + \Phi_{\text{cd-Ksec}} - \Phi_{\text{cd-Kreab}}. \quad (\text{B.13})$$

Aldosterone effects

To model [ALD], denoted by C_{al} , we use the approach developed by Maddah & Hallow [140]:

$$C_{\text{al}} = C_{\text{al}}^{\text{base}} \times e^{m_{\text{K-ald}} \times (K_{\text{ECF}}^{\text{total}} - K_{\text{ECF}}^{\text{base}})} \quad (\text{B.14})$$

where $m_{\text{K-ald}}$ is a fitting parameter and $K_{\text{ECF}}^{\text{total}}$ is the total extracellular fluid $[\text{K}^+]$ given by

$$K_{\text{ECF}}^{\text{total}} = \frac{K_{\text{plas}} \times V_{\text{plasma}} + K_{\text{inter}} \times V_{\text{inter}}}{V_{\text{plasma}} + V_{\text{inter}}}$$

and $K_{\text{ECF}}^{\text{baseline}}$ is the baseline extracellular $[\text{K}^+]$. The effect of [ALD] on Na^+ - K^+ -ATPase abundance is modeled by the scaling factor ρ_{al} (see Eq. B.6), which is represented linearly as:

$$\rho_{\text{al}} = \alpha_{\text{al}} + \beta_{\text{al}} \times C_{\text{al}} \quad (\text{B.15})$$

where α_{al} and β_{al} are parameters. The effect of [ALD] on distal tubule and collecting duct K^+ secretion are represented by γ_{al} and λ_{al} , respectively:

$$\gamma_{\text{al}} = A_{\text{dt-Ksec}} \times (C_{\text{al}})^{B_{\text{dt-Ksec}}} \quad (\text{B.16})$$

$$\lambda_{\text{al}} = A_{\text{cd-Ksec}} \times (C_{\text{al}})^{B_{\text{cd-Ksec}}} \quad (\text{B.17})$$

with fitting parameters $A_{\text{dt-Ksec}}$, $B_{\text{dt-Ksec}}$, $A_{\text{cd-Ksec}}$ and $B_{\text{cd-Ksec}}$.

Insulin effects

The concentration of plasma insulin, denoted by C_{insulin} , in the time after a meal is given by

$$C_{\text{insulin}} = \begin{cases} \frac{(325-22.6) \text{ pmol/L}}{90 \text{ min}} \times (t - t_0 \text{ min}) + 22.6 \text{ pmol/L} & \text{if } t_0 \leq t < t_0 + 90 \\ \frac{-(325-22.6) \text{ pmol/L}}{270 \text{ min}} \times (t - (t_0 + 360 \text{ min})) + 22.6 \text{ pmol/L} & \text{if } t_0 + 90 \leq t \leq t_0 + 360 \\ 22.6 \text{ pmol/L} & \text{otherwise.} \end{cases} \quad (\text{B.18})$$

where t_0 is the time at the beginning of the meal (see Ref. [214], Chap. 2). We insulin stimulation of $\text{Na}^+\text{-K}^+\text{-ATPase}$ by letting ρ_{insulin} (see Eq. B.6) be given by

$$\rho_{\text{insulin}} = \max(1, A_{\text{insulin}} \times (C_{\text{insulin}} - C_{\text{insulin}}^{ss}) + 1) \quad (\text{B.19})$$

where $C_{\text{insulin}}^{ss} = 123.4 \text{ pmol/L}$ denotes steady-state C_{insulin} and A_{insulin} is a fitting parameter. This function is bounded below by 1 so that insulin only impacts $\text{Na}^+\text{-K}^+\text{-ATPase}$ activity when C_{insulin} is larger than baseline insulin levels (C_{insulin}^{ss}).

Gastrointestinal feedforward effect

The gastrointestinal feedforward effect is a signal in which K^+ levels within the gut impact K^+ secretion in the nephrons before entering the bloodstream. This signal is represented via the term γ_{Kin} , which alters distal tubule K^+ secretion (see Eq. B.10) depending on M_{Kgut} where

$$\gamma_{\text{Kin}} = \max(1, A_{\text{Kin}} \times (M_{\text{Kgut}} - M_{\text{Kgut}}^{ss}) + 1) \quad (\text{B.20})$$

where A_{Kin} is a fitting parameter and M_{Kgut}^{ss} is the baseline value of M_{Kgut} . The function is bounded below by 1 so that M_{Kgut} only increases distal tubule K^+ secretion when M_{Kgut} is larger than M_{Kgut}^{ss} .

B.2 Additional figures

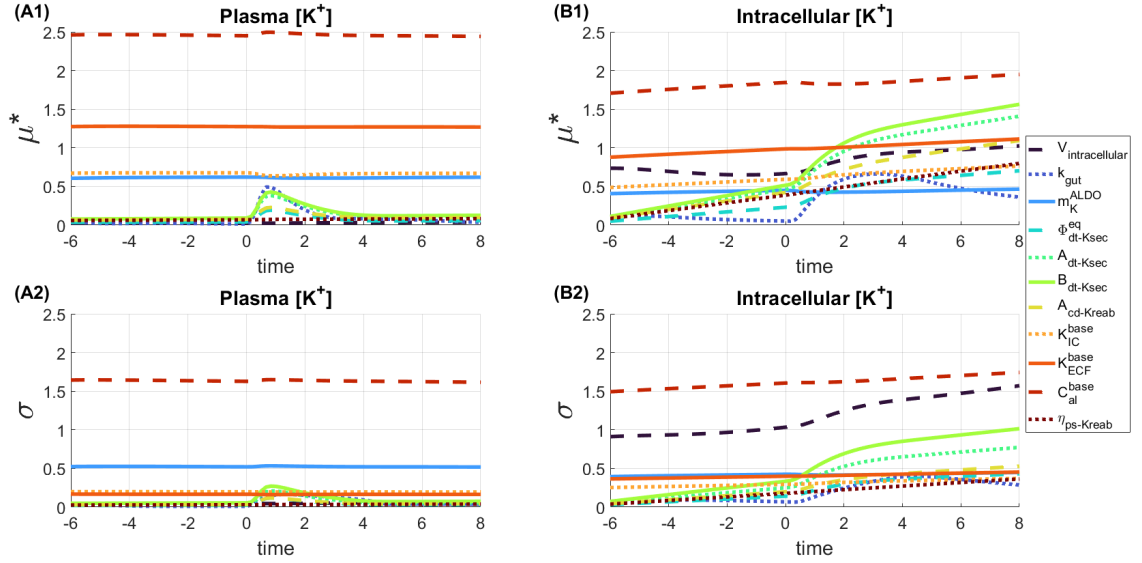


Figure B.2: Morris (A1-B1) μ^* and (A2-B2) σ values for time points along the “KCl only” simulation. Plasma $[K^+]$ effects are shown in panels (A1-A2) and intracellular $[K^+]$ effects are shown in (B1-B2). Elementary effects were compute for every 10 minutes of simulations. Only parameters that had significant μ^* and σ values through the simulations are shown.

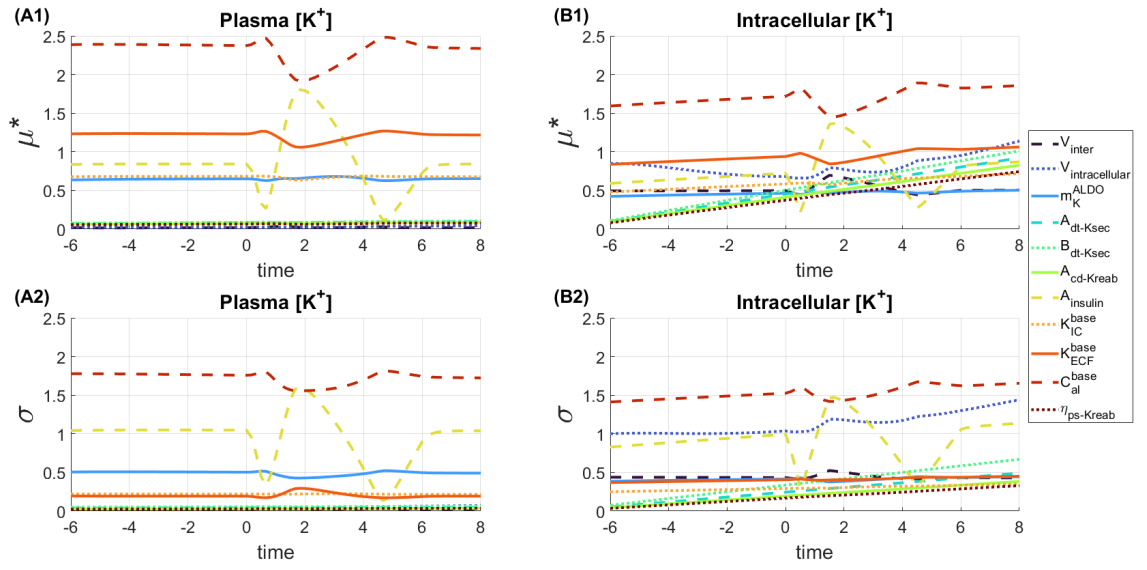


Figure B.3: Morris (A1-B1) μ^* and (A2-B2) σ values for time points along the “Meal only” simulation. Plasma $[K^+]$ effects are shown in panels (A1-A2) and intracellular $[K^+]$ effects are shown in (B1-B2). Elementary effects were compute for every 10 minutes of simulations. Only parameters that had significant μ^* and σ values through the simulations are shown.

Appendix C

Supplementary materials for Chap. 4

C.1 Model description

With the exception of the model equations changed in Section 4.2.2 (i.e., Eqs. 4.1–4.2), all model equations and parameters are the same as discussed in Appendix B.1 (Chap. 3). A detailed model schematic is shown in Fig. C.1. A list of the new parameters and their values used in the baseline model is given in Table C.2.

Name	Unit	Value	Description
$\Phi_{\text{GFR}}^{\text{base}}$	L/min	0.12	baseline glomerular filtration rate
$\eta_{\text{pt-Kreab}}^{\text{base}}$	–	0.67	baseline fractional PT reabsorption
$\eta_{\text{pt-Kreab}}$	–	0.36	fractional PT reabsorption (high K ⁺ diet)
α_{TGF}	ml/min	0.12	TGF parameter
$\eta_{\text{LoH-Keab}}$	–	0.25	loop of Henle fractional reabsorption

Table C.1: Baseline parameter values and descriptions as used in the model simulations. All other parameter values are listed in Table 3.1.

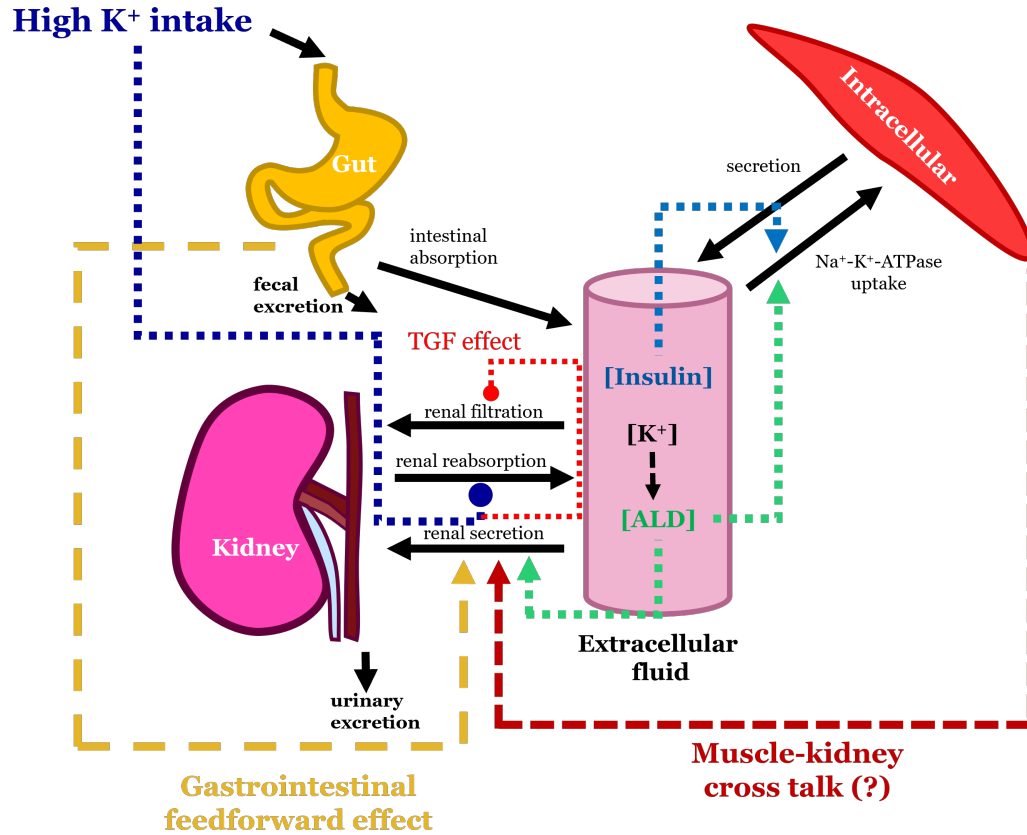


Figure C.1: Detailed model schematic under high K^+ intake. Black arrows represent transport of K^+ between the compartments. Green, light blue, and yellow arrows represent the stimulating effects of aldosterone, insulin, and the gastrointestinal feedforward mechanism, respectively. High K^+ intake inhibitory effects on the proximal tubule (i.e., renal reabsorption) reabsorption is shown by the blunted dark blue arrow. Tubuloglomerular feedback is shown by the blunted light red arrow. The hypothesized muscle-kidney cross talk signal is shown by the dark red arrow. The extracellular compartment is made up of plasma and interstitial fluid. ALD: aldosterone; TGF: tubuloglomerular feedback

C.2 Sensitivity analysis

We conducted a analysis with the Morris method in this study. A detailed description of the Morris method is provided in Chap. 3 (Section 3.2.4). In this study, the model output studied is the final plasma and intracellular $[K^+]$ after a 50 day simulation under high K^+ intake. The ranges for the parameters used in the sensitivity analysis are listed in Table C.2.

Name	Range	Original
k_{gut}	0.5e-2 - 1.5e-2	1.0e-2
A_{Kin}	0.13 - 0.38	0.25
$\Phi_{\text{GFR}}^{\text{base}}$	0.095 - 0.13	0.12
$\eta_{\text{pt-Kreab}}^{\text{base}}$	0.36 - 0.67	0.67
$\eta_{\text{pt-Kreab}}$	0.36 - 0.67	0.36
α_{TGF}	0.058 - 0.18	0.12
$\eta_{\text{LoH-Keab}}$	0.2 - 0.3	0.25
$\Phi_{\text{dt-Ksec}}^{\text{eq}}$	0.031 - 0.0672	0.041
$A_{\text{dt-Ksec}}$	0.17 - 0.52	0.35
$B_{\text{dt-Ksec}}$	0.12 - 0.36	0.24
$A_{\text{cd-Kreab}}$	0.3 - 0.75	0.50
A_{insulin}	0.27 - 0.82	0.55
$C_{\text{al}}^{\text{base}}$	42.5 - 127.5	85
$m_{\text{K}}^{\text{ALDO}}$	0.25 - 0.75	0.5
α_{al}	0.37 - 1.11	0.74
β_{al}	1.52e-3 - 4.6e-3	3.04e-3

Table C.2: Parameter ranges and baseline values (from Ref. [214], Chap. 2) used for the Morris analysis. Parameters without a range given were not used in the Morris analysis. Parameter descriptions are the same as in Tables 3.1 and C.1.

C.3 Muscle-kidney cross talk effects

In our previous study [214] (Chap. 2), we explored a hypothesized signal called “muscle-kidney cross talk” and predicted its effects under high and low K^+ intake. This signal is between intracellular fluid (driven by skeletal muscle) and kidneys, where the skeletal muscle intracellular $[K^+]$ directly affects urine K^+ excretion without changes in the extracellular $[K^+]$. In the same way as Ref. [214] (Chap. 2), muscle-kidney cross talk is not included in the baseline model because there has not been definitive evidence for this signal.

In this study, we further investigate the impacts of muscle-kidney cross talk in the context of a high K^+ diet by conducting “what-if” simulations, in addition to the baseline model simulations. The coupling function (ω_{Kic}) is taken to be a linearly increasing function of K_{IC} , bounded above 0:

$$\omega_{Kic} = \max(0, m_{Kic} (K_{IC} - K_{IC}^{\text{baseline}}) + 1). \quad (C.1)$$

where the baseline value for $m_{Kic} = 0.1$. At homeostasis, $K_{IC} = K_{IC}^{\text{baseline}} = 145$ mEq/L, thus $\omega_{Kic} = 1$. To model a muscle-kidney cross talk signal that targets distal tubule K^+ secretion, we add the impact of ω_{Kic} by replacing the equation for distal segment K^+ secretion with

$$\Phi_{dt-Ksec} = \Phi_{dt-Ksec}^{\text{eq}} \times \gamma_{al} \times \gamma_{Kin} \times \omega_{Kic}. \quad (C.2)$$

(We have previously found that the distal tubule target resulted in similar results to collecting duct reabsorption [214] (Chap. 2).)

Results for predicted intracellular $[K^+]$ during high K^+ diet simulations of muscle-kidney cross talk for varied m_{Kic} values with the PT + TGF effects are shown in Fig. C.2A. A stronger cross talk signal, i.e., larger m_{Kic} , results in lower intracellular $[K^+]$ levels. When $m_{Kic} = 0.1$, intracellular $[K^+]$ levels remain within normal range for the full simulation. However, the predicted intracellular $[K^+]$ are on the high end of the normal range. Results for the same simulations with the addition of PT + TGF effects ($\eta_{pt-Kreab} = 0.36$ and $\alpha_{TGF} = \alpha_{TGF}^{\text{base}}$) are shown in Fig. C.2B. We can see that with the PT + TGF effects and muscle-kidney cross talk parameter $m_{Kic} \geq 0.025$, intracellular $[K^+]$ levels remain within the normal range for the full simulation (Fig. C.2B).

The intracellular and plasma $[K^+]$ at the end of the 50 day high K^+ simulations with and without PT + TGF effects for varied m_{Kic} are shown in Fig. C.3. Regardless of PT + TGF effects, increasing m_{Kic} decreases both final intracellular and plasma $[K^+]$ levels (Fig. C.3). As expected, adding the PT + TGF effects improved final intracellular and

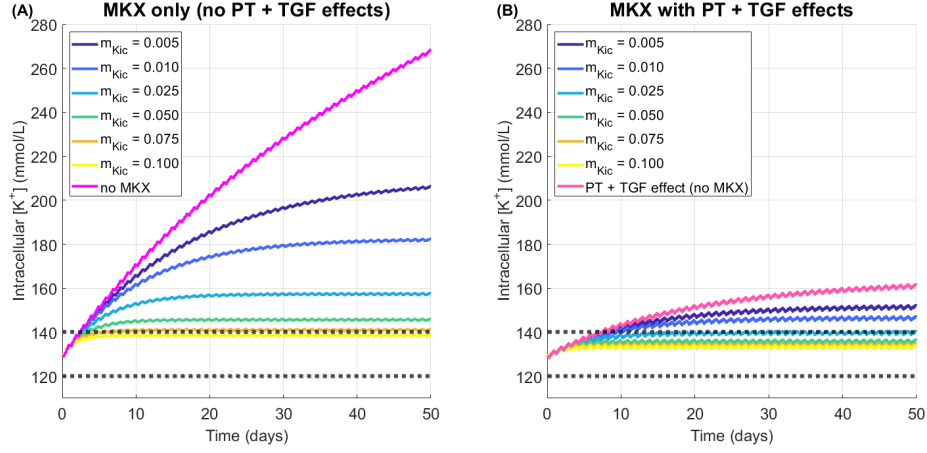


Figure C.2: Model predictions of a high K^+ diet for 50 days with and without muscle-kidney cross talk (MKX) and proximal tubule and tubuloglomerular feedback effects (PT + TGF). PT + TGF effects includes the “TGF effect” (Eq. 2.2 in main text) with $\eta_{pt-Kreab} = 0.36$ as in Wang et al. [230] and α_{TGF} is the baseline value. (A) Model predictions of intracellular $[K^+]$ with muscle-kidney cross talk only where parameter m_{Kic} is the value as given by legend. (B) Model predictions of intracellular $[K^+]$ with muscle-kidney cross talk with PT + GFR high K^+ effects where parameter m_{Kic} is as given by the legend. Normal range for intracellular $[K^+]$ indicated by gray dotted line (120-140 mmol/L). MKX: muscle-kidney cross talk; PT: proximal tubule; TGF: tubuloglomerular feedback; m_{Kic} : muscle-kidney cross talk parameter (see Eq. C.1).

plasma $[K^+]$ levels. This is especially true for the no muscle-kidney cross talk simulation or small values of m_{Kic} . Final plasma $[K^+]$ levels are within normal range with the addition of the muscle-kidney cross talk for $m_{Kic} \geq 0.005$ (Fig. C.3B), but final intracellular $[K^+]$ remains higher than normal range until $m_{Kic} \geq 0.025$ (Fig. C.3A).

In summary, while the impacts of high K^+ intake on PT reabsorption greatly improve the model’s plasma and intracellular $[K^+]$ predictions, the presence of a muscle-kidney cross talk signal prevents excess K^+ from being stored in the intracellular fluid.

In our previous study [214] (Chap. 2), we used our mathematical model to conduct “what-if” simulations of muscle-kidney cross talk under short-term K^+ loading or depletion. Specifically, we found that in our baseline model (i.e., no muscle-kidney cross talk) after 4 days of K^+ loading (high K^+ intake), plasma $[K^+]$ levels started to reach hyperkalemic levels and intracellular $[K^+]$ levels remained high for several days after returning to normal K^+ intake [214] (Chap. 2). When the muscle-kidney cross talk signal was included, model

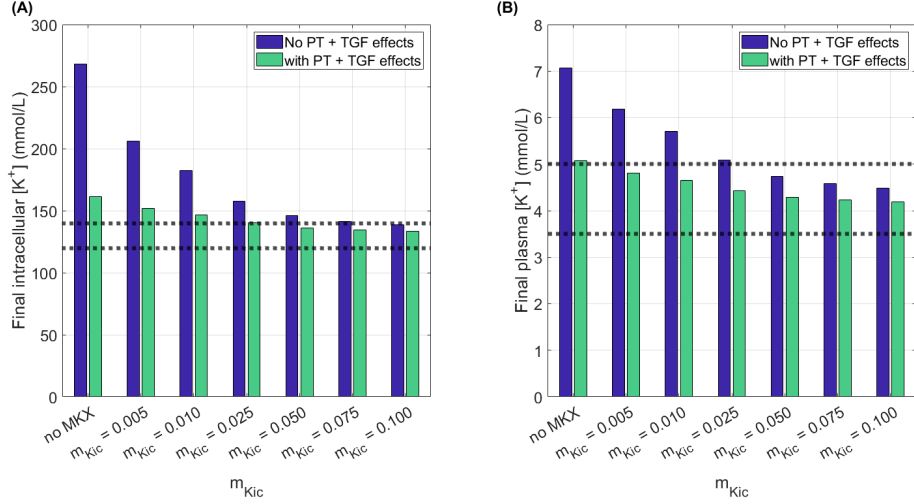


Figure C.3: (A) Intracellular and (B) plasma $[K^+]$ levels at the end of 50 day simulation under with and without proximal tubule and tubuloglomerular feedback effects (PT + TGF effects). Muscle-kidney cross talk as given by the parameter m_{Kic} with or without PT + GFR effects. Normal ranges for intracellular and plasma $[K^+]$ indicated by gray dotted line (120-140 mmol/L and 3.5-5.0 mmol/L, respectively). PT: proximal tubule; TGF: tubuloglomerular feedback; m_{Kic} : muscle-kidney cross talk parameter (see Eq. C.1).

simulations predicted that intracellular $[K^+]$ levels remained within normal range. In this study, we extended our model to consider PT and TGF effects reported by Wang et al. [230]. Without the muscle-kidney cross talk signal, these effects were unable to keep both plasma and intracellular $[K^+]$ levels within normal ranges on a chronic high K^+ diet, which suggests that the muscle-kidney cross talk signal may be one of the necessary regulatory mechanisms. To test this, we conducted “what-if” simulations of a high K^+ diet with varying strengths of the muscle-kidney cross talk signal (m_{Kic} ; Eq. C.1). Model results indicated that muscle-kidney cross talk improves K^+ balance (Fig. C.2; Fig. C.3).

Appendix D

Supplementary materials for Chap. 5

D.1 Additional figures for Chap. 5

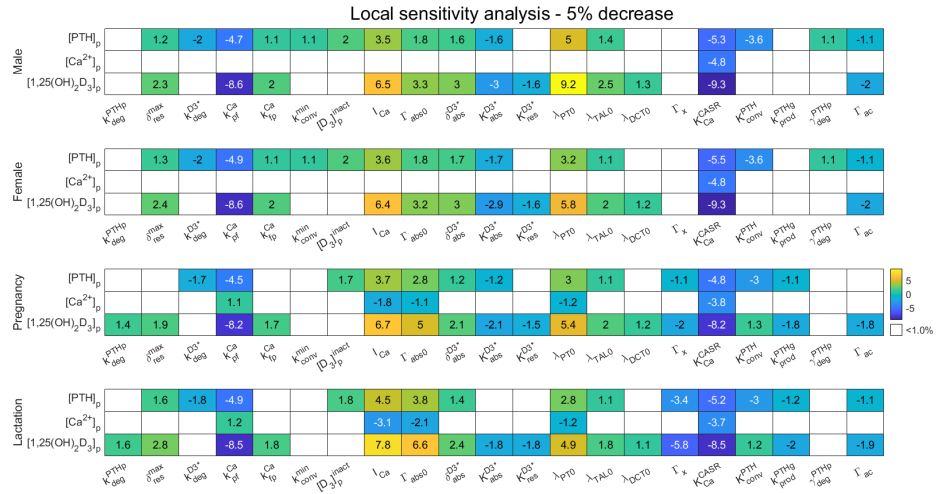


Figure D.1: Local sensitivity analysis for the male, female, pregnancy, and lactation models. Sensitivity analysis was conducted by decreasing individual parameters by 5%. The resulting percent change in model steady state concentrations from baseline is presented here. PTH: parathyroid hormone; 1,25(OH)₂D₃: calcitriol; D₃*: 1,25(OH)₂D₃

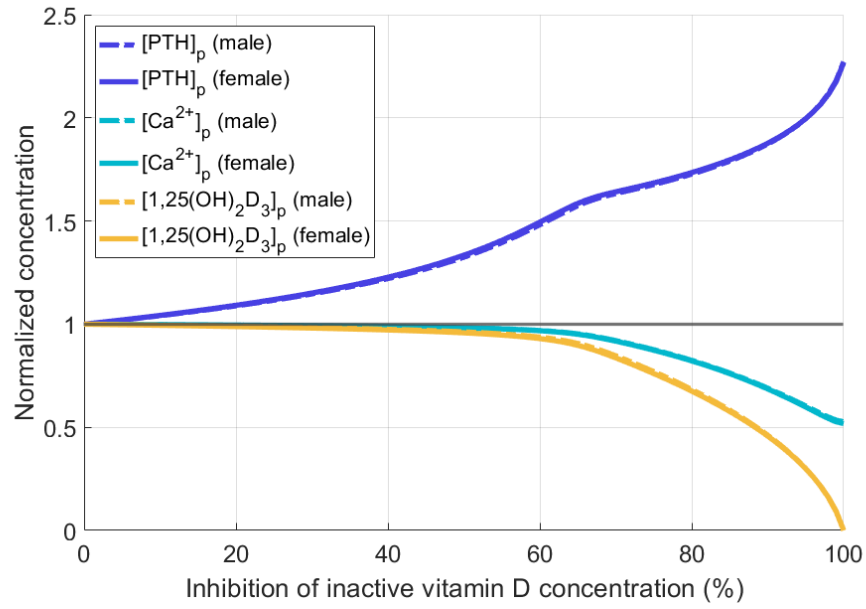


Figure D.2: Predicted normalized steady state plasma concentrations of parathyroid hormone (PTH), calcium (Ca^{2+}), and calcitriol ($1,25(\text{OH})_2\text{D}_3$) in male and female rats under decreased inactive vitamin D ($[\text{D}_3]_{\text{inact}}$) concentrations.

Appendix E

Supplementary materials for Chap. 6

E.1 Impact of declining estrogen levels on the RAS

How do the various impacts of estrogen on the RAS change RAS levels during post-menopausal estrogen decline? To investigate this question, we conducted a simulations of the estrogen-RAS model presented in this study (Section 6.2.1). The results are shown in Fig. E.1. We assume that estrogen decline starts at age 50 years (Fig. E.1A; Eq. 6.1). The decline in estrogen levels, decreases AGT production so that by age 80 years [AGT] is 60% below pre-menopausal levels (Fig. E.1B). Plasma renin secretion is inhibited by estrogen levels, which are rapidly decreasing. Renin secretion is inhibited by estrogen, so the lowered estrogen levels result in driving increased renin secretion so that ultimately plasma renin levels rise (Fig. E.1C).

While renin concentration increases, the decreased AGT levels result in decreased plasma renin activity, which drives Ang I production. This results in stable Ang I levels (Fig. E.1D). ACE activity is increased so we see a slight increase in Ang II levels (Fig. E.1E). Ang II then binds to AT1R and AT2R. Estrogen inhibits AT1R binding and activates AT2R binding (Fig. 6.1). When estrogen levels decrease, AT1R receptors are inhibited less, thus leading to increased AT1R-bound Ang II levels (Fig E.1F). For AT2R receptors, initially, AT2R-bound Ang II levels slightly increase from baseline due to the increased Ang II levels, but later AT2R-bound Ang II levels decrease from baseline due to the loss of activation by estrogen levels (Fig. E.1G).

We conducted a Sobol sensitivity analysis on the estrogen-RAS model to investigate which parameters impact the estrogen deficiency model simulation the most for AGT, Ang

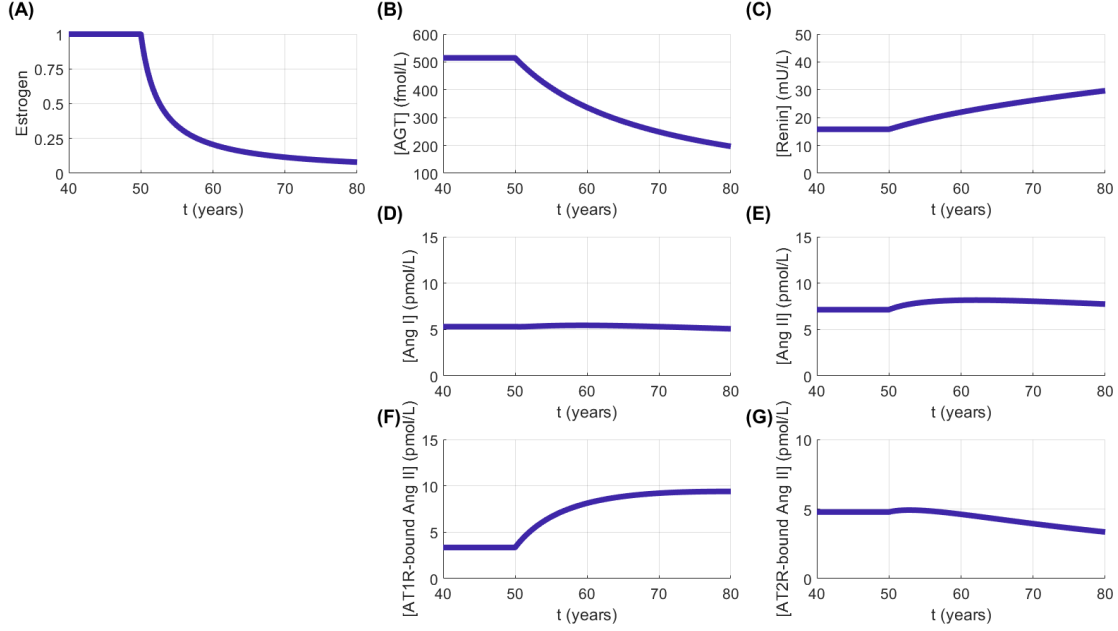


Figure E.1: Simulation results of the impact of decreasing (A) estrogen levels on the plasma concentration of (B) AGT, (C) renin, (D) Ang I, (E) Ang II, (F) AT1R-bound Ang II, and (G) AT2R-bound Ang II. AGT: angiotensinogen, Ang: angiotensin, AT1R: angiotensin type 1 receptor, AT2R: angiotensin type 2 receptor

II, AT1R-bound Ang II, and AT2R-bound Ang II. We varied the parameters that represent the rate of enzyme impacts on the RAS (i.e., parameters c_i) as well as the parameters that represent estrogen impact on the RAS (i.e., parameters e_i listed in Table 6.1). Each parameter was varied by a 2-fold increase and decrease from the baseline value. We then conducted a Sobol analysis at the time point of every 10 years for the estrogen deficiency simulation.

The results for the total order Sobol indices (S_{total}) over the estrogen decline RAS simulation are shown in Fig. E.2. Before the onset of estrogen decline due to menopause (i.e., $t < 50$), the parameters e_i (i = renin, AGT, ACE, etc), which represent the impact of estrogen on the RAS (Eqs. 6.2–6.3), do not have any impact on the model variation as expected because $E = 1$.

For renin, e_{renin} represents the impact of estrogen on renin secretion and leads to about 10% of the model output variation by $t = 70$ years (Fig. E.2A). The parameters with

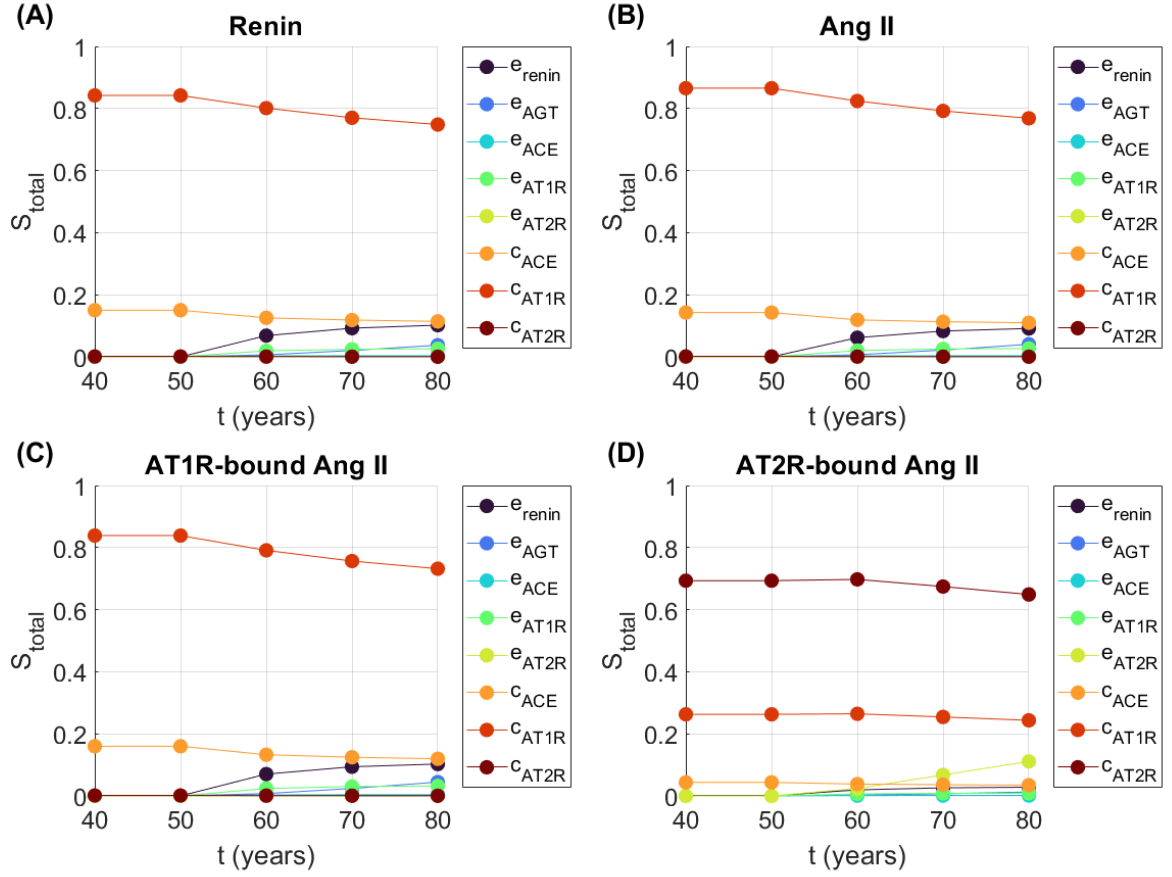


Figure E.2: Sobol total indices (S_{total}) for estrogen impact on (A) AGT, (B) Ang II, (C) AT1R-bound Ang II, and (D) AT2R-bound Ang II at different time points in the simulation. Parameters not shown did not have a S_{total} over 0.01 for any variable. AGT: angiotensinogen, Ang: angiotensin, AT1R, angiotensin type 1 receptor, AT2R: angiotensin type 2 receptor

the largest output on model output of Ang II are c_{ACE} and c_{AT1R} which correspond to ACE activity and AT1R binding, respectively (Fig. E.2B). After the decline in estrogen, the impact of estrogen on renin secretion, e_{renin} contributes to the variation in the model output of Ang II as well (Fig. E.2B). AT1R-bound Ang II is also impacted by c_{ACE} and c_{AT1R} the most due to the dependence on Ang II levels (Fig. E.2C). After the onset of estrogen decline, the impact of estrogen on renin and AT1R binding contribute to variation in the model output. By the end of the simulation time, about 10.2% and 3.2% of model output variation of AT1R-bound Ang II can be attributed to e_{renin} and e_{AT1R} , respectively (Fig. E.2C). For AT2R-bound Ang II, about 11% of the total variation can be attributed to the impact of estrogen on AT2R binding, e_{AT2R} , by the end of the simulation time (Fig. E.2D).

E.2 Additional figures

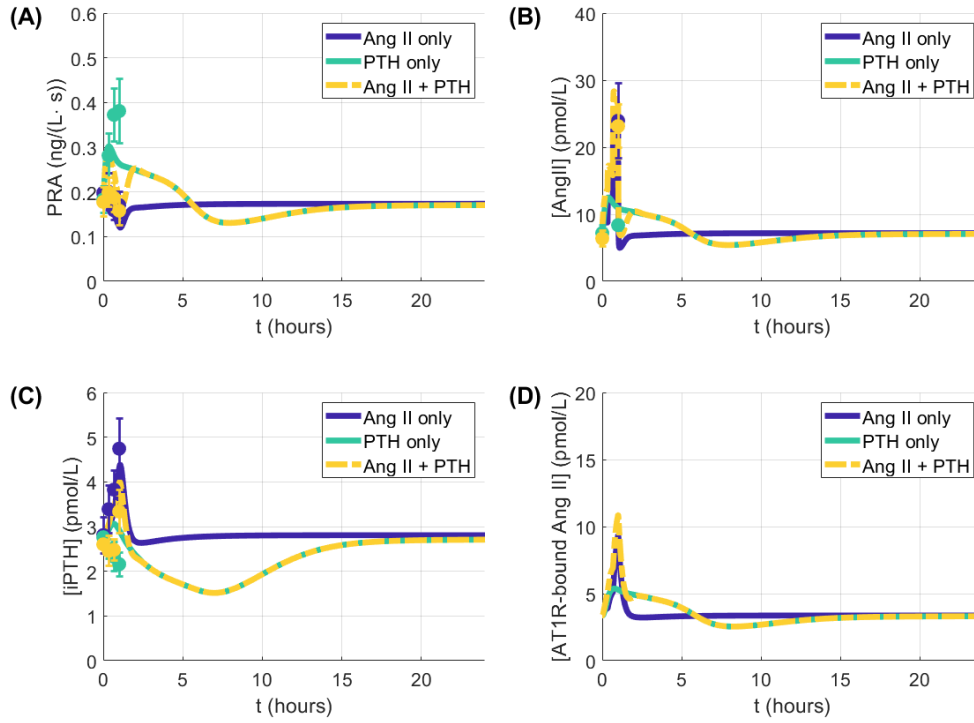


Figure E.3: Replication of Fig. 6.2 for a longer simulation time (24 hours). Simulation results for replication of the experiments in Grant et al. [80]. Results for (A) plasma renin activity, (B) angiotensin II (Ang II) plasma concentration, (C), parathyroid hormone (PTH) concentration, and (D) angiotensin receptor type II bound Ang II are shown for during and after 1 hour of infusions of Ang II only, PTH only, and Ang II and PTH infusions together. Lines indicate simulations, while the dots and error bars in panels A–C are from the relevant experimental data presented in Grant et al. [80]. AT1R: angiotensin type 1 receptor, PRA: plasma renin activity, iPTH: intact parathyroid hormone

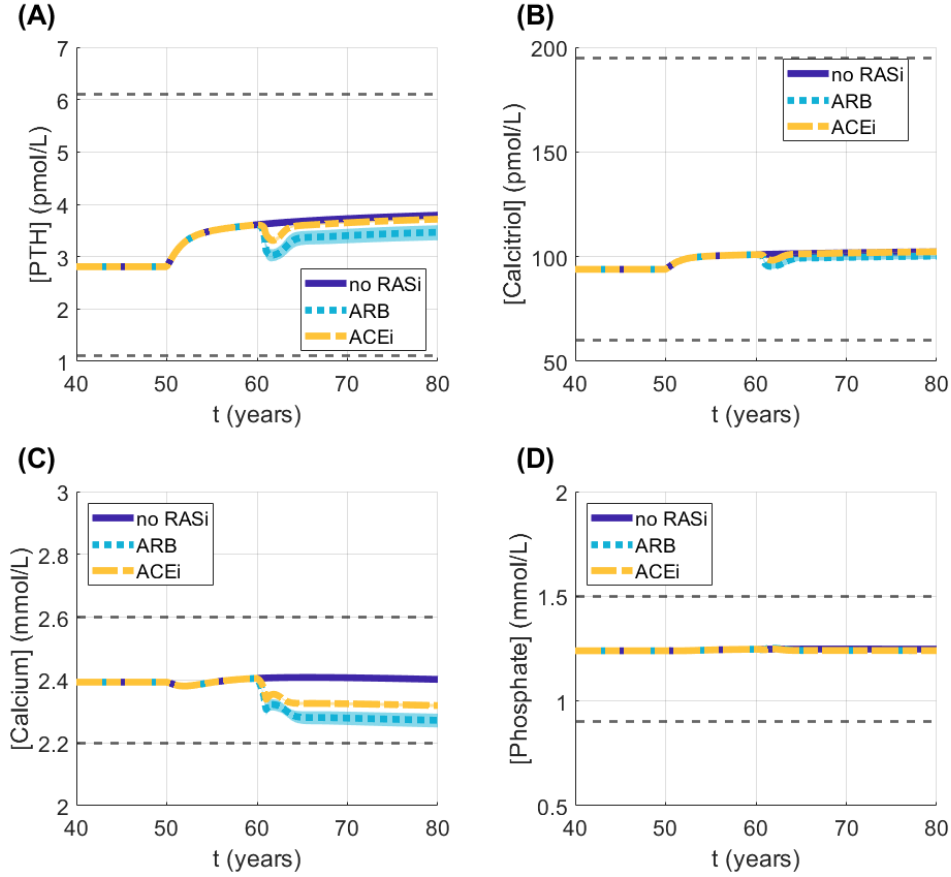


Figure E.4: Impact of estrogen deficiency and RAS inhibitors on extracellular levels of calcitropic hormones (A) parathyroid hormone and (B) calcitriol as well as electrolytes (C) calcium and (D) phosphate. For the ARB simulations (labeled “ARB” in the legend), σ_{ARB} was varied between 92–98.3% based on the ranges reported in Ref. [90]. For the ACEi simulations (labeled “ACEi” in the legend), σ_{ACEi} was varied between 94–97.2% as reported in Ref. [90]. Lines represent the mean values over the varied σ_i values for the respective treatment with shaded area representing the range. PTH: parathyroid hormone, RASi: RAS inhibitor, ARB: angiotensin receptor blocker, ACEi: angiotensin-converting enzyme inhibitor

E.3 Key parameter descriptions

Name	Description	Reference
$E_{\max,L-PTH}$	half-maximal response of effect of PTH on RANKL production	[165]
k_3	binding rate of RANK and RANKL	[165]
k_4	unbinding rate of RANK-RANKL	[165]
$k_{in,L}$	baseline RANKL production	[165]
$k_{out,L}$	degradation rate of RANKL	[165]
$k_{in,RNK}$	baseline RANK production	[165]
$k_{out,RNK}$	degradation rate of RANK	[165]
$\rho_{AT1R,L}$	minimum impact of AT1R on RANKL	Eq. 6.22
$\delta_{AT1R,L}$	half-maximal response of AT1R impact on RANKL	Eq. 6.22
$\alpha_{CaSR,renin}$	maximum impact of CaSR on renin	Eq. 6.18
$\delta_{CaSR,renin}$	half-maximal response of CaSR impact on renin	Eq. 6.18
$\Phi_{Plas2BoneCa0}$	baseline calcium exchange from extracellular fluid to bone	[165]
$\Phi_{Bone2PlasCa0}$	baseline calcium exchange from bone to extracellular fluid	[165]
c_{AT1R}	binding rate of Ang II to AT1R	[90, 131], Eq. 6.10
N_{rs}	baseline renin secretion	[90, 131], Eq. 6.4
X_{PRCPRA}	equilibrium ratio of PRA to PRA	[90]
$\delta_{PTH,renin}$	half-maximal response of PTH impact on renin	Eq. 6.19
$K_{Ca,PTH}$	half-maximal response of PTH secretion to CaSR	[165]
$k_{base,AOH}$	baseline AOH production	[165]
$k_{deg,AOH}$	degradation rate of AOH	[165]
$V_{\max,PTH-AOH}$	maximum effect of PTH on AOH production	[165]
$\delta_{AOH,Phos}$	half-maximal response of impact of phosphate on AOH	[165]
$V_{\max,Phos}$	maximum impact of phosphate on AOH production	[165]
$V_{\max,PTH-reabs}$	maximum impact of PTH on renal reabsorption	[165]

Table E.1: Descriptions of parameters shown in Figs. 6.6, 6.7, and 6.8. The reference is the original source of the parameter value. Ang II: angiotensin II, AOH: 1α -hydroxylase, AT1R: angiotensin type 1 receptor, CaSR: calcium sensing receptor, OC: osteoclast, PTH: parathyroid hormone

E.4 Model equations

A list of the ordinary differential equations in the model is given below.

E.4.1 Renin-angiotensin system

$$\frac{d[\text{PRC}]}{dt} = R_{\text{sec}} - \frac{\ln(2)}{h_{\text{renin}}} \times [\text{PRC}] \quad (\text{E.1})$$

where

$$R_{\text{sec}} = N_{\text{rs}} \times \nu_{\text{AT1R}} \times g_{\text{renin}}^-(E) \times H_{\text{CaSR,renin}}^- \times H_{\text{PTH,renin}}^+ \times H_{\text{Ctriol,renin}}^- \quad (\text{E.2})$$

$$\frac{d[\text{AGT}]}{dt} = g_{\text{AGT}}^+(E) \times k_{\text{AGT}} - \text{PRA} - \frac{\ln(2)}{h_{\text{AGT}}} \times [\text{AGT}] \quad (\text{E.3})$$

where

$$\text{PRA} = X_{\text{PRCPRA}} \times [\text{PRC}] \quad (\text{E.4})$$

$$\frac{d[\text{AngI}]}{dt} = \text{PRA} - (g_{\text{ACE}}^-(E) \times c_{\text{ACE}} + c_{\text{chym}} + c_{\text{nep}})[\text{AngI}] - \frac{\ln(2)}{h_{\text{AngI}}}[\text{AngI}] \quad (\text{E.5})$$

$$\begin{aligned} \frac{d[\text{AngII}]}{dt} &= (g_{\text{ACE}}^-(E) \times c_{\text{ACE}} + c_{\text{chym}})[\text{AngI}] \\ &\quad - (c_{\text{ACE2}} + c_{\text{II,IV}} + g_{\text{AT1}}^-(E) \times c_{\text{AT1}} + g_{\text{AT2}}^+(E) \times c_{\text{AT2}})[\text{AngII}] \\ &\quad - \frac{\ln(2)}{h_{\text{AngII}}}[\text{AngII}] + k_{\text{inf}} \end{aligned} \quad (\text{E.6})$$

$$\frac{d[\text{Ang}(1-7)]}{dt} = c_{\text{NEP}} \times [\text{AngI}] + c_{\text{ACE2}} \times [\text{AngII}] - \frac{\ln(2)}{h_{\text{Ang}(1-7)}}[\text{Ang}(1-7)] \quad (\text{E.7})$$

$$\frac{d[\text{AngIV}]}{dt} = c_{\text{AngII=AngIV}} \times [\text{AngII}] - \frac{\ln(2)}{h_{\text{AngIV}}} \times [\text{AngIV}] \quad (\text{E.8})$$

$$\frac{d[\text{AT1R} - \text{AngII}]}{dt} = g_{\text{AT1}}^-(E) \times c_{\text{AT1}}[\text{AngII}] - \frac{\ln(2)}{h_{\text{AT1}}}[\text{AT1R} - \text{AngII}] \quad (\text{E.9})$$

$$\frac{d[\text{AT2R} - \text{AngII}]}{dt} = g_{\text{AT2}}^+(E) \times c_{\text{AT2}}[\text{AngII}] - \frac{\ln(2)}{h_{\text{AT2}}}[\text{AT2R} - \text{AngII}] \quad (\text{E.10})$$

E.4.2 Calcium regulation

$$\frac{d[\text{Ca}]}{dt} = \Phi_{\text{Bone2Plas-Ca}} - \Phi_{\text{Plas2Bone-Ca}} - \Phi_{\text{Urine-Ca}} + \Phi_{\text{Gut2Plas-Ca}} \quad (\text{E.11})$$

with

$$\Phi_{\text{Gut2Plas-Ca}} = I_{\text{Ca}} \times \left(\Phi_{\text{Gut2Plas-Ca0}} + V_{\text{max-CtrlGut}} \times \frac{[\text{Ctrlol}]^{\gamma_{\text{CtrlGut}}}}{(K_{\text{CtrlGut-Ca}})^{\gamma_{\text{CtrlGut}}} + [\text{Ctrlol}]^{\gamma_{\text{CtrlGut}}}} \right) \quad (\text{E.12})$$

$$\Phi_{\text{Plas2Bone-Ca}} = \xi_{15} \times [\text{Ca}] \times (1 - \nu_{\text{J15}} + \nu_{\text{J15}} \times \text{HAp}) \quad (\text{E.13})$$

$$\Phi_{\text{Bone2Plas-Ca}} = Q_{\text{bone}} \times \left(\Phi_{\text{Bone2Plas-Ca0}} + V_{\text{max,OC-Ca}} \times \frac{\text{OC}^{\gamma_{\text{OCCa}}}}{K_{\text{OC}}^{\gamma_{\text{OCCa}}} + \text{OC}^{\gamma_{\text{OCCa}}}} \right) \quad (\text{E.14})$$

$$\Phi_{\text{Urine-Ca}} = \kappa_{\text{Ca}} \times \Phi_{\text{GFR}} \times [\text{Ca}] - \Phi_{\text{Reabs-Ca}} \quad (\text{E.15})$$

$$(\text{E.16})$$

where

$$\Phi_{\text{Reabs-Ca}}^{\text{max}} = \Phi_{\text{GFR}} \times [\text{Ca}] \times (1 + m_{\text{EST}}(E - 1)) \quad (\text{E.17})$$

$$\Phi_{\text{Reabs-Ca}} = \Phi_{\text{Reabs-Ca}}^{\text{max}} \times \frac{V_{\text{max-Ca}} \times [\text{Ca}]}{K_{\text{reabs-Ca}} + [\text{Ca}]} \times \frac{V_{\text{max-PTHreabs}} \times [\text{PTH}]}{K_{\text{PTH-reabs}} + [\text{PTH}]} \quad (\text{E.18})$$

E.4.3 Parathyroid hormone

$$\frac{d[\text{PTH}]}{dt} = S_{\text{PTH}} \times H_{\text{AT1R,PTH}}^+ - k_{\text{deg,PTH}} \times [\text{PTH}] \quad (\text{E.19})$$

$$S_{\text{PTH}} = H_{\text{CaSR,PTH}}^- \times \frac{\text{PTG}}{0.5} \times \text{PT}_{\text{max}} \quad (\text{E.20})$$

$$\frac{d\text{PTG}}{dt} = k_{\text{prod-PTG}} - k_{\text{deg-PTG}} \times \text{PTG} \quad (\text{E.21})$$

$$\frac{d\text{PT}_{\text{max}}}{dt} = \text{PT}_{\text{max-base}} \times H_{\text{Ctrlol,PTmax}}^- \times H_{\text{Phos,PTmax}}^+ - k_{\text{deg-PTmax}} \times \text{PT}_{\text{max}} \quad (\text{E.22})$$

E.4.4 Calcitriol

$$\frac{d[\text{AOH}]}{dt} = k_{\text{prod-AOH}} - k_{\text{deg}} \times [\text{AOH}] \quad (\text{E.23})$$

with

$$k_{\text{prod-AOH}} = k_{\text{base-AOH}} \times H_{\text{PTH,AOH}}^+ \times H_{\text{Phos,AOH}}^- \quad (\text{E.24})$$

$$\frac{d[\text{Ctriol}]}{dt} = \text{AOH} - k_{\text{deg-Ctriol}} \times [\text{Ctriol}] \quad (\text{E.25})$$

E.4.5 Phosphate regulation

$$\begin{aligned} \frac{d[\text{PhosECC}]}{dt} &= \Phi_{\text{Bone2Plas-Phos}} - \Phi_{\text{Plas2Bone-Phos}} - \Phi_{\text{Urine-Phos}} \\ &\quad + \Phi_{\text{Gut2Plas-Phos}} - \Phi_{\text{ECC2Int-Phos}} + \Phi_{\text{Int2ECC-Phos}} \end{aligned} \quad (\text{E.26})$$

$$\frac{d[\text{PhosInt}]}{dt} = \Phi_{\text{ECC2Int-Phos}} - \Phi_{\text{Int2ECC-Phos}} \quad (\text{E.27})$$

with

$$\Phi_{\text{Gut2Plas-Phos}} = I_{\text{Phos}} \times \left(\Phi_{\text{Gut2Plas-Phos0}} + V_{\text{max-CtriolGutPhos}} \times \frac{[\text{Ctriol}]^{\gamma_{\text{CtriolGut}}}}{(K_{\text{CtriolGut-Ca}})^{\gamma_{\text{CtriolGut}}} + [\text{Ctriol}]^{\gamma_{\text{CtriolGut}}}} \right) \quad (\text{E.28})$$

$$\Phi_{\text{Urine-Phos}} = \kappa_{\text{phos}} \times \Phi_{\text{GFR}} \times [\text{ECCPhos}] - \Phi_{\text{Phos-reabs}} \quad (\text{E.29})$$

$$\Phi_{\text{Plas2Bone-Phos}} = \eta_{\text{bone-phos}} \times \Phi_{\text{Plas2Bone-Ca}} \quad (\text{E.30})$$

$$\Phi_{\text{Bone2Plas-Phos}} = \eta_{\text{bone-phos}} \times \Phi_{\text{Bone2Plas-Ca}} \quad (\text{E.31})$$

where

$$\Phi_{\text{Phos-reabs}} = \Phi_{\text{Phos-reabs0}} \times \Phi_{\text{GFR}} \quad (\text{E.32})$$

E.4.6 Bone remodeling

$$\begin{aligned}\frac{dOC}{dt} &= k_{\text{prod-OC}} \times H_{\text{RANK-RANKL}}^+ \times OC_{\text{base}} \\ &\quad - k_{\text{deg-OC}} \times H_{\text{TGFbact,OC}}^+ \times H_{\text{RNK-RNKL}}^- \times OC\end{aligned}\quad (\text{E.33})$$

$$\frac{dpOB}{dt} = k_{\text{prod-pOB}} \times \left(\frac{1}{E}\right)^{\gamma_{\text{pOB,EST}}} - k_{\text{deg,pOB}} \times H_{\text{TGFbact,pOB}}^+ \times pOB \quad (\text{E.34})$$

$$\frac{dOB}{dt} = k_{\text{prod-OB}} \times H_{\text{TGFbact,OB}}^- \times pOB - k_{\text{deg-OB}} \times OB \quad (\text{E.35})$$

$$\begin{aligned}\frac{dRNK}{dt} &= -k_3 \times RNK \times L + k_4 \times RNK - RNKL \\ &\quad + k_{\text{prod-RNK}} \times H_{\text{TBFbact,RNK}}^+ - k_{\text{deg-RNK}} \times RNK\end{aligned}\quad (\text{E.36})$$

$$\frac{dRNKL}{dt} = -k_3 \times RNK \times L + k_4 \times RNK - RNKL \quad (\text{E.37})$$

$$\begin{aligned}&+ k_{\text{prod-L}} \times \left(\frac{OB}{OB_0}\right)^{\gamma_{\text{OBeff}}} \times H_{\text{PTH,L}}^+ \times H_{\text{AT1R,RANKL}}^+ \\ &- k_{\text{deg-L}} \times RNKL - k_1 \times OPG \times L + k_2 \times RNK - OPG\end{aligned}\quad (\text{E.38})$$

$$\frac{dRNK - RNKL}{dt} = k_3 \times RNK \times L - k_4 \times RNK - RNKL \quad (\text{E.39})$$

$$\begin{aligned}\frac{dOPG}{dt} &= -k_1 \times OPG \times RNKL + k_2 \times RNK - OPG + \\ &\quad k_{\text{prod-OPG}} \times H_{\text{PTH,OPG}}^+ \times H_{\text{AT1R,OPG}}^+ - k_{\text{deg-OPG}} \times OPG\end{aligned}\quad (\text{E.40})$$

$$\frac{dRNK - OPG}{dt} = k_1 \times OPG \times RNKL - k_2 \times RNK - OPG \quad (\text{E.41})$$

$$\frac{dTGFb}{dt} = k_{\text{prod-TGF}} \times \left(\frac{OB}{OB_{\text{base}}}\right)^{\gamma_{\text{OBtgf}}} \times \left(\frac{1}{E}\right)^{\gamma_{\text{TGFb,E}}} \quad (\text{E.42})$$

$$- k_{\text{act,TGFb}} \times TGFb \times \left(\frac{OC}{OC_{\text{base}}}\right)^{\gamma_{\text{OCtgf}}} \times E^{\gamma_{\text{tgfbactE}}} \quad (\text{E.43})$$

$$\begin{aligned}\frac{dTGFb_{\text{active}}}{dt} &= k_{\text{act,TGFb}} \times TGFb \times \left(\frac{OC}{OC_{\text{base}}}\right)^{\gamma_{\text{OCtgf}}} \times E^{\gamma_{\text{tgfbactE}}} \\ &\quad - k_{\text{deg-TGFact}} \times TGFb_{\text{active}}\end{aligned}\quad (\text{E.44})$$

$$\begin{aligned}\frac{dBMD}{dt} &= k_{\text{BMD-form}} \times \left(\frac{OB}{OB_{\text{base}}}\right)^{\gamma_{\text{OBform}}} - k_{\text{BMD-res}} \times \left(\frac{OC}{OC_{\text{base}}}\right)^{\gamma_{\text{OBres}}} \times BMD \\ &\quad (\text{E.45})\end{aligned}$$

E.4.7 List of variable names

- AGT: angiotensinogen
- Ang I: angiotensin I
- Ang II: angiotensin II
- Ang (1-7): angiotensin (1-7)
- Ang IV: angiotensin IV
- AOH: 1α -hydroxylase
- AT1R-AngII: angiotensin type 1 receptor bound Ang II
- AT2R-AngII: angiotensin type 2 receptor bound Ang II
- BMD: bone mineral density
- Ca: plasma calcium
- Ctrial: calcitriol in plasma
- E : normalized estrogen level
- OB: osteoblasts
- OC: osteoclasts
- OPG: osteoprotegerin
- PhosECC: extracellular phosphate
- PhosInt: intracellular phosphate
- pOB: pre-osteoblasts
- PRA: plasma renin activity
- PRC: plasma renin concentration
- $PT_{\max}$: maximal parathyroid gland secretion
- PTG: parathyroid hormone in the parathyroid glands

- PTH: parathyroid hormone
- RNK: RANK
- RNKL: RANKL
- TGFB: latent $\text{TGF}\beta$
- $\text{TGFB}_{\text{active}}$: active $\text{TGF}\beta$

Glossary

1 α -hydroxylase Enzyme that produces calcitriol in the proximal tubules of the kidneys.

accretion Bone formation facilitated by osteoblasts.

angiotensin-converting enzyme Enzyme that converts angiotensin I to angiotensin II

calcitriol A calcitropic hormone that is the active form of vitamin D that made in the proximal tubules of the kidneys by 1 α -hydroxylase. It is also known as 1,25-dihydroxycholecalciferol or 1,25(OH)₂D₃.

collecting duct collecting ducts

collecting ducts The collecting ducts are at the end of the nephrons where the luminal fluid is collected for excretion through urine. Some reabsorption and secretion of solutes happen in these segments for final fine tuning.

distal tubule distal tubules

distal tubules The distal tubules are the segments after the macula densa of the nephrons and before the collecting ducts. In this thesis, the distal tubules are made up of the distal convoluted tubule and connecting tubule. For potassium, (typically) secretion primarily happens along these segments.

gastrointestinal feedforward effect Signal from the gastrointestinal system to the kidneys to increase potassium excretion

glomerular filtration rate The volume of fluid filtered into the kidneys per unit time (typically minutes).

glomeruli Plural of glomerulus

glomerulus The main filtering unit of the kidney. Fluid filtered through the glomerulus enter into Bowman's capsule to enter the nephrons.

heptatoportal circulation Specialized venous system that transports blood from the gastrointestinal tract (including the stomach, intestines, spleen, and pancreas) to the liver before returning to the heart.

hydroxyapatite The main structural component of bone in mammals. It is a stable compound made up of calcium and phosphate groups.

hypercalcemia High serum calcium levels.

hyperkalemia High serum potassium levels.

hypocalcemia Low serum calcium levels.

hypokalemia Low serum potassium levels.

loop of Henle loops of Henle

loops of Henle Nephron segments after the proximal tubules and before the macula densa.

macula densa A specialized group of epithelial cells in the distal tubule of the kidney that monitors the sodium, chloride, and potassium concentration in the luminal fluid. These cells control glomerular filtration rate through the tubuloglomerular feedback signal.

nephron nephrons

nephrons The functional units of the kidneys. Nephrons are tiny tubules lined with epithelial cells that selectively reabsorb and secrete essential nutrients, such as electrolytes, so what is excreted at the end of the nephrons is waste.

osteoblast osteoblasts

osteoblasts A type of bone cell that strengthens existing bone and forms new bone tissue.

osteoclast osteoclasts

osteoclasts A type of bone cell that breaks down (i.e., resorbs) bone tissue.

proximal segment Nephron segments before the macula densa. In this thesis, the proximal segments include the proximal tubules and loops of Henle.

proximal tubule proximal tubules

proximal tubules Proximal tubules are the first segments of the nephrons of the kidneys. Most of the reabsorption of solutes and volume happen along the proximal tubule.

resorption Break down of bone tissue.

thick ascending limb A segment in the loop of Henle.

tubuloglomerular feedback Feedback signal where the macula densa detects changes in the luminal fluid sodium, chloride, and potassium concentration and adjusts the glomerular filtration rate accordingly. For example, when sodium, chloride, and potassium concentration are high, GFR is decreased.