DEVELOPMENT OF A MATHEMATICAL MODEL OF HUMAN POTASSIUM-ALDOSTERONE HOMEOSTASIS AND CARDIORENAL FUNCTION AND APPLICATION TO UNDERSTAND POTASSIUM RESPONSES TO DRUG THERAPY

by

ERFAN MADDAH

(Under the Direction of K. Melissa Hallow)

ABSTRACT

Regulation of plasma potassium (K^+) within a narrow range is essential for life. The kidney maintains K^+ homeostasis by matching K^+ intake and excretion, in part through the action of aldosterone (ALDO). K^+ regulation is altered by disease states such as kidney dysfunction and by therapies that directly or indirectly alter ALDO such as mineralocorticoid receptor antagonists (MRAs). These conditions increase the risk for hyperkalemia and/or hypokalemia, serious complications that can be deadly.

Predicting the effects of disease and therapies on plasma K^+ levels is difficult. To address this challenge, a mathematical model was developed that integrates K^+-ALDO regulation and therapeutic mechanisms.

This model mechanistically describes processes of renal K^+ filtration, reabsorption, secretion, and ALDO regulation by K^+ and Na^+. K^+- ALDO feedback was calibrated by fitting data from human subjects on high/low K^+ and Na^+ diets following K^+ infusion.

The model describes observed baseline changes in plasma K^+ and ALDO with changes in K^+/Na^+ intake, as well as dynamic changes in these variables with initiation and cessation of K^+ infusion. The model was also fit to urinary K^+ excretion data following spironolactone treatment.

As validation, the model predicted steady-state changes in plasma/urinary ALDO and K^+ with sustained MRA spironolactone treatment in human subjects with hyperaldosteronism.

This K^+- ALDO homeostasis model was then integrated into a well-established cardiorenal model of Na^+ and blood pressure (BP) homeostasis.

The integrated model mechanistically describes processes of renal K^+ and Na^+, filtration, reabsorption, secretion, and ALDO regulation by K^+ and Na^+. The model was recalibrated to reproduce previously published plasma K^+ and ALDO responses to K^+ infusion during low/high K^+ and Na^+ diets and to reproduce the urinary K^+, Na^+ to spironolactone. It was then validated by predicting the chronic plasma K^+ and BP response data for MRA antagonists.

The model was also used to describe the K^+ response to sodium-glucose co-transporter inhibitors (SGLT2i), which was previously unclear. This model is a valuable tool for mechanistically understanding the effects of therapies on electrolyte homeostasis in both normal and impaired kidney function. It can aid in determining optimal drug dosing for balancing safety and efficacy.

INDEX WORDS: Potassium, Kidney, Sodium, Model Calibration, Model Validation, Drug Safety, Drug Efficacy, Hyperkalemia

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DEDICATION

To my beloved family

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ABBREVIATIONS

ACE	**Angiotensin converting enzyme**
ALDO	**aldosterone**
ASCLoH	**ascending loop of Henle**
ANGI	**angiotensin I**
ANGII	**angiotensin II**
AT1	**angiotensin receptor type 2**
AT2	**angiotensin receptor type 2**
CCD	**cortical collecting duct**
CD	**collecting duct**
CKD	**chronic kidney disease**
CNT	**connecting tubule**
DCT	**distal convoluted tubule**
DT	**distal tubule**
DSCLoH	**descending loop of Henle**
ECF	**extracellular fluid**
ENAC	**epithelial sodium channel**
GFR	**glomerular filtration rate**
K^+	**potassium**
Kir	**potassium inward rectifier channel**
LoH	**loop of Henle**
MAP	**mean arterial pressure**
MCD	**medullary collecting duct**
MRA	**mineralocorticoid receptor antagonist**
Na^+	**sodium**
PRA	**plasma renin activity**
PT	**proximal tubule**
RAAS	**renin angiotensin aldosterone system**
RBF	**renal blood flow**
RIHP	**renal interstitial hydrostatic pressure**
ROMK	**renal outer medullary potassium channel**
RVR	**renal vascular resistance**
SGLT2i	**sodium-glucose co-transporter inhibitor**
SNGFR	**single nephron glomerular filtration rate**

1. Chapter 1: Introduction and Background

1.1. Introduction

The kidneys are bean-shaped organs that exist in all vertebrates and are responsible for many important regulatory roles. The kidneys not only filter toxins and wastes from the body, but they also have a significant role in water and electrolytes homeostasis. Potassium (K^+) is one of the critical electrolytes which is essential to normal cellular function. The K^+ level of the human body is regulated through changes in renal K^+ excretion [1]. Low blood K^+ (hypokalemia) can lead to weakness, fatigue, muscle cramps, and cardiac arrhythmias. On the other hand, high blood K^+ (hyperkalemia) affects the cardiovascular system and can lead to sudden cardiac death [2]. Hyperkalemia risk in patients with kidney diseases is increased because excess K^+ remains in blood instead of leaving the body through the urine.

Consequently, renal function has a vital role in K^+ homeostasis. The complexity of the renal system brings noticeable challenges in the interpretation of the effects of novel and existing therapeutics that act through the renal system. Despite having a large body of information and data on both normal renal K^+ handling and disease- and drug-induced alterations in its regulation, the complexities of renal physiology, pathophysiology, and pharmacology often make it challenging to understand and interpret this data. Moreover, hormonal feedback regulation (e.g., potassium/aldosterone (K^+-ALDO) regulation) and interaction with other systems such as cardiovascular physiology brings more complications to our understanding and ability to make predictions.

1.1.1. Significance of electrolyte balance

In normal physiology, electrolytes are essential to maintain a stable equilibrium in fluid balance, myocardial function, neurological function, and much more. Electrolytes maintain resting voltages (the Nernst potential) across cell membranes and action potentials are generated by the movement of K^+ and Na^+ ions across the cell membrane. Thus, deviations in K^+ or Na^+ concentration from normal can impair the ability of the nervous system to function or the ability of the heart to generate contractions. The most common reason for electrolyte disturbances is renal dysfunction. The kidneys work to keep the electrolyte concentrations in blood constant despite changes in electrolyte and fluid intake rates or other changes within the body.

1.1.2. Significance and physiology of potassium homeostasis

Abnormality in the level of extracellular (blood and interstitium) K^+ is one of the most severe electrolyte perturbations. K^+ is mainly an intracellular electrolyte. Intracellular concentration is typically 140-150 mEq/l, while the concentration in the blood and interstitial fluid is typically around 4.2 mEq/l. The accurate control of plasma potassium is vital since cell viability is incredibly sensitive to extracellular fluid K^+ concentration changes. Hyperkalemia or high plasma K^+ (plasma $K^+ > 5$mEq/l) is associated with renal impairment, such as glomerular or tubular disorders. Hypokalemia or low plasma K^+ (plasma $K^+<3.5$ mEq/l) is often due to excess renal K^+ losses. Both conditions can cause cause cardiac dysfunction, and higher concentrations can lead to cardiac arrest or fibrillation [3].

K^+ regulation occurs in nephrons, the smallest functional and structural unit of the

kidney (**Fig 1.1**) [4]. Each healthy human kidney has more than one million nephrons. Each nephron begins with a glomerulus (a cluster of capillaries that forms the filtering component) that filters blood entering the kidney. Thus, glomerular filtration is the first step in electrolyte balance through the nephron. The glomerulus freely filters K^+. Kidney filtration is evaluated by a variable called glomerular filtration rate (GFR). Normal GFR level is from 90 to 120 $\frac{mL}{min.1.73m^2}$, decreasing with age or kidney diseases. From the glomerulus, filtered water and solutes enter the tubule, where most of the filtrate is reabsorbed and returned to the bloodstream.

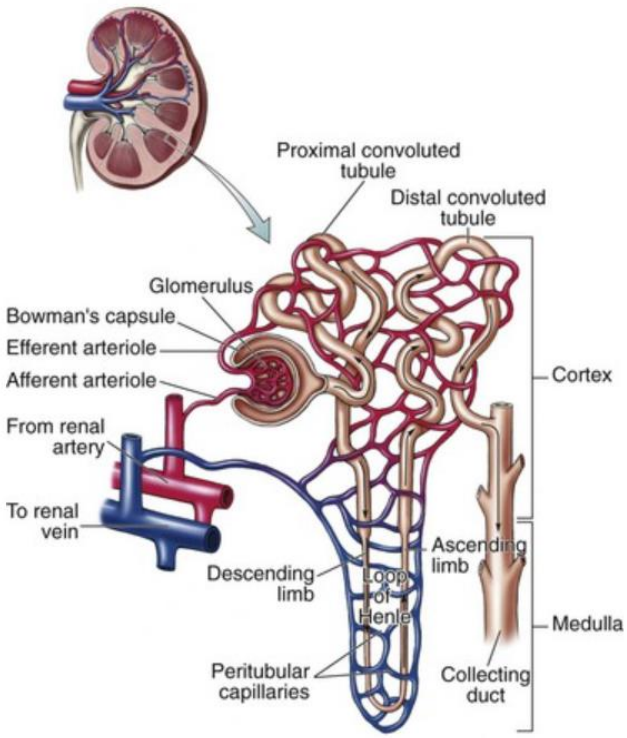

Fig 1.1. Nephrons as the smallest functional units of the kidney, consisting of a blood supply and particular connected ducts named a tubule. The glomerulus is a cluster of capillaries supplied by a

high-pressure arteriole (afferent arteriole) and leads blood to the efferent arteriole. Fluid filtered across the glomerular membrane flows into the tubule system. The major tubule segments are proximal tubule (PT), loop of Henle (LoH), distal tubule (DT), and collecting duct (CD), and each of them is responsible for electrolyte reabsorption or secretion [5, 6] . The nephron figure is taken from [7].

K^+ in the plasma and interstitial fluid is regulated over time by control of renal K^+ excretion. Filtered K^+ is mainly reabsorbed in the proximal tubule [8] (65%) and loop of Henle's ascending loop [LoH] (27%), respectively. Consequently, almost 8% of the filtered K^+ load is delivered to the distal tubule [DT]. Secreted K^+ (4% of filtered load) in DT and collecting ducts [CD] adds to the amount leaving the tubule. The daily excreted K^+ is about 12% of the K^+ filtered at the glomerular capillaries [9].

Adjustments in K^+ secretion is a key mechanism by which the kidney prevents abnormal K^+ elevation in the blood. It plays a significant role in keeping the plasma K^+ level constant. The most crucial cells involved in K^+ secretion are principal cells located in the connecting [CNT] and cortical collecting duct [CCD] (**Fig 1.2**). K^+ secretion is coupled with Na^+ reabsorption through a mechanism that involves three main processes: 1) active exchange of Na^+ and K^+ on the basolateral side of the cell through Na^+/K^+-ATPase pumps, 2) passive flux of K^+ out of the cell into the lumen through ROMK and BK channels[10], and 3) passive flux of Na^+ into the cell from the lumen through ENaC[11](**Fig 1.2**).

Aldosterone (ALDO), a steroid hormone secreted by the adrenal gland, regulates this process through its effect on (mineralocorticoid) MR receptors. MR receptors upregulate ENaC, increasing the flux of Na^+ into the cells. This, in turn, increases the exchange of Na^+ and K^+ through Na^+/K^+ ATPase pumps, leading to an increase in K^+ concentration inside the principal cells and

increasing secretion rate into the lumen through ROMK and BK channels. MR may also directly increase the activity of Na^+/K^+ ATPase[12, 13]. Moreover, MR's effect on ROMK channels is essential in mediating ALDO's effects on K^+ secretion[14].

Downstream of the DCT and CCD, the medullary collecting duct (MCD) plays a vital role in the reabsorption of K^+ from the filtrate, rather than secretion. In the MCD, K^+ is reabsorbed through the activity of several transporters, including the ROMK channel and the Na^+-K^+-chloride cotransporter (NKCC2)[15].

The net result of the filtration, secretion, and reabsorption processes determines the amount of K^+ that is excreted from the body. This is important because the balance between K^+ intake and excretion is what determines the total body K^+ and plasma K^+ concentration.

When the excretion rate of K^+ is too low, it can lead to an accumulation of K^+ in the body, a condition known as hyperkalemia. On the other hand, when the excretion rate of K^+ is too high, it can lead to a deficiency of K^+ in the body, a condition known as hypokalemia.

In conclusion, the processes of filtration, secretion, and reabsorption all work together to determine the excretion rate of K^+. The balance between intake and excretion of K^+ is crucial for maintaining optimal total body K^+ and plasma K^+ concentration.

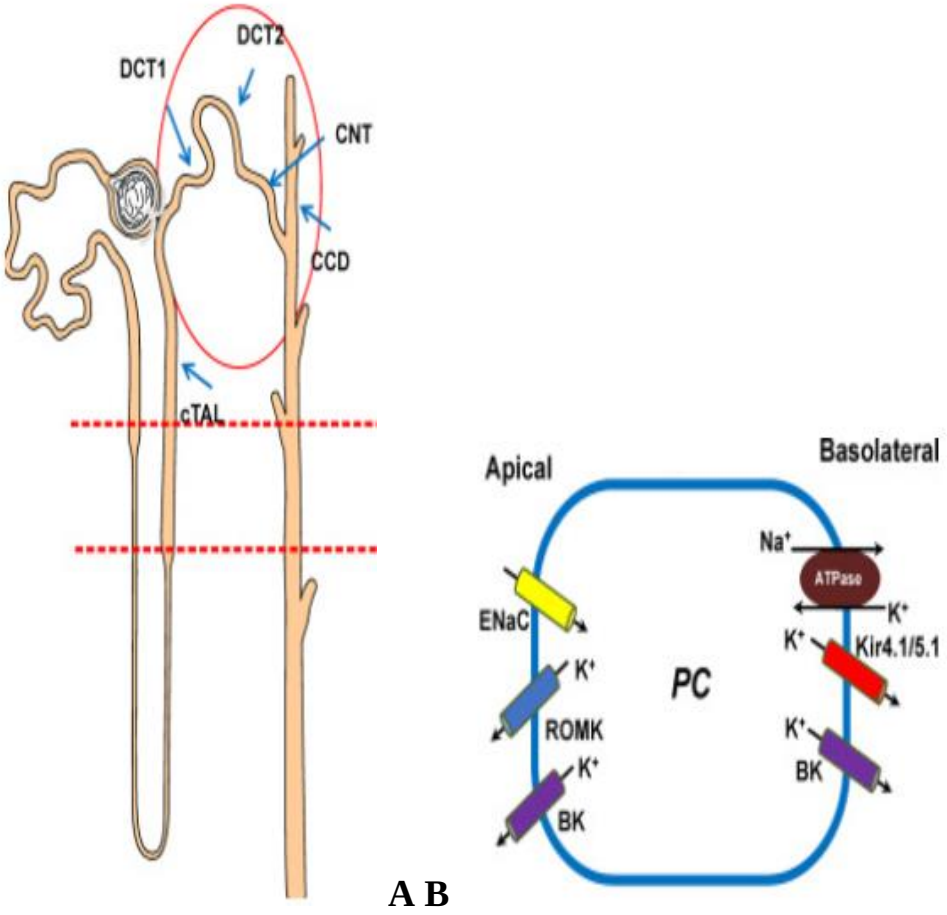

Fig 1.2. (A) Principal cells (PC) are located in distal convoluted tubule segments DCT1 and DCT2, connecting tubule (CNT), and cortical collecting duct (CCD). (B) The process of moving Na^+ and K^+ ions across the principal cell membrane. The extracellular fluid has a higher concentration of Na^+ and lower concentration of K^+ than the principal cells. The extra positive charges in the intracellular fluid are decreasing by moving two K^+ ions to the cell and moving out three Na^+ ions to the extracellular fluid.Figure taken from[16].

1.1.3. Potassium – aldosterone (K^+- ALDO) regulation feedback

Aldosterone (ALDO) is a mineralocorticoid (MR) hormone produced by the adrenal glands, which are located above the kidneys. ALDO's primary function is to affect the late distal tubule and collecting duct of nephrons in the kidney by stimulating Na^+ reabsorption and K^+ secretion. Moreover, ALDO enhances the activity of potassium-adenosine triphosphatase pumps (ATP), which are located on the basolateral membrane of principal cells in connecting segment (CNT) and collecting duct (CD) of the tubule to increase intracellular K^+ concentration (**Fig 1.3**). Thus, ALDO acts to increase K^+ excretion and lower plasma K^+ levels.

ALDO secretion by the adrenal gland is in turn directly controlled by plasma K^+, forming a closed feedback loop. Thus, elevated plasma K^+ directly enhances aldosterone secretion, leading to increased K^+ secretion in the CNT and CD. Accordingly, secreted K^+ into the lumen is excreted by the urine, and plasma K^+ concentration becomes regulate.

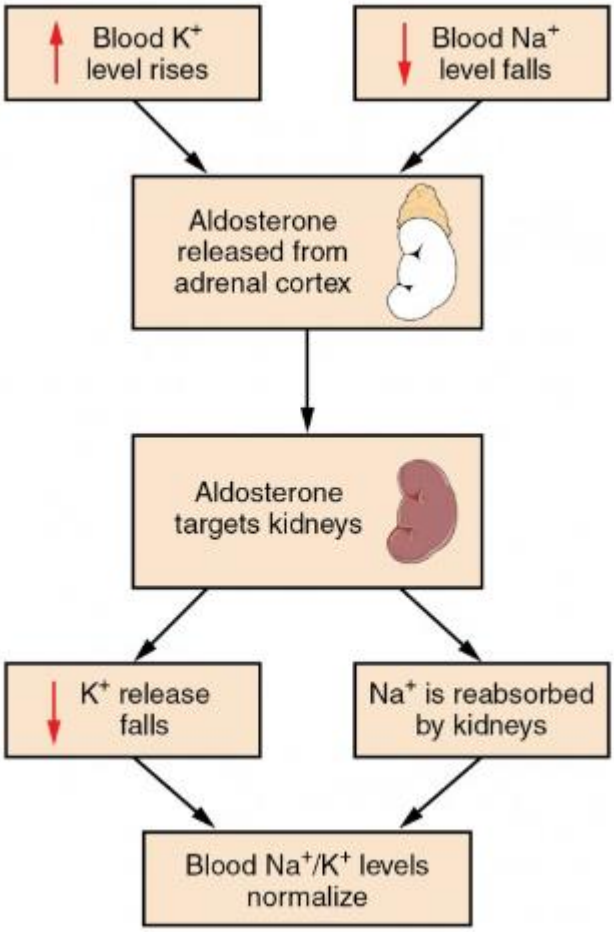

Fig 1.3. Aldosterone(ALDO), which is secreted by adrenal glands, facilitates secretion of K^+ reabsorption of Na^+ and water in order to make sure K^+ and Na^+ in the blood are remained at the normal value.Figure taken from[17].

1.1.4. Mechanism of sodium (Na^+) handling in the kidney

Control of plasma Na^+ concentration is one of the most significant functions of the kidney. A stable Na^+ concentration is vital in how muscles and nerves work, and thus the kidneys must remove the excess Na^+ and fluid from the body. When Na^+ intake increases, the normal kidney responds by quickly adjusting Na^+ reabsorption so that the excretion rate matches the intake rate.

However, the kidney does not eliminate the excess quickly enough, water is retained instead. This maintains plasma Na^+ concentration at a constant level, but may lead to a rise in blood pressure[18].

Renin-angiotensin-aldosterone system (RAAS) plays an important role in helping the kidney regulate Na^+ excretion and blood pressure, as illustrated in **Fig 1.4** The RAAS is activated by a drop in blood pressure. Renin, secreted by the kidney, combines with an angiotensin-converting enzyme (ACE) to produce angiotensin II (AngII). Receptors in the adrenal gland sense AII and respond by secreting aldosterone. In the kidney, AII and aldosterone both act to increase Na^+ reabsorption, and water follows Na^+ passively through osmosis, causing fluid volume and arterial pressure to return to normal [19].

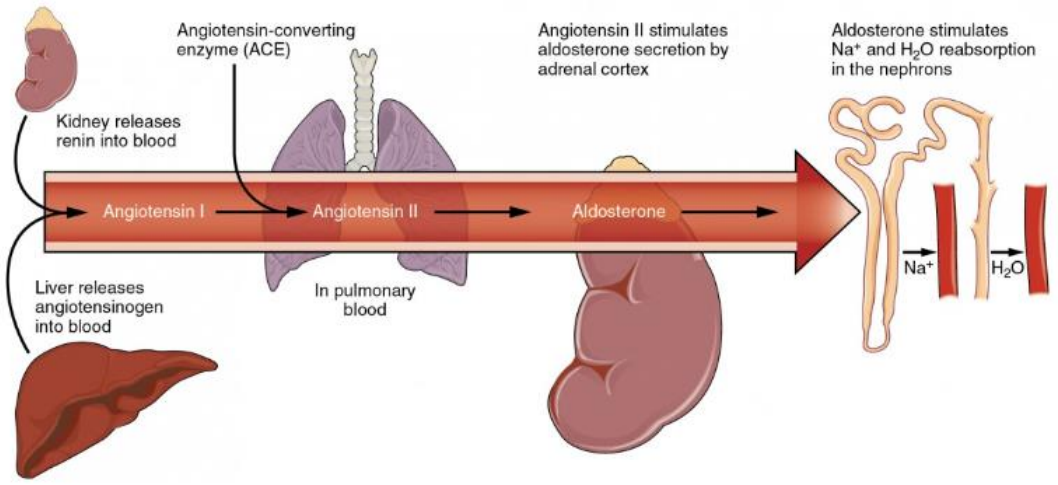

Fig 1.4. Secreted renin and angiotensin by the kidney and liver make angiotensin I. Angiotensin I combined with Angiotensin-converting enzyme make Angiotensin II which stimulates ALDO. ALDO secretion increases Na^+ and water reabsorption in the nephron which elevates blood pressure[17, 19].

1.1.5. Physiological effects that may perturb sodium and potassium homeostasis

Aldosterone (ALDO): ALDO affects renal Na^+ and K^+ transport through its interaction with the Na^+/K^+ ATPase, the ROMK channel, and ENaC.

ALDO elevates the activity of Na^+/K^+ ATPase in the distal tubules of the kidney (i.e. the distal convoluted tubule (DCT), connecting tubule (CNT), and cortical and medullary collecting duct (CCD and MCD)), leading to increased Na^+ reabsorption and K^+ secretion (**Fig 1.2**). This effect of ALDO on the Na^+/K^+ ATPase is mediated by the mineralocorticoid receptor (MR), which binds to ALDO and translocates to the nucleus, where it regulates gene expression[20]. ALDO may also increase the expression of ROMK channels (the apical channels through which K^+ is passively secreted), leading to increased K^+ secretion into the urine. This effect of ALDO on ROMK channels is also mediated by the MR[21]. ALDO also increases the expression of ENaC (the apical channels through which Na^+ passively moves into the cell) and its activity, leading to increased Na^+ reabsorption. This effect of ALDO on ENaC is also mediated by the MR[22]. Therefore, all these effects of ALDO on renal transporters play a crucial role in the body's fluid regulation balance and blood pressure.

Consequently, K^+ and Na^+ regulations mainly depend on K^+ secretion of ALDO-sensitive segments of the nephron. [9, 23].

Cell lysis: Major cell death cause serious perturbations in plasma K^+ regulation by releasing intracellular K^+ into the blood and interstitial fluid.

Acid-base irregularities: Na^+/K^+ ATPase pump activity is inhibited when hydrogen concentration increases. Therefore, the low pH can disrupt K^+ homeostasis [9].

Renin-Angiotensin-Aldosterone System (RAAS): The RAAS is an important regulator of sodium homeostasis. Changes in Na^+ intake cause a change in Na^+ delivered to the macula densa

the part of the nephron just downstream of the Loop of Henle. The cells of the macula densa sense Na^+ delivery – when it decreases, they response by secreting more renin, and when it increases, they secrete less renin. Renin is the enzyme responsible for the formation of Angiotensin II, which has powerful sodium-retaining effects by constricting the renal blood vessels and increasing proximal tubule sodium reabsorption. Moreover, AngII also stimulates ALDO secretion [9, 23, 24].

Blood volume: Elevation in blood pressure due to decrease in blood volume can alter the amount of Na^+ excretion from the renal tubule.

Diets: dietary interventions are crucial in managing electrolyte imbalances in individuals with impaired kidney function. A low Na^+ diet can manage hypertension, while a low K^+ diet can avoid hyperkalemia. It is vital to follow the dietary recommendations of a healthcare professional to maintain proper Na^+ and K^+ homeostasis in patients with kidney impairment[25].

Kidney impairment: impaired kidney function causes an imbalance in Na^+ and K^+ levels, leading to potentially detrimental health consequences (e.g. hyperkalemia, and hypernatremia.). Consequently, proper management of these electrolyte imbalances is essential in patients with kidney dysfunction[26].

1.1.6. Pathologies of altered sodium and potassium regulation

Hypernatremia occurs when plasma Na^+ concentration exceeds the normal range (>145 mEq/L) [23, 27]. This can cause various conditions from mild to severe, followed by chronic renal dysfunction, hypocalcemia, anemia, and hypokalemia. **Table 1.1** presents pathologies associated with hypernatremia.

Table 1.1. Causes and consequences of hypernatremia [9, 23, 28]

Hypernatremia causes	Pathologic consequences of hypernatremia
1. Diarrhea 2. Heart failure 3. Water loss 4. High Na^+ intake	1. Muscle weakness 2. Seizures (severe cases) 3. Coma 4. Excessive sweating

Hyponatremia occurs when plasma Na^+ concentration is lower than the normal value (< 136 mEq/L). Hyponatremia is the most common ion dysfunction. **Table 1.2** presents the disease states linked to hyponatremia.

Table 1.2. Causes and consequences of hyponatremia [9, 23]

Hyponatremia causes	Pathologies followed by hyponatremia
1. Excessive water intake 2. Kidney disease 3. Liver dysfunction 4. Heart failure	1. Respiratory arrest 2. Seizures 3. Edema 4. Rhabdomyolysis

Hyperkalemia occurs when the blood concentration of K^+ exceeds 5 mEq/L, this can lead to serious consequences such as ventricular tachycardia, fibrillation, and in severe cases, cardiac arrest[23, 29]. **Table 1.3** presents the hyperkalemia causes and diseases associated with hyperkalemia.

Table 1.3. Causes and consequences of hyperkalemia[9, 23].

Hyperkalemia causes	Pathologies followed by hyperkalemia
1. Addison's disease 2. Angiotensin-converting enzyme (ACE) inhibitors 3. Dehydration 4. Type 1 diabetes	1. Abnormal heart rhythms 2. Cardiac arrest 3. Muscle weakness 4. Paralysis

Hyperkalemia is less common than hypokalemia. However, it still affects about 8% of patients in US hospitals [29]. Redistribution hyperkalemia may be caused by K^+ shifting from the intracellular space into the extracellular space, thus raising serum K^+. It may also be due to ALDO deficiency such as Addison's disease or tubular unresponsiveness to ALDO (e.g., chronic renal diseases).

Hypokalemia is defined as low plasma K^+ concentration. This is happening when K^+ concentration drops below the 3.5 mEq/L. This condition can be caused by gastrointestinal and renal disorders. Hypokalemia can lead to neuromuscular dysfunctions, affecting the muscles and nerves. Moreover, atrioventricular block and cardiac arrest can result from hypokalemia[30]. The **table 1.4** represents the disease states linked to hypokalemia.

Table 1.4. Causes and consequences of hypokalemia[9].

Hypokalemia Causes	Pathologies resulting from hypokalemia
1.Renal failure 2. K^+ moving into cells 3. Vomiting	1. Cardiac dysfunction 2. Muscle weakness 3. Gastrointestinal dysfunction

Hyperaldosteronism: Hyperaldosteronism is an endocrine irregularity that occurs when adrenal glands secrete too much ALDO (>0.59 nmol/L). High ALDO levels usually lead to high

blood pressure and hypokalemia. This disability can be caused by a tumor in the adrenal gland or may be an outcome of other diseases. If a tumor exists, it can be surgically removed. Alternatively, mineralocorticoid receptor antagonists (MRA), which belong to the diuretic drug class, can antagonize the action of aldosterone at MR receptors. Spironolactone, as the first member of this drug class, is commonly used for hyperaldosteronism management. Furthermore, it can be combined with other drugs to treating chronic heart failure and hypertension. Eplerenone, a more recent MRA, is more selective over spironolactone and causes fewer side effects (gynecomastia, breast pain, and impotence)[31].

1.1.7. Therapies that alter sodium and potassium homeostasis

Angiotensin-converting enzyme (ACE) inhibitors: ACEi are a class of drugs that inhibit ACE, which is involved in the RAAS. By inhibiting this enzyme, ACEi decreases ALDO level and increase the Na^+ and water excretion. ACEi can also lead to an increase in K^+ concentration, especially in cases with renal insufficiency[32].

Loop diuretics: Medications such as such as furosemide and bumetanide inhibit Na^+ reabsorption in the ascending loop of Henle of the kidney. Loop diuretics lead to a remarkable elevation in urinary K^+, leading to a decrease in plasma K^+ concentration. Loop diuretics can also cause a decrease in serum Na^+ levels, especially in cases with impaired kidney function or on concomitant medications that can raise urinary Na^+ excretion[33].

Thiazide diuretics: Medications such as hydrochlorothiazide are a class of medications that inhibit Na^+ reabsorption in the distal convoluted tubule of the kidney. Thiazide diuretics can cause a mild increase in K^+ level, especially in patients with impaired kidneys or on concomitant potassium-sparing diuretics[34].

Mineralocorticoid antagonists (MRA): Medications such as spironolactone and eplerenone block the effect of ALDO on the MR receptor in the distal tubules of the kidney. By blocking ALDO effects, MRAs can decrease Na^+ reabsorption and increase K^+ retention[35].

Sodium-glucose cotransporter inhibitor (SGLT2i): Medications that inhibit the Na^+ and glucose cotransporter-2 in the proximal tubules of the kidney decrease glucose reabsorption and increase urinary glucose, leading to osmotic diuresis and natriuresis. The effects of SGLT2i on plasma potassium are not well understood, but they may cause a fall in serum K^+ levels, especially in patients with impaired renal function[36].

SGLT2 is a protein that plays a vital role in glucose reabsorption in the cardiorenal system. SGLT2 transporters are located in the kidney, specifically in the proximal convoluted tubules(PCT)[37]. Glucose is filtered out of the blood by the glomerulus in the kidney and enters the renal tubules (**Fig1.5**). However, in the PCT, SGLT2 transporters reabsorb nearly all of the filtered glucose back into the bloodstream [38]. SGLT2 transporters work by simultaneously transporting both Na^+ ions and glucose molecules from the tubular fluid into the PT cells, and then from the tubular cells into the bloodstream.

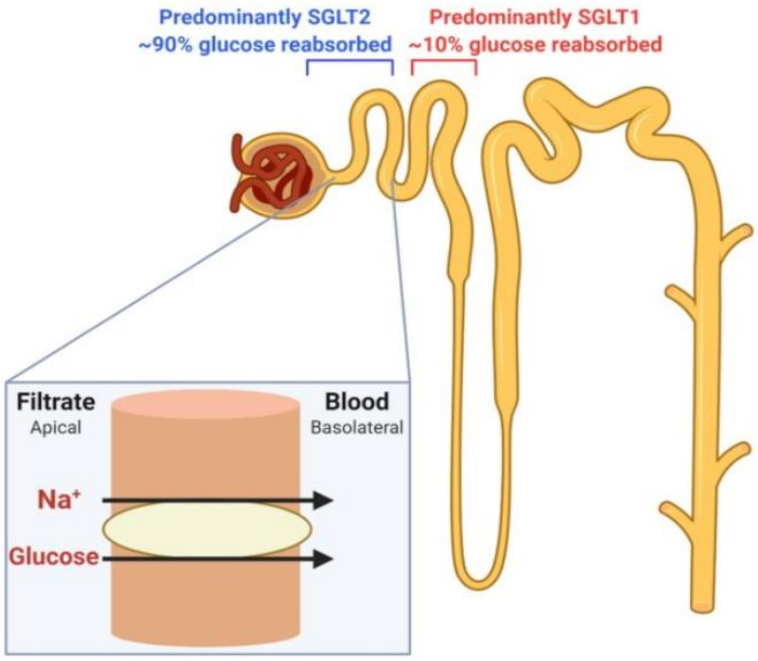

Fig1.5.The SGLT1 and SGLT2 transporters are located in the proximal segment of the nephron, and the mechanism by which SGLT2 inhibitors promote renal glucose excretion[39].

SGLT2 inhibitors (SGLT2i) are a class of medications that block the action of SGLT2, which leads to elevated urinary glucose excretion and decreased blood glucose concentration. SGLT2i is commonly used in the treatment of type 2 diabetes, but they increasingly used to treat heart failure (HF) and chronic kidney diseases (CKD). Some examples of SGLT2i include canagliflozin and dapagliflozin[40, 41].

The effect of SGLT2i on K^+ levels is not well studied. The limited clinical data available suggests that it may reduce the risk of hyperkalemia, but the underlying mechanism for this is not understood, and no study to date has directly investigated this question. SGLT2i increase the amount of glucose and Na^+ excreted in the urine, which can lead to an osmotic diuresis and could result in a slight increase in urinary K^+ excretion. However, in most cases, this effect is not

clinically significant and does not lead to significant alterations in serum K^+ concentration(e.g. Canagliflozin)[42]

Interestingly, an investigation of co-administration of an MRA antagonist esaxerenone and a sodium-glucose cotransporters 2 (SGLT2) inhibitor in Japanese patients with diabetic kidney diseases[43] found that SGLT2i administration reduced the rise in plasma K^+ that occurred with esaxerenone alone, supporting a potential protective effect against hyperkalemia. A meta analysis [43, 44] found that SGLT2i reduced the risk of serious hyperkalemia and did not increase the risk of hypokalemia.

1.2. Current state of experimental studies and mathematical modeling for potassium homeostasis

1.2.1. Experimental studies

Micropuncture experiments have attempted to quantify K^+ homeostasis physiology by measure K^+ flux rates along the nephron. The renal micropuncture technique allows direct access to the superficial nephrons in *in vivo* rodent models. Tubular filtrate flow rates, concentrations, and pressures can be measured. Accordingly, a renal micropuncture study on a single nephron leads to a better understanding of glomerular filtration, tubular transport, reabsorption, and secretion[45].

Experimental perturbations have also been used to understand K^+ handling and its regulation by ALDO in adrenalectomized rats. Since ALDO concentration level is constantly changing, adrenal glands were removed in rats to control ALDO changes, and a constant rate of ALDO was infused subcutaneously in some adrenalectomized rats. This allowed the determination of the relationship between ALDO and K^+ reabsorption and secretion through the nephron[46]. These experimental studies have increased our understanding of K^+ handling, but there are still many challenges in understanding and predicting system behavior in various situations in human

disease and therapy.

1.2.2. Mathematical modeling of K^+-ALDO

The complexities of K^+- ALDO regulation, renal physiology, pathophysiology, and pharmacology often make it challenging to understand and predict the integrated behavior of this system. Mathematical models that integrate the current state of knowledge of K^+ regulation physiology with experimental data may assist in better understanding this complexity.

Previous mathematical models have described processes of K^+ transport in segments of the nephron (**Fig1.6**) [47-50]. These studies identified mathematical relationships for transporters on the luminal and basolateral side of the cell membrane and simulated fluxes across the cell membrane. While these models provided important insights on local processes of K^+ transport, they do not allow the impact of local alterations in K^+ handling on systemic K^+ concentrations to be evaluated, and have not considered the dynamics of ALDO regulation of K^+ excretion[51].

Other models have simulated systemic K^+ regulation by ALDO, but have not mechanistically modeled the role of renal processes (reabsorption, secretion, and its reabsorption by ALDO)[52, 53].

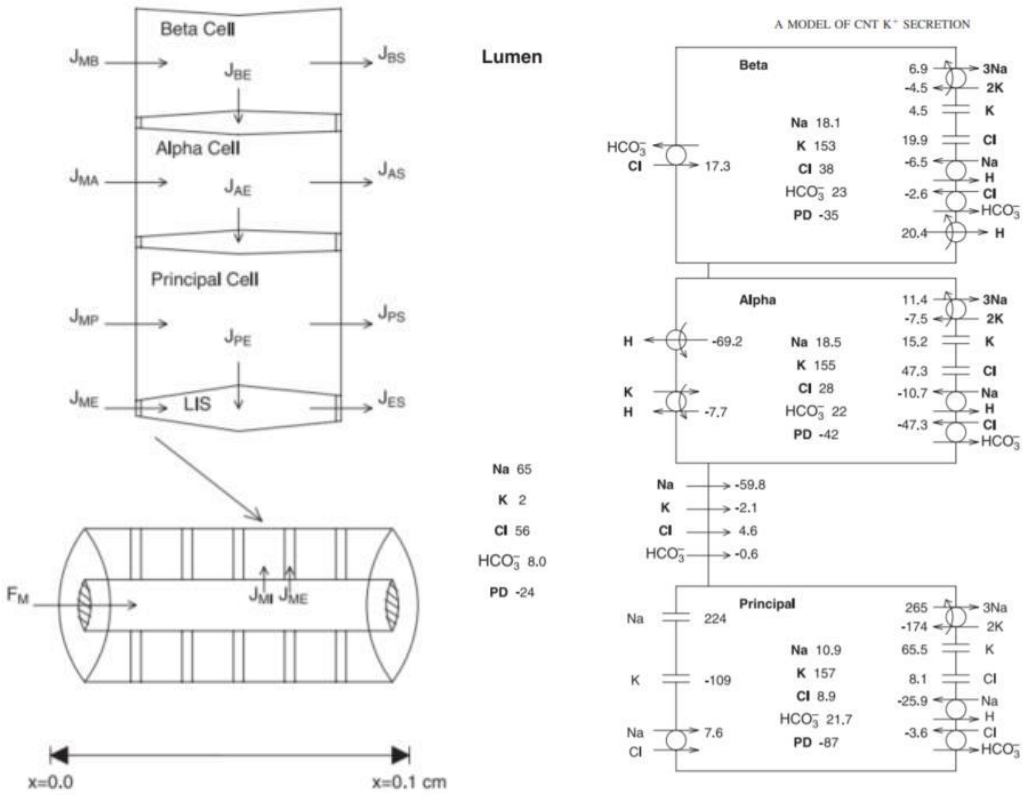

Fig 1.6. Connecting segment epithelium model, principal and intercalated cells and lateral intercellular space (LIS). This epithelium lines the model tubule lumen. Intraepithelial fluxes are designated Jαβ(i). MP, MA, MB referred to fluxes from the lumen to intracellular space, and lateral cell membranes (PE, AE, BE), basal cell membranes (PS, AS, BS), tight junction (ME), or interspace basement membrane (ES). Along the tubule lumen, axial flows are designated FM(i), and JMI represents a generic flux from lumen to cell(A). Connecting tubule (CNT) transport pathways along with the model concentrations(mmol) and fluxes (pmol.mm^{-1} .min^{-1}) calculated by the model, using the baseline solution for electrolytes and PD, potential difference(B) [50].

1.2.3. Mathematical modeling of Na^+/Water homeostasis

Previously, a detailed mathematical representation of the physiological mechanisms involved in blood pressure regulation through the relationship between the renal (kidneys) and cardiovascular system was developed (**Fig 1.6** top right) [54, 55]. The model mainly focuses on the role of renal function in Na^+ and water homeostasis. The kidneys are responsible for the regulation of Na^+ and water in the body, which leads to control of blood pressure. The model simulates the processes of glomerular filtration and tubular reabsorption, which determine Na^+ and water balance (**Fig 1.7** bottom left)[55].

The renin angiotensin aldosterone system (RAAS) is also considered in the model and plays a vital role in regulating blood pressure and Na^+ balance. When blood pressure decreases, the enzyme renin is released from the kidneys, stimulating production of angiotensin II (ANGII), which has direct effects on sodium retention by the kidney, and also stimulates the release of ALDO, which has additional effects on Na^+ reabsorption in the kidneys, causing increases blood volume and and eventually restoring blood pressure (**Fig 1.7** bottom right).

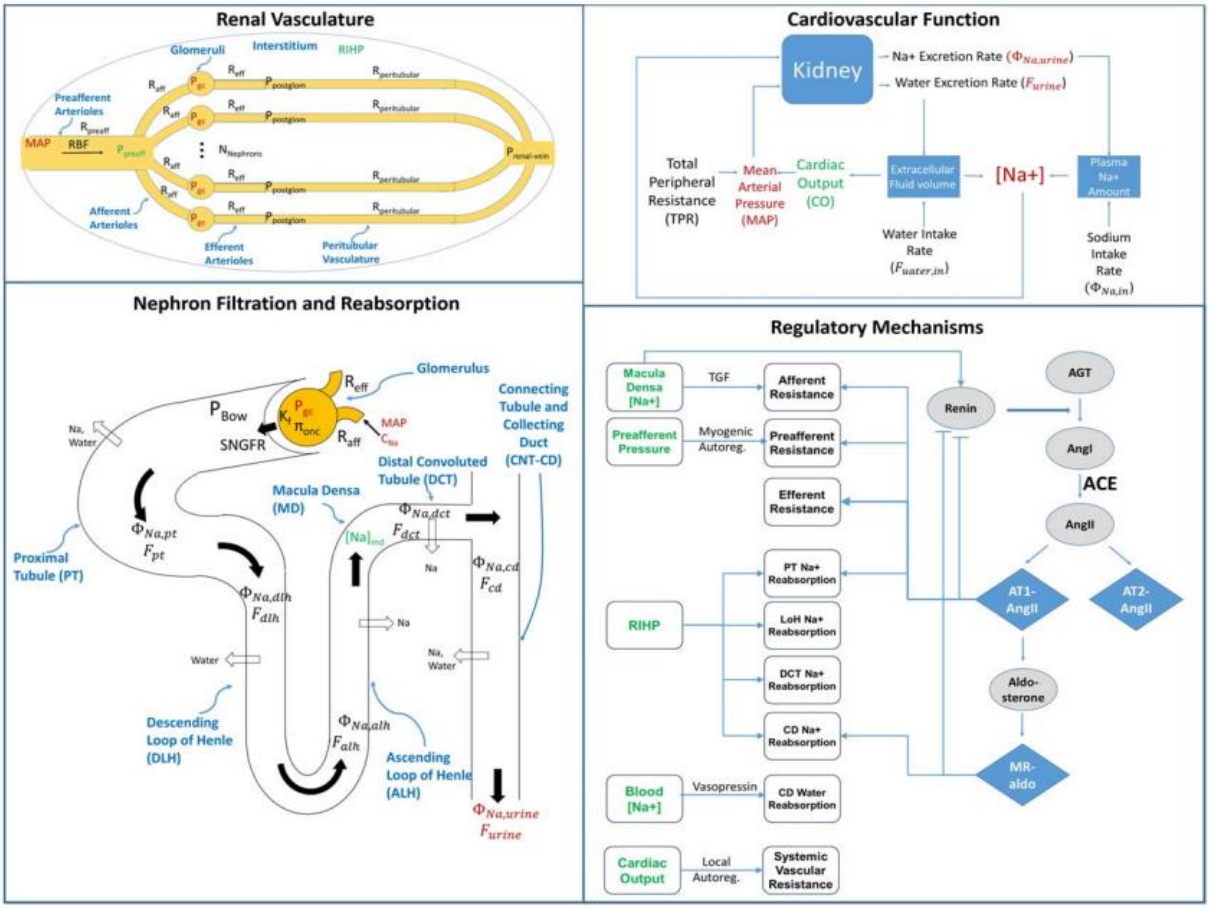

Fig 1.7. The model is represented schematically. Top left: One preafferent resistance vessel feeds N parallel nephrons. Bottom left: The glomerulus is modeled according to Starling's law for Na^+ and water filtration. The PT, LoH, DCT, and CNT/CD resorb Na^+ and water at different fractional rates, and Na^+ and water excretion rates are calculated from unabsorbed Na^+ and water. Top right: The balance between Na^+ excretion and water intake determines extracellular fluid volume, plasma Na^+ concentration, and eventually cardiac output and mean arterial pressure (MAP). The renal model (left) is closed by a feedback of Na^+ and MAP. Bottom right: this diagram shows RAAS, TGF, myogenic autoregulation, RIHP regulation of tubular Na^+ reabsorption, vasopressin regulation of tubular water reabsorption, and local blood flow autoregulation[54].

1.3. Knowledge gaps

Previous studies described above significantly help in understanding the relationship between plasma K^+ and ALDO regulation. However, there are still many challenges that current data and tools cannot address. Regulation of K^+ is a dynamic feedback control system, and plasma ALDO levels dynamically change response to changes in factors like Na^+ intake, K^+ intake, fluid levels, function, and therapies that impact any of these factors. Current methods cannot describe these dynamic changes or predict the effect of therapies on them. Cell-based mathematical models described above gave a clear idea about the K^+ diffusion through the membrane in connecting the segment and collecting duct of the nephron. However, while they describe a detailed description of electrolyte transportation across the cell membrane and intercellular paths, they can only describe K^+ handling at the cellular level. The effect of local cell/tubule-level changes on whole body homeostasis and systemic plasma electrolyte concentrations is not considered. Therefore, by modeling the K^+- ALDO regulation, we aim to understand quantitatively:

- How processes of kidney filtration, reabsorption, and secretion of the K^+ alter systemic plasma K^+ concentration.
- The dynamic feedback between ALDO and plasma Na^+ and K^+.
- Effect of renal impairment and therapies that alter ALDO, K^+ handling, or Na^+ handling on plasma K^+ regulation.

In the next chapters, we describe the development, calibration, and validation of a model of renal and systemic K^+- ALDO regulation. The model mechanistically describes renal K^+ handling, systemic K^+ balance, and feedback control of potassium by ALDO, facilitating quantitative investigation of K^+ regulation. We apply this model to investigate the effect of mineralocorticoid receptor (MR) antagonists, changes in renal function, and changes in K^+ intake

on control of plasma K^+ concentration. Lastly, we integrated our validated K^+- ALDO homeostasis model to the previously validated cardiorenal Na^+/Water homeostasis model[54], and apply it to investigate the mechanistic behavior of poorly understood sodium-glucose co-transporters inhibitor (SGLT2i). This ultimately demonstrates the model's ability in investigation of the impacts of novel therapies or combinations of therapies on K^+ levels in healthy subjects and patients.

1.4. Specific Objectives and Organization of Chapters

Chapter 2:

Specific aim 1

Develop a mathematical model which explains potassium handling in the kidney and potassium-aldosterone regulation feedback.

a) Mechanistically describe processes of renal K^+ filtration, reabsorption, and secretion.
b) Mathematically model the regulation of ALDO by K^+ and Na^+, and subsequently ALDO regulation of renal K^+ secretion.
c) Calibration of K^+-ALDO feedback to fit acute response data following perturbation of K^+ intake and K^+ infusion.

Chapter 3:

Specific aim 2

Calibrate the pharmacodynamic mechanism of action of mineralocorticoid antagonists in the model, and validate the therapeutic response by simulating clinical studies.

a) Illustrate the pharmacologic effect by calibrating the pharmacokinetic

and pharmacodynamic mechanism of action of MR antagonists.

b) Simulating clinical studies to validate the therapeutic response.

c) Investigation of the model ability to describe the factors that alter K^+ concentration.

Chapter 4:

Specific aim 3

Integrate developed potassium homeostasis model into the existing sodium and water homeostasis model and investigate the effect of drug administration (MRAs and SGLT2is) alone and in combination on plasma potassium concentration.

a) Recalibrate the integrated model with experimental data.

b) Validate the integrated model by testing its ability ot predict plasma K^+ and blood pressure response to the MRA therapy.

c) Apply the model to investigate mechanisms underlying the effects of SGLT2i and MRAs, alone and in combination, on potassium homeostasis, in states of health and disease (diabetes and chronic kidney disease).

Chapter 5:

Conclusion and future direction

CHAPTER 2

SPECIFIC AIM 1

DEVELOP A MATHEMATICAL MODEL WHICH EXPLAINS POTASSIUM HANDLING IN THE KIDNEY AND POTASSIUM-ALDOSTERONE REGULATION FEEDBACK

This chapter contains text from the following publication:

Maddah, Erfan, and K. Melissa Hallow. "A quantitative systems pharmacology model of plasma potassium regulation by the kidney and aldosterone." *Journal of Pharmacokinetics and Pharmacodynamics* 49.4 (2022): 471-486.

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2. Chapter 2: Specific aim 1, Develop a mathematical model which explains potassium handling in the kidney and potassium-aldosterone regulation feedback

2.1. Introduction

The objective of this chapter is to develop a mechanistic model that describes renal potassium (K^+) filtration, reabsorption, secretion along the tubule and excretion. The complexities of potassium/aldosterone regulation, renal physiology, pathophysiology, and pharmacology often make it challenging to understand and predict the integrated behavior of this system. Mathematical models that integrate the current state of knowledge of K^+ regulation physiology with experimental data may assist in better understanding this complexity. Previous mathematical models have described processes of K^+ transport in segments of the nephron [47-49, 56], and have provided important insights on local processes of K^+ transport, but they do not allow the impact of local alterations in K^+ handling on systemic K^+ concentrations to be evaluated, and have not considered the dynamics of aldosterone regulation of K^+ excretion. Other models have simulated systemic K^+ regulation by aldosterone, but have not mechanistically modeled the role of renal processes (reabsorption, secretion, and its reabsorption by aldosterone) [52, 53, 57]. Thus, there is currently a gap in our ability to mathematically understand the dynamic interactions between plasma K^+ concentration, K^+ handling in the kidney, and regulation by aldosterone, as well as to predict the effect of alterations through therapies, changes in K^+ intake, or changes in renal function on K^+ levels.

Here we describe the development, calibration, and validation of an ordinary differential equations (ODE) model of renal and systemic potassium/aldosterone regulation. The model mechanistically describes renal K^+ handling, systemic K^+ balance, and feedback control of K^+ by aldosterone, facilitating quantitative investigation of K^+ regulation.

2.2. Methods

In brief, as illustrated in **Fig 2.1**, the model describes renal potassium (K^+) filtration, reabsorption, and/or secretion of K^+ in each tubule segment, and K^+ excretion in the urine. Extracellular K^+ concentration is determined by the balance between K^+ intake and excretion. Aldosterone (ALDO) secretion is controlled by plasma K^+ concentration, and plasma ALDO concentration in turn alters K^+ secretion in the distal nephron. The effects of mineralocorticoid receptor (MR) antagonists on distal K^+ secretion and reabsorption are also modeled. The model development and calibration are described in detail below.

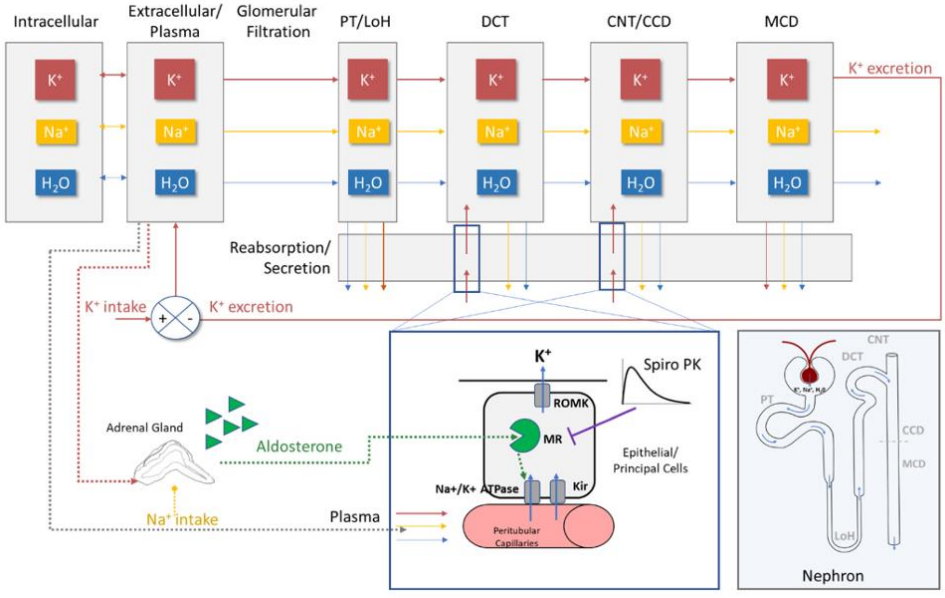

Fig 2.1. Schematic of K^+ regulation model. The kidney is modeled as a set of N nephrons. K^+, along with sodium and water, is filtered across each glomerulus. K^+ is reabsorbed

proportional to Na^+ and water in the proximal tubule and Loop of Henle (LoH). In the distal convoluted tubule (DCT), connecting tubule (CNT), and cortical collecting duct (CCD), K^+ is secreted, and the rate is determined by the net flux through basolateral Na^+/K^+ ATPase and Kir channels and luminal ROMK channels. K^+ is reabsorbed in the medullary collecting duct (MCD) and excreted in the urine. The balance between K^+ intake and excretion determines extracellular K^+ concentration. K^+ is also exchanged between the extracellular and intracellular space. ALDO secretion by the adrenal gland is controlled by plasma K^+ and Na^+ intake, and in turn, controls activity of Na^+/K^+ ATPase in the DCT, CNT, and CCD through the action of mineralocorticoid receptors (MR). Mineralocorticoid receptor antagonists (MRAs) inhibit MR and thus reduce K^+ secretion[51, 58].

2.2.1. Renal filtration and proximal reabsorption

The kidney is modeled as a set of N nephrons. Each nephron includes a glomerulus and a tubule consisting of the PT/LoH, DCT, CNT/CCD, and MCD (**Fig 2.1**).

K^+ and Na^+ are freely filtered across the glomerular membrane so that concentration in the tubular filtrate is the same as the concentration in the plasma. The single nephron K^+ and Na^+ filtration rates are given by:

$$\varphi_{k,f} = \left(\frac{GFR}{N_{nephrons}}\right) * [K^+]_P \quad (1)$$

$$\varphi_{Na,f} = \left(\frac{GFR}{N_{nephrons}}\right) * [Na^+]_P \quad (2)$$

In the PT and LoH, a large fraction of filtered K^+ is reabsorbed passively and proportional to Na^+ and water reabsorption [9].

The bulk of K^+ and Na^+ reabsorption occurs in the PT and LoH. Na^+ reabsorption in these segments is highly regulated through neurohormonal mechanisms to maintain a

small and stable flow of Na^+ into the distal nephron[59]. While in chapter 4, this K^+ model may be coupled with a detailed model of renal Na^+ regulation, for the current model we are interested in Na^+ only as a factor in determining rates of K^+ reabsorption and excretion. We assume that 1) Na^+ intake and excretion are in balance, 2) the distal nephron reabsorbs 95% of delivered Na^+, and 3) the PT/LoH adjusts its reabsorption rate so that when 95% of distal Na^+ is reabsorbed, Na^+ balance is maintained (Na^+ excretion equals intake).

Thus, Na^+ flow from the LoH into the DCT is given by:

$$\varphi_{Na,DCT,in} = \frac{\varphi_{Na,intake}}{(1-0.95)N_{nephrons}} \quad (3)$$

And fractional Na^+ reabsorption in the PT/LoH is:

$$\gamma_{Na+,PT-LoH} = \frac{(\varphi_{Na,f} - \varphi_{Na,DCT,in})}{\varphi_{Na,f}} \quad (4)$$

K^+ and water are reabsorbed in the PT proportionally to Na^+. Thus, K^+ and water delivery to the DCT are then given by:

$$\varphi_{K,DCT,in} = \varphi_{k,f}(1 - \gamma_{PT-LoH}) \quad (5)$$

$$\varphi_{water,DCT,in} = \frac{GFR}{N_{nephrons}}(1 - \gamma_{PT-LoH}) \quad (6)$$

Thus, an increase in Na^+ intake will decrease fractional reabsorption in the PT/LoH, and so will increase the rate of K^+ and water delivery to the DCT.

2.2.2. Potassium secretion in DCT, CNT, and CCD

Downstream of the LoH, net K^+ secretion occurs in the DCT, CNT, and CCD[60]. On the luminal cell membrane, K^+ is secreted from the tubule cells into the lumen through ROMK channels across an electrochemical gradient. For each segment $_i$, this flux can be described by the Goldman equation[50]:

$$J_{L,i} = h_L * \vartheta_L * \left[\frac{[K^+]_{cell,i}e^{-\vartheta_L} - [K^+]_{lumen,i}}{1-e^{-\vartheta_L}}\right] \qquad (7)$$

Here, h_L is the luminal cell membrane permeability to K^+ through ROMK channels, $[K]_{cell}$ and $[K]_{lumen}$ are the intracellular and luminal K^+ concentrations, respectively, and ϑ_L is the normalized membrane potential, given by:

$$\vartheta_L = \frac{FV_L}{RT} \qquad (8)$$

F is the Faraday constant, R is the universal gas constant, T is body temperature, V_L is the electrical potential across the luminal membrane.

On the basolateral side, K^+ flux from the plasma in the peritubular capillaries into the cell, J_p, occurs through both passive diffusion across the electrochemical gradient through Kir channels, and active transport by exchange through Na^+/K^+ ATPase pumps.

$$J_{B,i} = J_{B,i}^{passive} + J_{B,i}^{active} \qquad (9)$$

Passive flux through Kir is again given by Goldman's equation:

$$J_{B,i}^{passive} = h_B * \vartheta_B * \left[\frac{[K^+]_{P,i} - [K^+]_{cell,i}e^{-\vartheta_B}}{1-e^{-\vartheta_B}}\right] \qquad (10)$$

Here h_B is basolateral cell membrane permeability to K^+ through Kir channels, $[K]_{cell}$ and $[K]_P$ are the intracellular and plasma K^+ concentrations, respectively, and ϑ_B is the normalized membrane potential, given by:

$$\vartheta_B = \frac{FV_B}{RT} \quad (11)$$

Because the intracellular concentration is higher than the plasma concentration, this flux is negative, meaning that K^+ diffuses passively out of the cell into the peritubular capillaries. The active flux of K^+ through Na^+/K^+ ATPase has been described previously[49], and is given by:

$$J_{B,i}^{active} = -\frac{2}{3} J_{Na+,max}^{active} \left[\frac{[Na^+]_{cell}}{[Na^+]_{cell}+K_{Na}}\right]^3 \left[\frac{[K^+]_P}{[K^+]_P+K_K}\right]^2 \quad (12)$$

$J^{active}_{Na+,max}$ is the maximum Na^+ flux and K_{Na} and K_K are the concentrations of Na^+ and K^+, respectively that produce half the maximum flux. Thus, active transport of K^+ into the cell depends on plasma K^+ concentration and intracellular Na^+ concentration. In addition, the K_{Na} increases linearly with plasma K^+ concentration and K_K increases linearly with plasma Na^+ concentration [49], as determined by [13].

$$K_{Na} = 0.2\left[1 + \frac{[K^+]_{cell}}{8.33}\right] \quad (13)$$

$$K_K = 0.1\left[1 + \frac{[Na^+]_P}{18.5}\right] \quad (14)$$

For each tubule segment, intracellular K^+ concentration is determined by the net flux of K^+ into the cell from the basolateral side and out of the cell on the luminal side:

$$\frac{d[K^+]_{cell.i}}{dt} = \frac{(J_{B,i} - J_{L.i})}{SV_{ratio,i}} \quad (15)$$

Here, SV_{ratio} is the surface to volume ratio of tubule cells in each segment. For each tubule

segment, the lumen is approximated as a well-mixed compartment, and axial gradients in concentration within the segment are not considered. Luminal K^+ concentration is determined from the K^+ flow in from the previous segment, K^+ secretion rate, and K^+ flow out:

$$\frac{d[K^+]_{lumen.i}}{dt} = \varphi_{K,i-1,out} + J_{L,i}A_{L,i} - [K^+]_{lumen,i}\varphi_{water,i-1,out}(1-\gamma_{water,i}) \quad (16)$$

A_L is the luminal surface area available for secretion:

$$A_{L,i} = \pi r_i^2 L_i Fr_{c,i} \quad (17)$$

Here, $Fr_{c,i}$ is the fraction of the luminal surface covered by cells involved in secretion.

2.2.3. Potassium reabsorption/secretion in the MCD

K^+ is reabsorbed actively in the MCD through the action of H^+/K^+-ATPase. In addition, K^+ may be secreted in the MCD in exchange with Na^+ through Na^+/K^+ ATPase. Under normal K^+ and Na^+ intake conditions, there is a small net MCD K^+ reabsorption. Under conditions of dietary Na^+ depletion or hypokalemia, H^+/K^+ ATPase activity is greatly increased[61-64]. Thus, MCD K^+ reabsorption is modeled as a constant rate that increases exponentially as plasma K^+ concentration falls below normal.

$$\varphi_{K,reabs,MCD} = \varphi_{K,reabs.MCD0} + e^{\frac{m_{K,P_{MCD}}([K^+]_{P,0}-[K^+]_p)}{[K^+]_{p,0}}} \quad (18)$$

Here, $\varphi_{K,reabs,MCD0}$ is the rate of K^+ reabsorption calculated under steady-state conditions with normal Na^+ and K^+ intake, $[K^+]_{P,0}$ is the normal setpoint for plasma K^+, and $m_{K,P\text{-}MCD}$ is a fitting parameter, estimated by fitting experimental data as described later. K^+ secretion in the MCD is assumed to change proportional to Na^+ reabsorption,

but only a portion of Na^+ reabsorption occurs through Na^+/Ka^+ ATPase. Thus, MCD K^+ secretion is described as a linear function of Na^+ reabsorption.

$$\varphi_{K,sec,MCD} = m_{Na-K,MCD}(\varphi_{Na,reabs,MCD} - \varphi_{Na,reabs,MCD0}) \quad (19)$$

Here, $\varphi_{Na,reabs,MCD0}$ is the rate of Ka^+ reabsorption calculated under steady-state conditions with normal Na^+ and Ka^+ intake, and $m_{Na\text{-}K,MCD}$ is a fitting constant, estimated by fitting experimental data as described later. The rate of Na^+ reabsorption in the MCD is tightly regulated by hormonal and humoral mechanisms in order to maintain Na^+ balance, since the MCD, as the final tubule segment, finely controls Na^+ excretion so that it is matched to intake over the long term[65].

We assume the Na^+ reabsorption rate in the MCD is regulated so that the amount of Na^+ leaving the MCD is equal to the Na^+ intake rate, and the rate of Na^+ reabsorption is given by:

$$\varphi_{Na,reabs,MCD} = \varphi_{Na,out,CCD} - \frac{\varphi_{Na,intake}}{N_{nephrons}} \quad (20)$$

Since no further reabsorption occurs after the MCD, the total urinary Ka^+ excretion rate across all nephrons is the sum of the Ka^+ rate that leaves the MCD in all nephrons of the kidney, which is given by:

$$\varphi_{k,out} = N_{nephrons} * (\varphi_{K,out,CCD} - \varphi_{K,reabs,MCD} + \varphi_{K,sec,MCD}) \quad (21)$$

2.2.4. Systemic potassium balance

Whole-body K^+ is modeled as a two-compartment model consisting of an extracellular and intracellular compartment. Intracellular Ka^+ concentration is much

higher than extracellular concentration, and movement between the intracellular and extracellular compartment serves as a buffer against rapid changes in extracellular concentration. Ka^+ is assumed to move from the ECF into the intracellular compartment when plasma concentration is elevated, and to move from the intracellular space into the ECF when intracellular concentration is elevated, so that the flux between ECF and the intracellular compartment is given by:

$$\varphi_{ecf,-ic} = Q_{K,IC}\left(\left([K^+]_p - [K^+]_{P0}\right) - \left([K^+]_{ic} - [K^+]_{ic0}\right)\right) \quad (22)$$

The rate constant $Q_{K,IC}$ was estimated as described later. Plasma and extracellular Ka^+ concentrations are assumed to reach near-instantaneous equilibrium so that plasma/ECF Ka^+ concentration is determined by the balance between Ka^+ intake($\varphi_{k,in}$), excretion ($\varphi_{k,out}$), and movement between the ECF and intracellular compartments ($\varphi_{ecf\text{-}ic}$):

$$\frac{d[K^+]_P}{dt} = \frac{(\varphi_{k,in} - \varphi_{k,out} - \varphi_{ecf-ic})}{V_{ecf}} \quad (23)$$

Intracellular Ka^+ concentration is given by:

$$\frac{d[K^+]_{ic}}{dt} = \frac{\varphi_{ecf-ic}}{V_{ic}} \quad (24)$$

V_{ecf} and V_{ic} are the extracellular and intracellular fluid volumes, respectively.

2.2.5. Steady-State model parameterization

Before considering feedback responses to perturbations, the model was parameterized to produce a state in which plasma and intracellular potassium are stable and at their normal levels (4.1 mEq/L and 150 mEq/L [9]), and so that fractional reabsorption/secretion rates in each tubule section are consistent with established values (PT reabsorption: 65%, LoH reabsorption: 27%,

DCT/CNT/CCD secretion: 8%, MCD reabsorption: 4% [9]) as a percentage of filtered load.

These parameters are given in **Table 2.1** Parameters and initial conditions for parameters defining concentrations, geometrical dimensions, intake rates, and filtration rates were determined from literature values for normal human physiology. Luminal and basolateral potassium permeabilities (h_L and h_B) and Na^+/K^+ ATPase max flux were first approximated from studies in rats and then adjusted to produce the expected steady state plasma and intracellular concentrations (**Fig2.2**).

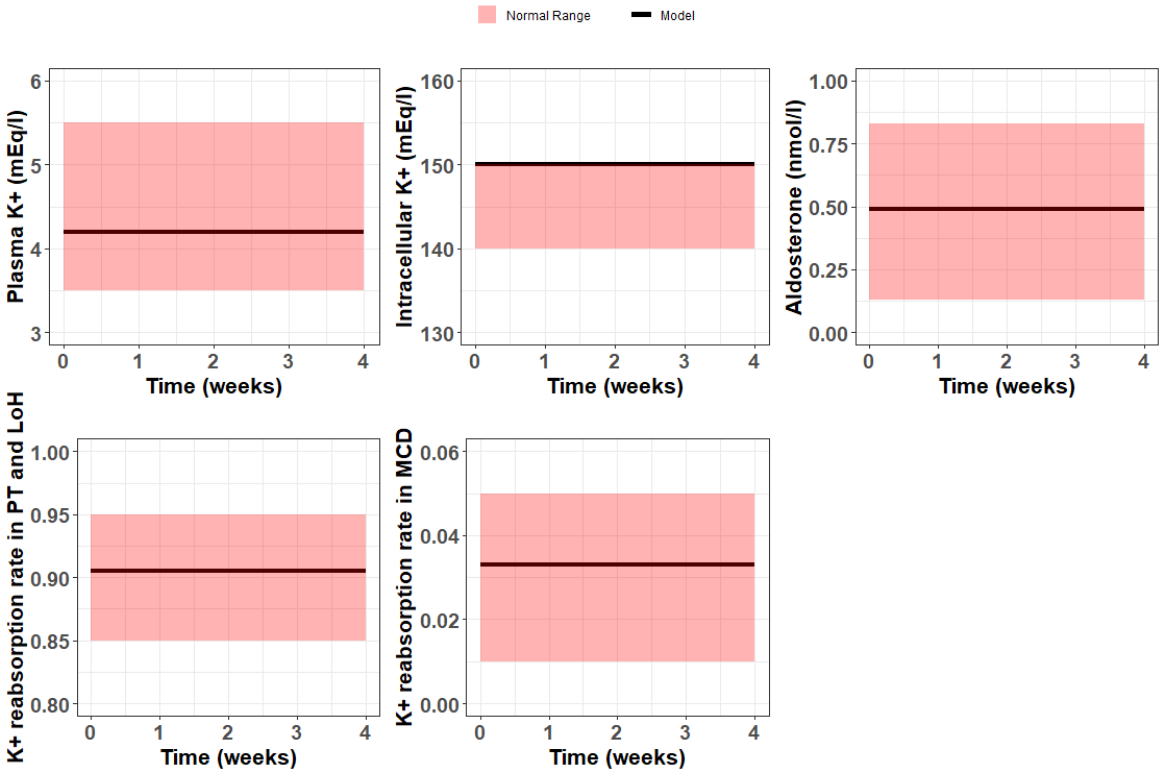

Fig 2.2. Keymodel variables are stable at steady state and fall within the normal ranges in physiological references [9]

Table 2.1. Model parameters and initial conditions

Parameter	*Definition*	*Normal Range*	*Value*	*Unit*	*Source*
$[Aldo]_0$	Baseline aldosterone concentration	0.13-0.83	0.49	*nmol/L*	[66]
D_{CCD}	Cortical collecting duct diameter	-	25	*µm*	[48]
D_{CNT}	Connecting segment diameter	-	24	*µm*	[50]
D_{DCT}	Distal convoluted tubule diameter	-	15	*µm*	[49]
F	Faraday Constant	-	96,485	*C/mol*	-
GFR	Glomerular filtration rate	90-130	105	*ml/min*	[9]
$Fr_{c,CCD}$	Fraction of CCD luminal surface area covered by secreting cells	-	0.6		[67, 68]
$Fr_{c,CNT}$	Fraction of CNT luminal surface area covered by secreting cells	-	0.6		[67, 68]
$Fr_{c,DCT}$	Fraction of DCT luminal surface area covered by secreting cells	-	1		[67, 68]
h_B	Basolateral potassium permeability through Kir	-	3.43e-5	*cm/min*	Calculated at steady-state
h_L	Luminal potassium permeability through ROMK	-	2.4935×10^{-5}	*cm/min*	Calculated at steady-state
$J_{Na+,max}^{active}$	maximum sodium flux through Na^+/K^+ ATPase	-	14.66×10^{-5}	*mmol/min/cm²*	[48], Calculated at steady-state
$[K^+]_{cell,0}$	Potassium principal cell concentration, initial condition and setpoint	-	150	*mEq/L*	[49]
$[K^+]_{p,0}$	Plasma potassium concentration initial condition and setpoint	3.5-5	4.2	*mEq/L*	[69]
L_{CCD}	Cortical collecting duct length	-	0.2	*cm*	[48]
L_{CNT}	Connecting tubule length	-	0.4	*cm*	[67]
L_{DCT}	Distal convoluted tubule length	-	0.5	*cm*	[49]

$[MR]_{norm}$	Normalized Mineralocorticoid receptor concentration	1	1	-	-
$N_{nephrons}$	Number of nephrons	-	$2x10^6$	-	[9]
$[Na^+]_P$	Plasma sodium concentration	135-145	140	*mEq/L*	[9]
R	Universal gas constant	-	8.3145	$J\ mol^{-1}\ K^{-1}$	-
$SV_{ratio-CCD}$	CCD volume to surface area ratio	-	0.004	cm^3/cm^2	[48]
$SV_{ratio-CNT}$	CNT volume to surface area ratio	-	0.006	cm^3/cm^2	[50]
$SV_{ratio-DCT}$	DCT volume to surface area ratio	-	0.0075	cm^3/cm^2	[49]
T	Body Temperature		310.6	*K*	-
V_{ecf}	Extracellular fluid volume	-	15	*L*	[7]
V_{ic}	Intracellular fluid volume	-	25	*L*	[62]
V_B	Electrical potential across the basolateral membrane	-	-78.2	*mV*	[48]
V_L	Electrical potential across the luminal membrane	-	-18.4	*mV*	[48]
$\gamma_{water,DCT}$	DCT fractional water reabsorption	-	0	-	[9]
$\gamma_{water,CNT}$	CNT fractional water reabsorption	-	0.7	-	[9]
$\gamma_{water,CCD}$	CCD fractional water reabsorption	-	0.75	-	[9]
$\varphi_{K,reabs,MCD0}$	Single-nephron MCD baseline potassium reabsorption rate	-	7.293e-9	*mEq/min*	Calculated at steady-state
φ_{K-in}	Potassium intake rate	100 -200 *mEq/day*	0.08	*mEq/min*	[7]
$\varphi_{NA-intake}$	Sodium intake rate	100 -200 *mEq/day*	0.07	*mEq/min*	[7]
$\varphi_{Na,reabs,MCD0}$	Single-nephron MCD baseline sodium reabsorption rate	-	3.125e-7	*mEq/min*	Calculated at steady-state

2.2.6. Modeling and calibration of feedback loop between aldosterone and potassium

2.2.6.1. Experimental data

To determine and calibrate the mechanisms of feedback between plasma K^+ and plasma ALDO, we utilized published measurements of the plasma K^+ and ALDO response during and following a 2-hour infusion of potassium chloride in normal human subjects in a study by Dluhy et al [70]. In this study, subjects were divided into 4 groups, and placed on controlled diets for 6-10 days prior to the start of the study period, with fixed level of Na^+ and K^+ intake: low K^+/low Na^+, high K^+/low Na^+, low K^+/high Na^+, and high K^+/high Na^+ intake. Low and high K^+ intakes were 40 and 200 mEq daily, and low and high Na^+ intakes were 10 and 200 mEq daily. In all subjects, at the start of the study period, potassium chloride was infused at a rate of 0.62 mEq/min for 120 minutes, followed by a 180-minute recovery period. Plasma K^+ and ALDO were measured at 0, 30, 90, 120, and 300 minutes.

2.2.6.2. Determination of feedback model structure and parameters

In response to perturbations, K^+ homeostasis is maintained through regulatory feedback control by the hormone ALDO. Changes in plasma K^+ concentration control ALDO secretion by the adrenal gland. ALDO in turn binds to mineralocorticoid receptors (MR) in the DCT, CNT, and CCD, increasing Na^+ influx into the cell by upregulating basolateral Na^+/K^+ ATPase expression and activity [70]. The increased basolateral exchange flux through Na^+/K^+ ATPase in turn increases K^+ secretion in the DCT, CNT, and CCD (See Eqs. 7 and 12). To describe this feedback system, mathematical relationships were introduced to describe 1) an effect of plasma K^+ on plasma ALDO,

and 2) an effect of ALDO on Na^+/K^+ ATPase activity in the DCT/CNT/CCD.

In addition, although understanding the effect of Na^+ on K^+ regulation is not the aim of the current model, it is necessary to account for effects of Na^+ intake on ALDO secretion, in order to fully reproduce the experimental studies by Dluhy et al at varying Na^+ and K^+ intakes [70]. Increased sodium intake suppresses plasma aldosterone, either through increases in plasma sodium or indirectly through suppression of renin. Thus, an effect of sodium intake on aldosterone secretion were included.

Changes in Na^+ intake also changes tubular Na^+ delivery along the tubule in the kidney. These effects are captured by the model structure described earlier (Eqs. 2-5 and Eq. 19), although the magnitude of the effect of MCD Na^+ reabsorption on MCD K^+ secretion ($m_{Na\text{-}K,MCD}$) was determined by fitting the experimental data at different Na^+ intakes. Likewise, the magnitude of the effect of changes in plasma K^+ on MCD K^+ reabsorption ($m_{Na\text{-}K,MCD}$) was determined by fitting the experimental data at different K^+ intakes.

For each mechanism, linear, exponential, and sigmoidal relationships were evaluated by simulating the Dluhy study [20] protocol. When two mechanisms affect the same parameter, additive and multiplicative relationships were evaluated. Parameters were estimated by minimizing the least square error between the observed and model-predicted plasma ALDO and plasma K^+ concentrations, and the parsimonious model structure that minimized the error was selected. The final best fit model structure is described here, and estimated parameters are given in **Table 2.2**.

Plasma ALDO concentration is modeled as an additive exponential function of plasma K^+ and Na^+ intake.

$$[Aldo] = [Aldo]_0 e^{m_{K,Aldo}([K]_p - [K]_{p,o})} + \left(e^{m_{Na,Aldo}(Na_{in,0} - Na_{in})} - 1\right) \quad (25)$$

Here, $m_{K,Aldo}$ and $m_{Na,Aldo}$ are fitting coefficients (**Table 2.2**). The effect of ALDO on Na^+/K^+ ATPase activity through MR in the DCT, CNT, and CCD was modeled as a linearly increasing effect on the maximum pump flux $J_{Na+,max}^{active}$ from Eq. 12.

$$J_{Na+,max}^{active} = J_{Na+,max,0}^{active} [MR]_{norm} \left(1 + m_{aldo,K} ([Aldo] - [Aldo]_0)\right) \quad (26)$$

Here, $[MR]_{norm}$ is the normalized MR receptor concentration (1 under baseline conditions) and $m_{aldo,K}$ is a fitting coefficient. An additional effect of ALDO on intracellular Na^+ concentration through increased ENAC activity did not further improve the model fit, and was not included in the final model.

Table 2.2. Estimated parameters for the regulatory feedback of potassium aldosterone, determined by fitting Dluhy et al[70]

Parameter	***Definition***	***Value***	***Unit***	***%SE***
Aldosterone and sodium effect				
$\mathbf{m_{aldo-K}}$	Fitting constant for aldosterone effect on luminal potassium permeability	102.3	L/nmol	0.2 %
$\mathbf{m_{K,Aldo}}$	Slope of plasma potassium effect on plasma aldosterone	0.9697	L/mEq	0.4 %
$\mathbf{m_{K-P,MCD}}$	Fitting constant for effect of plasma potassium on MCD K^+ reabsorption	7.2e-7	-	5.2%
$\mathbf{m_{Na,Aldo}}$	Fitting constant for sodium intake effect on plasma aldosterone	13.24	Min/mEq	0.5%
$\mathbf{Q_{K-ic}}$	Rate constant for interstitial and intracellular potassium exchange	0.3306	L/min	3%

2.3. Software

The model runs in R v1.4.1103 using the RxODE package[71]. The presented model equations, code, and parameters are available in the appendix A.

2.4. Results and discussion

Estimated parameter values for the effect of plasma K^+ on ALDO and the effect of ALDO on tubular K^+ secretion and reabsorption, fit to Dluhy et al.[70], are given in Table 2. As shown in **Fig 2.3A**, the model reasonably reproduced the effects of changes in K^+ and Na^+ intake on baseline K^+ and ALDO, as well as the magnitude and time-course of changes following initiation and then cessation of K^+ infusion. While the plasma K^+ response fits well, the ALDO response to K^+ infusion at low K^+ intake is slightly over estimated. This suggests that sustained potassium scarcity could weaken the ALDO response to plasma K^+ changes, but this mechanism was not included in the model.

Fig 2.3B illustrates the fate of K^+ during and after infusion, at different intake levels. Note first that prior to infusion, the system is in K^+ balance – excretion equals intake at time zero for both low and high K^+ groups. the net flux of K^+ between the intracellular and extracellular space is zero. In the kidney, excretion is the sum of filtration and secretion minus reabsorption in each intake combination (second row). Both tubular reabsorption and secretion of K^+ are increased at low sodium intake compared to high Na^+ intake. This increased reabsorption of K^+ is due to increased Na^+ retention in the PT as the kidney retains sodium to maintain sodium balance. However, because Na^+ intake also increases ALDO, which stimulates K^+ secretion, the distal nephron compensates by increasing K^+ secretion so that K^+ balance is also maintained.

Then, during K^+ infusion (represented as an increase in intake), a portion of the increased

K^+ in the system moved into the intracellular space, which serves as a buffer that limits changes in plasma K^+. Intracellular concentration remains unchanged (not shown), since its volume is large and existing store of K^+ is high relative to the amount of K^+ entering the cells. As plasma K^+ rises, ALDO secretion rises, and filtered K^+ also increases (since the rate of filtration is proportional to plasma K^+). ALDO stimulates increased secretion of K^+, and K^+ excretion rises. Then, when the K^+ infusion is stopped, plasma K^+ begins to fall, ALDO and K^+ secretion return toward normal, and K^+ excretion falls back toward baseline.

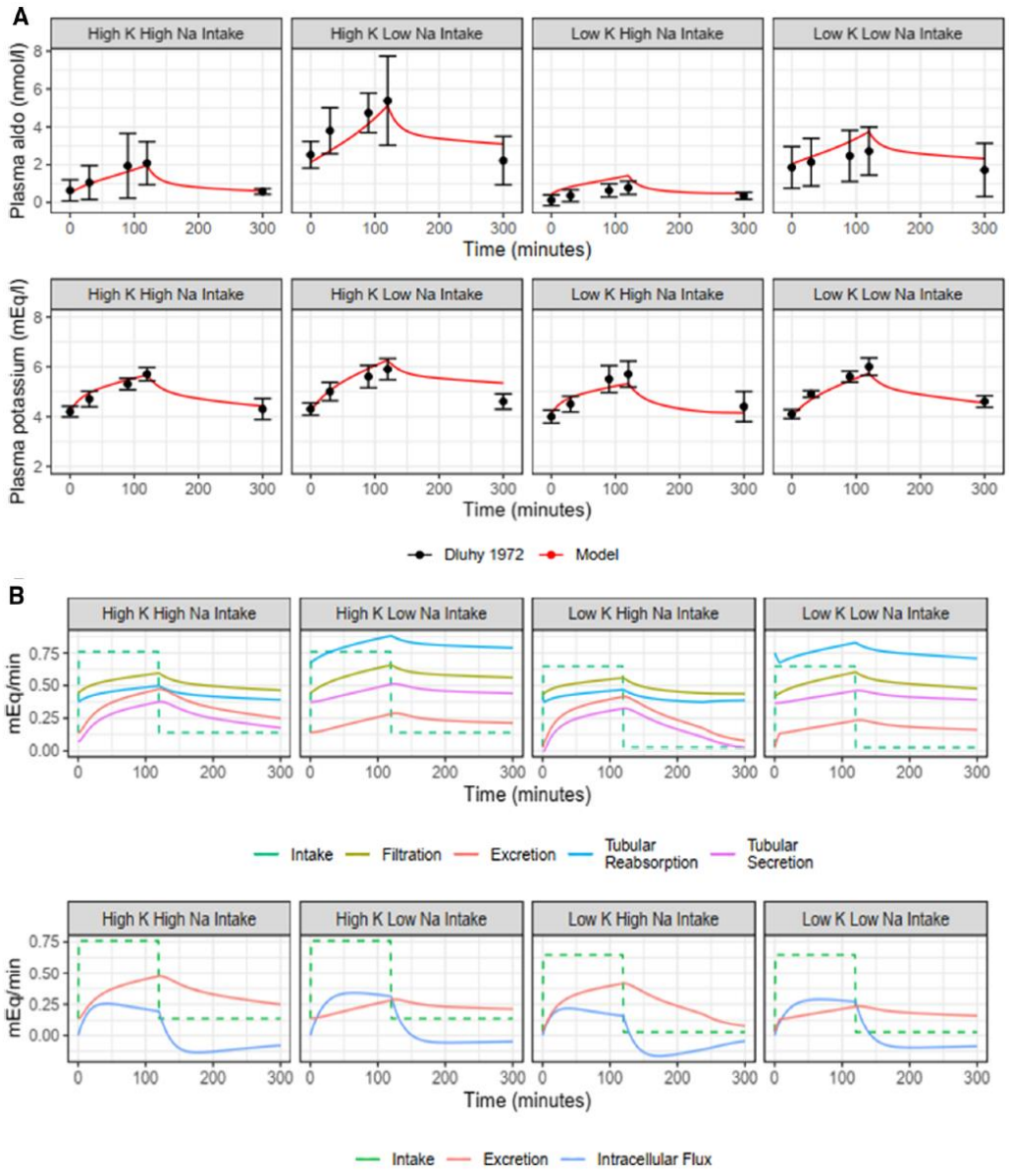

Fig. 2.3. A) Model fit to observed plasma ALDO (top row) and plasma K^+ (bottom row) response to K^+ in humans on a low/high K^+ (40 and 200 mEq/day) and low/high Na^+ (10 and 200 mEq/day). Data are mean ± SD from[70].Model reasonably reproduces the effects of diet on baseline K^+ and ALDO, as well as the dynamic changes in plasma ALDO and K^+ during and following a 120-minute K^+ infusion of 0.62 mEq/min. B) Fate of K^+ during infusion and after cessation. As K^+ is increased, increases in excretion and intracellular flux limit the rise in plasma K^+. The increased excretion is due to both increased filtration and increased secretion, which is driven in part by increased ALDO.

2.5. Conclusion

The mathematical model presented here describes the role of the kidney and ALDO feedback in maintaining K^+ homeostasis, and after calibration with short-term perturbation (acute K^+ infusion). While previous models have described potassium transport in the kidney[47-50] or systemic potassium balance[52, 53, 57], this model describes the integrated effects of renal transport, systemic K^+ balance, and ALDO feedback. This key advance allows simulation of the dynamic response to perturbations and prediction of the effect of changes in renal function or MRA therapy on plasma K^+, something that cannot be achieve when renal K^+ handling and systemic K^+ homeostasis are considered separately.

The functional form of equations describing the steady-state system are based on first principles and our current understanding of physiological processes of K^+ handling within the kidney. Nearly all parameters defining the system at steady state are known from the literature with reasonable certainty. For the parameters with the most uncertainty, namely the Na^+/K^+ ATPase max pump flux, luminal and basolateral permeabilities, and MCD K^+ reabsorption rate, their values could be calculated from other parameters in order to produce stable plasma and intracellular K^+ concentrations under the constraints of steady-state Na^+ and K^+ balance. On the other hand, the functional form of the equations describing homeostatic feedback mechanisms are less well-established. A local sensitivity analysis showed the plasma K^+ and ALDO are most sensitive to the parameters $m_{aldo\text{-}K}$ and $m_{K,aldo}$ (Appendix B), and both of these parameters were able to be estimated with good precision. The equations presented

here provided the best fit to the experimental data with the minimal number of parameters (6) required to be estimated. It is possible that these equations may not be generalizable far outside the conditions for which they were calibrated.

Several limitations should be noted. First, the model slightly overpredicts the ALDO response to plasma K^+ changes under low K^+ intake. We found that estimating separate values for the strength of the effect of plasma K^+ on ALDO secretion, $m_{k,aldo}$, at high vs low K^+ intake could improve the fit. One explanation for this is that, with sustained K^+ scarcity, the adrenal gland may adapt by reducing its ALDO response when ALDO does become available, thus helping to retain K^+ in the face of fluctuating variability. Such a mechanism could be incorporated in the model, but given the limited data available and to avoid overparameterizing the model based on a single study, the mechanism was not included.

Humoral factors that alter ALDO regulation, such as angiotensin II and adrenocorticotropic hormone (ACTH), were not considered [72].

In addition, we assumed a direct effect of Na^+ intake on ALDO secretion, but this mechanism is likely mediated indirectly through changes in renin induced by changes in Na^+ intake. In future, coupling of this model with a model of Na^+ homeostasis may allow a more mechanistic description of the interrelationship between K^+ and Na^+. We are currently working to integrate this model with our previously published model of Na^+ homeostasis [54, 55]. After integration with the K^+ homeostasis model, it could be particularly useful in evaluating and predicting benefit/risk when combining therapies that have both natriuretic and kaliuretic effects. This is particularly a common and significant problem in both patient management and drug development in chronic kidney disease, hypertension, and heart failure, in which several standard-of-care medicines (RAAS blockers, diuretics, MR antagonists, etc.) both improve cardiovascular

outcomes and increase hyperkalemia risk.

CHAPTER 3

SPECIFIC AIM 2

INTEGRATE THE POTASSIUM QSP MODEL (AIM 1)
WITH THE PHARMACOLOGIC MECHANISTIC
COMPONENT
OF MINERALOCORTICOID RECEPTOR ANTAGONISTS

ADMINISTRATION

This chapter contains text from the following publication:

Maddah, Erfan, and K. Melissa Hallow. "A quantitative systems pharmacology model of plasma potassium regulation by the kidney and aldosterone." *Journal of Pharmacokinetics and Pharmacodynamics* 49.4 (2022): 471-486.

3. Chapter 3: Specific aim 2, Integrate the potassium qsp model (aim 1) with the pharmacologic mechanistic component of mineralocorticoid receptor antagonists administration

3.1. Introduction

Studies over 50 years ago led to the introduction of the first mineralocorticoid receptor (MR) antagonist spironolactone, which remains in widespread use. This MR antagonist is mainly used in conditions of ALDO excess that are associated with primary and secondary aldosteronism, as a potassium-sparing diuretic[73]. Spironolactone and eplerenone, a newer FDA-approved MR antagonist, both inhibit the epithelial and non-epithelial receptors to ALDO. These therapies are using in the treatment of hypertension with or without hyperaldosteronism and different phases of heart failure (HF). The common benefits of MR antagonist include reduced fluid retention, cardiorenal protective effects, and anti-inflammatory effects[74].The benefits of MR antagonist in treatment of HF and high blood pressure have led to their routine use [75, 76], although they are not part of the standard-of-care regimen, due to their potential for inducing hyperkalemia.

3.1.1.Spironolactone mechanisms of action

Spironolactone as a specific pharmacologic antagonists of ALDO's MR receptor helps to block excessive Na^+ reabsorption in the kidney and assists the renal system to excrete more Na^+ and water. This occurs by binding to the MR receptors in epithelial cells in DCT, CNT, and CD segments of the nephron[77].

Spironolactone actually exerts its pharmacologic effects through its active metabolite, Canrenone. Canrenone binds to the MR receptors located in distal and collecting tubule segments of the nephron, blocking endogenous ALDO from binding [78]. **Fig 3.1** illustrates the schematic of the combination of the renal K^+-ALDO homeostasis model with the mechanistic component of MR antagonists.

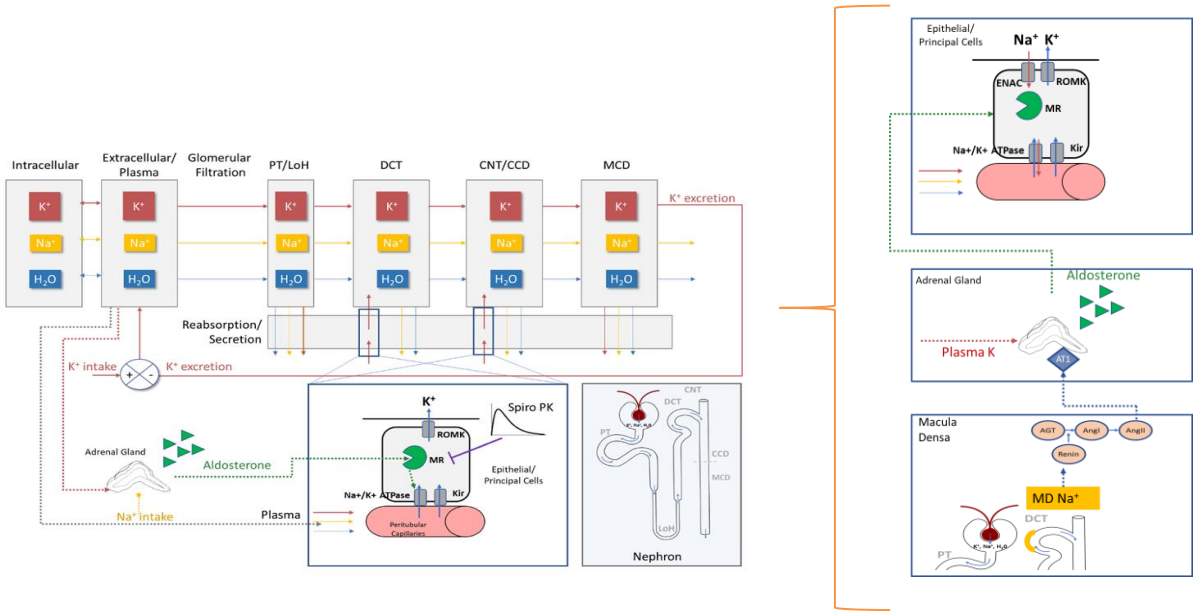

Fig 3.1. the schematic describes the combination of the calibrated model of K^+ and ALDO regulation and MR receptors that are located in epithelial and principal cells of DCT, CNT, and CCD segments of the nephron. Blocking the MR receptors by spironolactone enhances K^+ retention and Na^+ excretion from the kidneys.Figure belongs to [51].

3.2. Calibration of pharmacologic effects of mineralocorticoid antagonists

3.2.1. Experimental data

The concentrations of spironolactone and its active metabolite canrenone for 24 hours following a single 100mg given with a meal were obtained from Gardiner et al[79]. The response to spironolactone was calibrated by fitting clinical data from a published study[80] in which healthy subjects were administered a single dose of spironolactone (placebo, 25mg, 50mg, 100mg, 200mg, or 400mg dose levels), and urinary K^+ excretion was measured over 2-10 hours and12-16 hours post-dose.

3.2.2. Pharmacokinetic-Pharmacodynamic model structure

The pharmacokinetic (PK) profiles of spironolactone and its active metabolite canrenone were modeled with a one and two compartment model, respectively. For spironolactone, two transit compartments were added to account for the delayed rise in concentration[81]. Spironolactone PK were best described with a one compartment model with an absorption delay represented by series of transit compartments. a_{s0}, a_{s1}, and a_{s2} are the amount of spironolactone in each transit consecutive transit compartment, and K_a is the transfer rate constant between transit compartments.

$$\frac{d(a_{S0})}{dt} = -K_a a_{S0} \quad (27)$$

$$\frac{d(a_{S1})}{dt} = K_a(a_{S0} - a_{S1}) \quad (28)$$

$$\frac{d(a_{S2})}{dt} = K_a(a_{S1} - a_{S2}) \quad (29)$$

Spironolactone is transferred from the last transit compartment to the central compartment at a rate K_a, and cleared with a clearance rate CL_S.S is the concentration of spironolactone, and V_S is the spironolactone central compartment volume.

$$\frac{1}{V_S}\frac{d(S)}{dt} = K_a a_{s2} - CL_S S \quad (30)$$

A fraction F_m of spironolactone is cleared by conversion into the metabolite canrenone. Canrenone is best modeled with a two-compartment model. Spironolactone metabolized into canrenone is transferred into the central canrenone compartment at a rate $CLSF_m$. Canrenone is cleared from the central compartment with a clearance CLC, and the intercompartment clearance is Q_C. C_C and Cp are the concentrations of canrenone in the central and peripheral compartments, respectively, and $V_{C,c}$ and $V_{C,p}$ are the volumes of the central and peripheral canrenone compartments, respectively.

$$\frac{1}{V_{C,c}}\frac{d(C_C)}{dt} = CL_S F_m S_C - CL_C C_c - Q_c(C_c - C_p) \quad (31)$$

$$\frac{1}{V_{C,p}}\frac{d(C_p)}{dt} = Q_c(C_c - C_p) \quad (32)$$

Fig3.2 presents the designed PK structure for the spironolactone and the active metabolite canrenone.

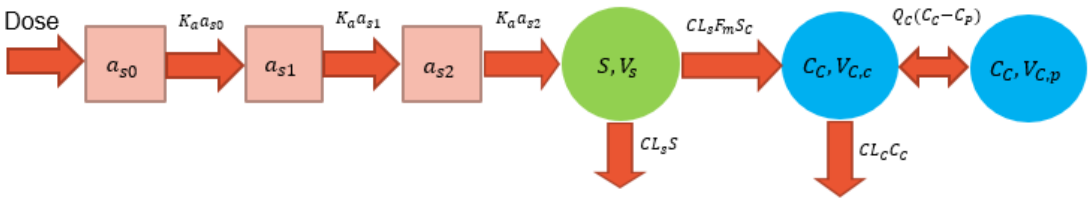

Fig3.2. represents the schematic of PK profile for the spironolactone and diuretic metabolite canrenone. After leaving the depot compartment, two transient compartments with same transfer

rate K_a were applied to avoid instant rise in spironolactone concentration. One central compartment for spironolactone and two compartments (central and peripheral) were designed to account for the main clearance and intercompartment clearance in central and peripheral compartment of canrenone.

Pharmacokinetic parameters for each drug were determined by minimizing the least square error between the observed and model-predicted plasma concentration. These resulting pharmacokinetic parameters were then fixed for pharmacodynamic simulations.

The pharmacodynamic effect of mineralocorticoid antagonists was best described with an indirect response model[82].

$$\frac{d(E_{MRA})}{dt} = K_{on}\left(1 - \frac{I_{max} * C_{MRA}}{\mathrm{C_{MRA}} + IC50}\right) - Koff * E_{MRA} \qquad (33)$$

Here, I_{max} and IC50 are the maximum drug effect and the concentration to reach half of the maximum inhibition, respectively, and C_{MRA} is the concentration of the active metabolite canrenone. When simulating MRA, the effect of ALDO on Na^+/K^+ ATPase (Eq. 34) was modified to include this effect:

$$\mu_{aldo-NaK} = MR_{norm}(1 - E_{MRA})\left(1 + m_{aldo,K}\left([Aldo] - [Aldo]_0\right)\right) \quad (34)$$

The McInnes et al[80] study protocol was simulated, and the parameters governing the spironolactone exposure-response (**Table 3.1**) were estimated by minimizing the least square error between the observed and model-predicted urine K^+ excretion.

Table 3.1. Estimated parameters for pharmacokinetics/pharmacodynamics of spironolactone, determined by fitting in [79, 80].

Parameter	*Definition*	*Value*	*Unit*	*%SE*
Spironolactone PK-PD				
K_a	Spironolactone absorption rate	4.816	ng/mi	0.53 %
CL_S	Spironolactone clearance	8.68	L/min	4.0 %
F_m	Fraction of spironolactone metabolized to canrenone	0.253	-	6.3%
V_S	Spironolactone central volume	6.2	L	282%
$V_{C,c}$	Canrenone central volume	74.2	L	6.0%
$V_{C,p}$	Canrenone peripheral volume	6.9	L	31.3%
CL_c	Canrenone clearance	0.277	L/min	5.9%
Q_c	Canrenone intercompartmental clearance	0.124	L/min	33.3%
I_{MAX}	Maximum spironolactone MR inhibition	0.9978	-	0.008 %
IC50	Spironolactone concentration that provides 50% of maximum inhibition	1.83	mg	0.76%
K_{off}	First-order dissociation rate constant	3.4	ml/ng	20.4%
K_{on}	Second-order association rate constant	3.4	-	

3.3. Model validation

To validate the model, we tested the model's ability to predict the chronic response to spironolactone in hyperaldosteronism patients, reported by Karagiannis et al [83] . In this study, patients were administered increasing doses of either spironolactone (50, 100, 200, and 400 mg b.i.d.), with the dose increasing every 4 weeks, for a total of 16 weeks. Plasma K^+ concentration

was measured every four weeks.

To simulate this study, hyperaldosteronism was first modeled as an increase in ALDO concentration sufficient to produce the decreased baseline plasma K^+ concentration in each arm of (Karagiannis, Tziomalos et al.). This was done by adding a constant concentration increase $[Aldo]_{hyperaldo}$ to Eq. 25 in chapter 2.

$$[Aldo] = [Aldo]_0 e^{m_{K,Aldo}([K]_p - [K]_{p,o})} + \left(e^{m_{Na,Aldo}(Na_{in,0} - Na_{in})} - 1\right) + [Aldo]_{hyperaldo} \quad (35)$$

$[Aldo]_{hyperaldo}$ was increased to 0.6 nmol/L, which reduced baseline plasma K^+ concentration to 3 mEq/L, as reported by the study. The dose of spironolactone was increased every four weeks.

3.4. Results and discussion

3.4.1. Calibration of spironolactone PK-PD response

Estimated spironolactone PK-PD parameters, fit to Gardiner 1989 and McInnes et al[79, 80], are given in table 3.1. The model reproduces the pharmacokinetic profile of spironolactone's main active metabolite canrenone (**Fig 3.3**), as well as the dose-dependent decrease in K^+ excretion with spironolactone treatment at 2-10 hours (**Fig3.4**). Interestingly, the model also reproduced the rebound in K^+ excretion observed at 12-16 hours post-dose at lower doses (**Fig 3.4**), although it does not perfectly reproduce the mean data. The response at 100mg was surprisingly lower than even the 200 and 400 mg doses, and the model does not capture this. For the 25 and 50mg doses, the model predictions fall well within the standard deviation, which is quite large, but does not show the same mean decrease from 25-50mg. Fig 3.5 illustrates the mechanisms underlying this effect. Initially, spironolactone suppresses K^+ secretion. However, this causes a rise in plasma K^+

which in turn stimulates an increase in ALDO (consistent with studies showing that MR antagonists increase plasma ALDO reference). As spironolactone concentrations begin to fall and the inhibition of MR wears off, there is more ALDO around to bind the MR receptors and stimulate secretion. Thus, at lower doses, the secretion stimulated by ALDO later in the day exceeds the inhibition by spironolactone, and K^+ excretion increases past baseline.

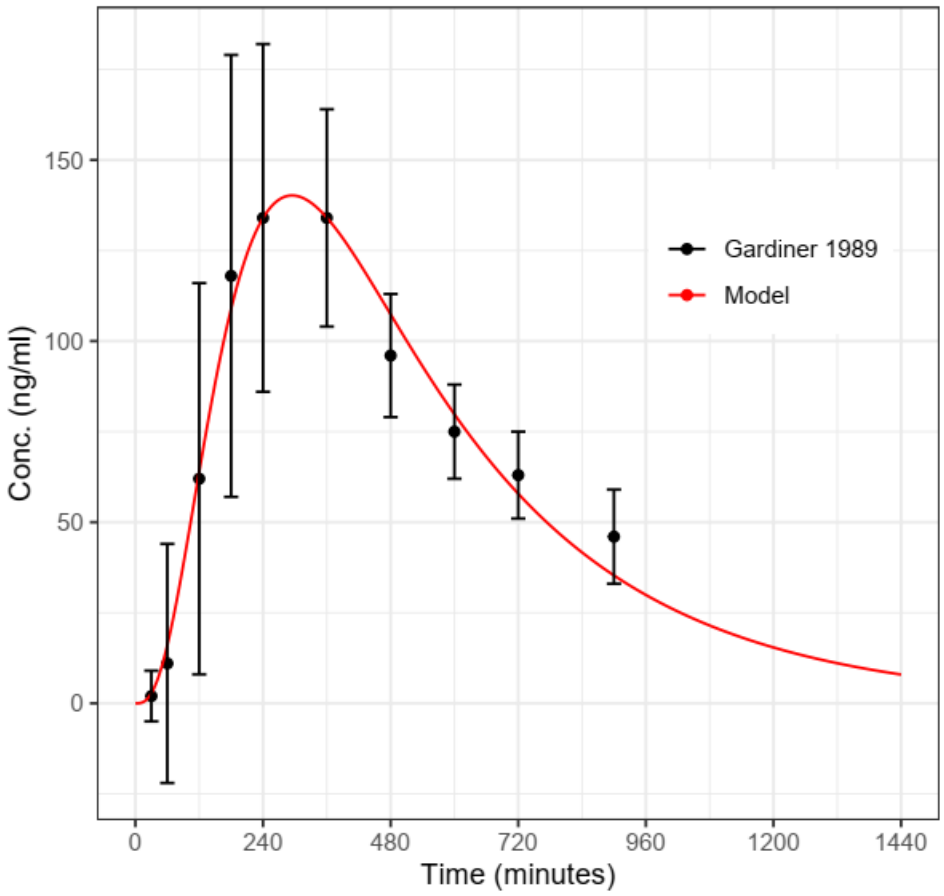

Fig3.3. Pharmacokinetic model of spironolactone reproduces the concentration profile of spironolactone's active metabolite canrenone. Date are mean ± SD from Gardener et al 1989[79].

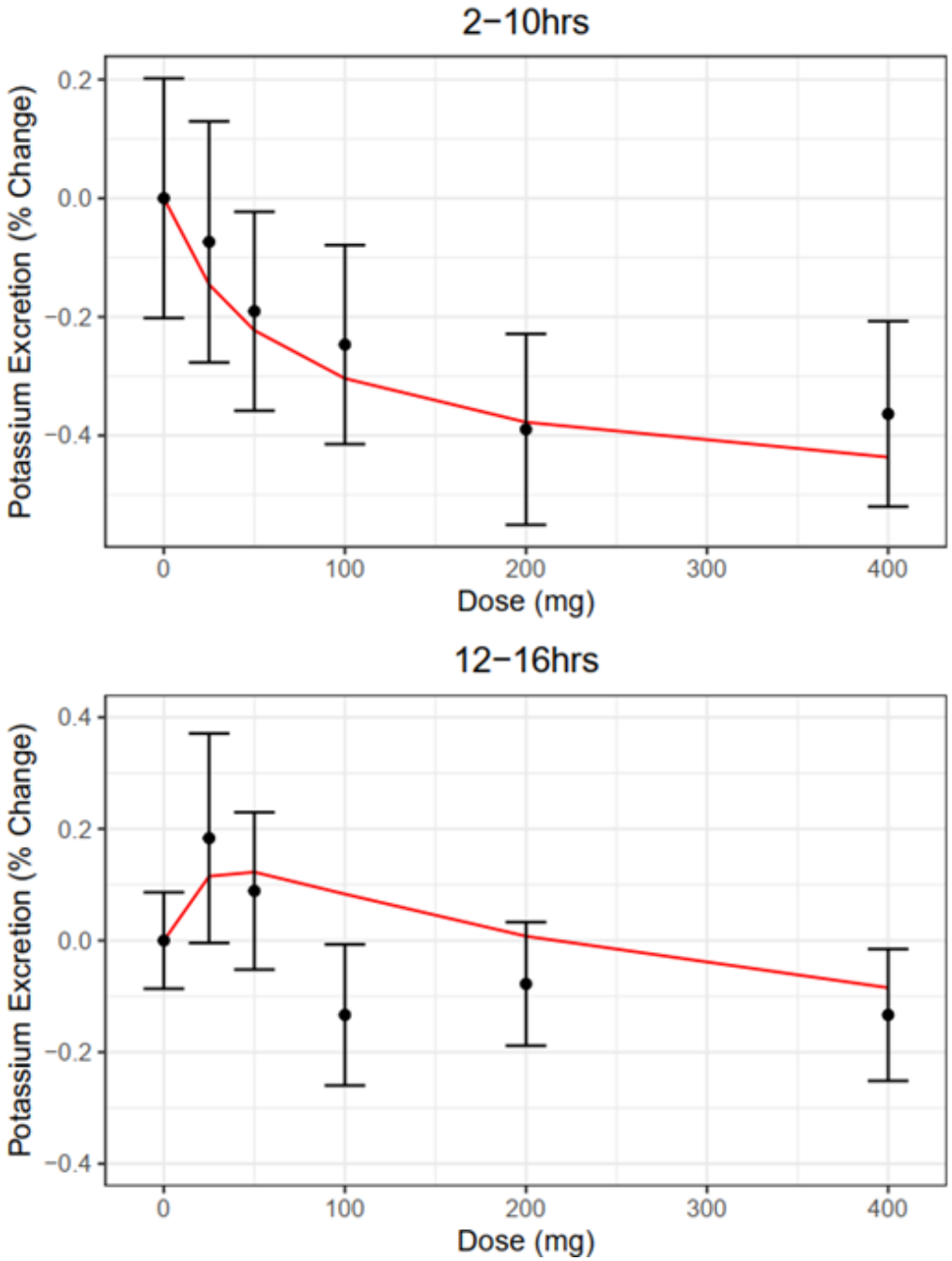

Fig 3.4. Model fit to urinary K^+ excretion dose-response 2-10 hours and 12-16 hours following a single dose of spironolactone in human subjects. Data are mean ± SD from McInnes et al 1982[80].

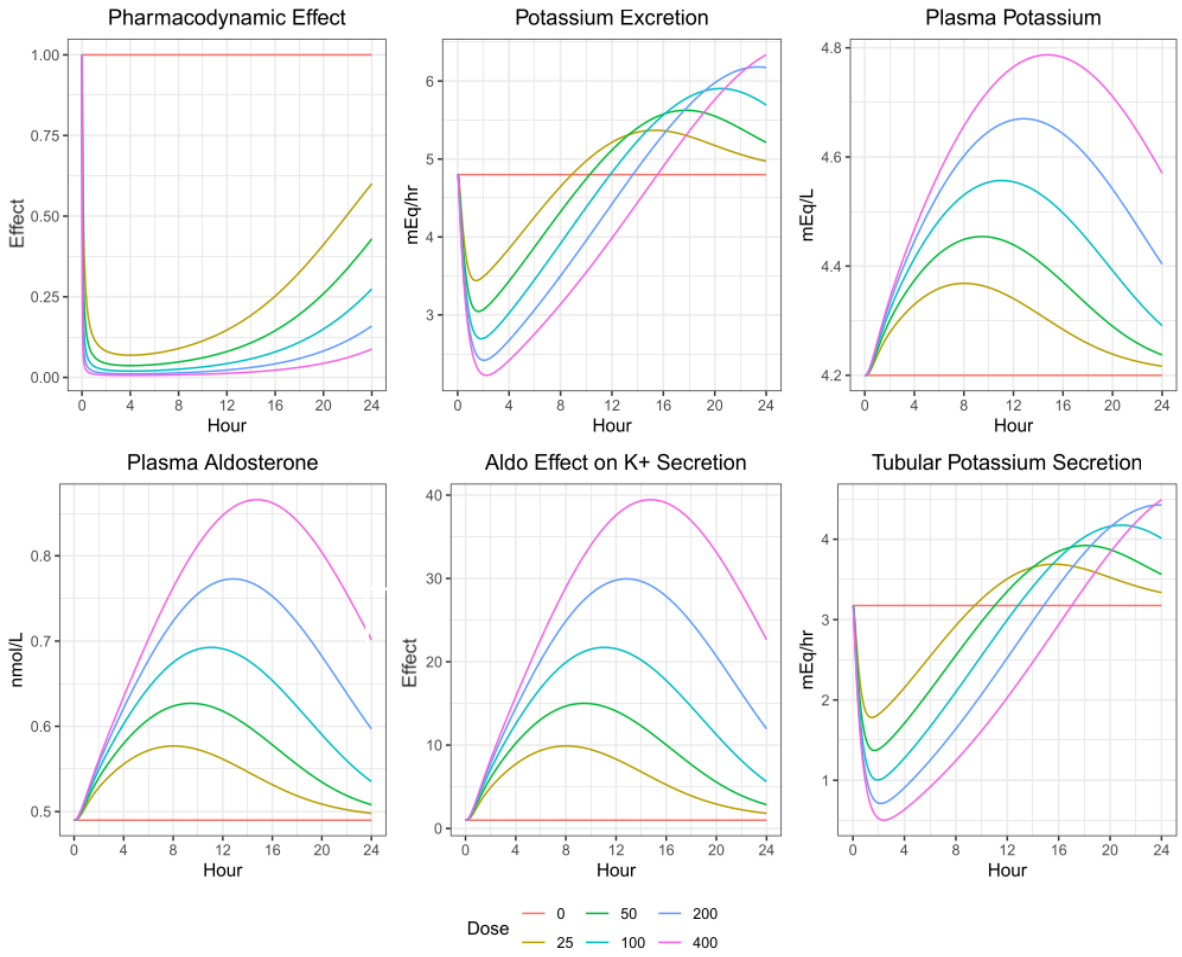

Fig3.5. Simulated response of model variables to different doses of spironolactone illustrate the mechanisms underlying the rebound in K^+ excretion at 12-16 hours observed in McInnes et al 1982[80]. Spironolactone reduced K^+ excretion, increasing plasma K^+, thus increasing plasma ALDO. Then, as spironolactone concentration falls and its inhibitory effect on the MR receptor wears off, the higher level of ALDO results in greater MR activation and increased secretion, causing K^+ excretion to rebound above baseline levels.

3.4.2. Calibration of spironolactone PK-PD response

Fig 3.6 compares the model-predicted and clinically observed responses to spironolactone in patients with hyperaldosteronism [83]. First, by altering ALDO production as described above in Eq. 29, the model was able to reproduce the lower baseline plasma K^+ concentration in

hyperaldosteronism subjects. When plasma ALDO is increased, it stimulates more tubular K^+ secretion, resulting in increased loss of K^+ and decreased plasma K^+ concentration. The model naturally captures this effect. In addition, the model also reproduces the rise in plasma K^+ over time with chronic treatment of increasing doses of spironolactone. Thus, the model calibrated using the acute K^+ excretion response to a single dose of spironolactone is able to reasonably predict the chronic plasma K^+ response to b.i.d. dosing.

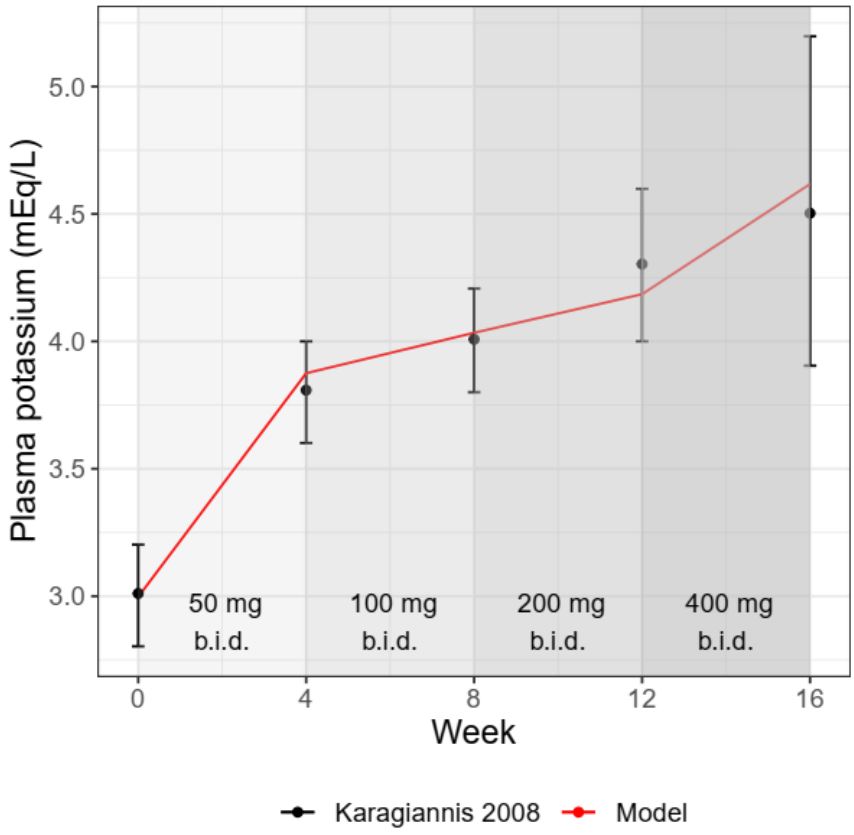

Fig 3.6. The model reproduces the chronic plasma K^+ dose-response to increasing doses of spironolactone in 17 human subjects with hyperaldosteronism. The model also reproduces the baseline decrease in plasma K^+ as a result of increased ALDO secretion. Data are mean ± SD from Karagiannis et al 2008[83].

3.4.3. Effects of GFR, potassium intake, or spironolactone treatment on plasma potassium

After calibration and validation, the model was used to investigate the relationship between GFR and plasma K^+, at normal and high K^+ intake (**Fig 3.7A**), and with and without spironolactone treatment (**Fig 3.7B**).

As shown in **Fig 3.7A**, plasma K^+ remains in the normal range (less than 5.5 mEq/L – green line) over a wide range of GFR. At a typical plasma K^+ intake of 115 mEq/day (Health agencies recommend a K^+ intake of 90 – 130 mEq/ day [84]), plasma K^+ changes minimally as GFR decreases from 130 down to 30 ml/min. However, as GFR decreases further, plasma K^+ increases slightly. At normal GFR, doubling K^+ intake has only a small effect on plasma K^+. As GFR decreases into the Stage 3-5 Chronic Kidney Disease (CKD) range (GFR 15-60), increasing K^+ intake causes plasma K^+ to rise substantially, especially for GFR < 30. On the other hand, lowering K^+ intake as minimal effect on plasma K^+, although at very low GFR, there is a slight decrease (still well above the hypokalemia range). For reference, the national kidney foundation recommends a restricted K^+ intake of 70-80 mEq/day in patients on hemodialysis [85].

As shown in **Fig 3.7B**, clinical doses of spironolactone is expected to have minimal effect on plasma K^+ when GFR is normal. Once-daily dosing has almost no effect, while b.i.d. dosing causes a small rise. However, as GFR falls in the CKD range, the increase in plasma K^+ with spironolactone treatment becomes much larger. Interestingly, at all GFR levels, 25 b.i.d. is predicted to increase plasma K^+ more than the same total dose (50mg) given q.d.

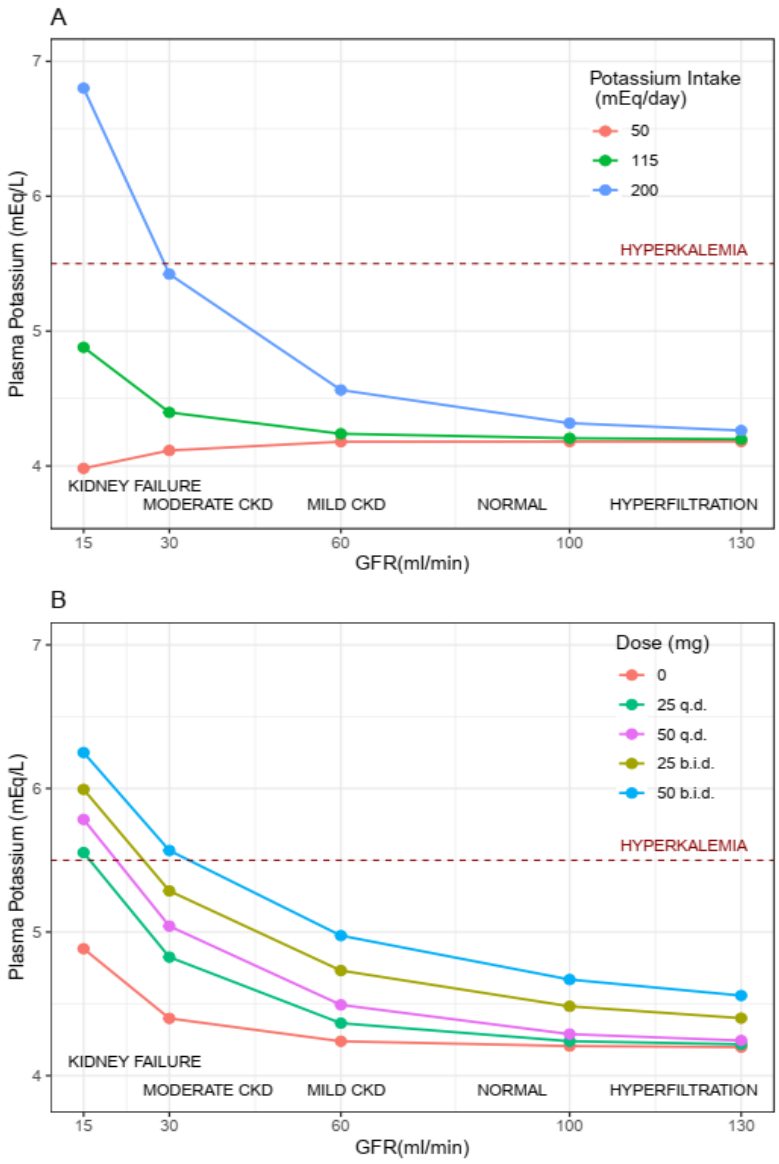

Fig 3.7. The simulated effect of GFR, K^+ intake, and spironolactone treatment on plasma K^+. At normal K^+ intake around 115 mEq/day (90-130 mEq is typical in western diets), loss of renal function only affects plasma K^+ as GFR approaches stage 5 CKD range (GFR 15-30ml/min), but stays well within the normal range. However, as GFR falls below 60, plasma K^+ becomes much more sensitive to increases in K^+ intake, as well as to the K^+ retaining effects of spironolactone, indicating that loss of renal function limits the system's ability to respond robustly to perturbations.

3.5. Conclusion

The model, which was calibrated with data from healthy subjects, performed well in reproducing the reduction in plasma K^+ in hyperaldosteronism patients, as well as the rise in plasma K^+ concentration when these patients were treated with spironolactone, providing some confidence in the generalizability of the model. In addition, the model was able to reproduce and mechanistically explain the rebound in K^+ excretion in the second half of the day following a single dose of spironolactone. This emergent behavior suggests that the underlying model structure is reasonable. Thus, we believe the model represents a reasonable tool for exploration of therapeutic, pathophysiologic, and lifestyle factors that alter K^+ homeostasis.

To this end, we investigated the effect of declining renal function, changes in K^+ intake or treatment with spironolactone on plasma K^+ levels. It is well established that the risk of hyperkalemia increases with worsening CKD [86, 87], but only subset of CKD patients ultimately develop hyperkalemia. Our simulations are consistent with this. They demonstrate that decreasing GFR alone only slightly elevates plasma K^+, consistent with a large observational cross-sectional study that found only small difference in median plasma K^+ between subjects with estimated GFR (eGFR) > 90 (3.98 [3.49–4.59] mEq/L) and eGFR < 15 mL/min/1.73 m^2 (4.43 [3.22–5.65] mEq/L)[88]. Our simulations also indicate that when perturbations are introduced (such as changes in K^+ intake or inhibition of mineralocorticoid receptors), lower GFR reduces the robustness of the feedback system in accommodating these changes and maintaining normal plasma K^+. This decreased robustness combined with various lifestyle and therapeutic differences likely explain why some develop hyperkalemia and others with similar loss of renal function do not. For example, in a population of nondiabetic CKD patients, 15% with eGFR less than 30 and 8% with eGFR 30-40 ml/min experienced a hyperkalemic event, while less than 2% of patients with eGFR > 40

experienced an event[89].

The model predicts that the effect of increasing K^+ intake on plasma K^+ is small when GFR is normal, but more substantial as GFR progresses toward end stage renal failure. While physicians typically recommend dietary K^+ restriction for dialysis patients effort to prevent hyperkalemia, the impact of K^+ intake on hyperkalemia risk has not been rigorously studied[90, 91]. A cross-sectional study found no relationship between K^+ intake and serum K^+ in CKD patients[92]

On the other hand, the NIH-AARP Diet and Healthy study found that in patients already on dialysis, higher K^+ intake was associated with higher mortality, although it is not clear whether the increase in mortality was due to hyperkalemia[93]. It should be noted that the simulations do not consider differences in gut K^+ absorption that may affect bioavailability of ingested K^+.

Similarly, the model predicts an increasing effect of MRAs on plasma K^+as renal function worsens. Because of their known effect on hyperkalemia, patients with existing CKD are typically excluded from clinical studies of MRAs, and thus very little data is available. However, in the RALES clinical trial, patients with severe heart failure (average eGFR 62 ml/min) treated with spironolactone 25-50 mg q.d. saw an average plasma K^+ increase of 0.3 mEq/L compared to pretreatment[94], quite consistent with the model-predicted changes of up to 0.25 mEq/L at 60 ml/min (**Fig 3.7B**). In RALES, twice as many of patients treated with spironolactone experienced serum $K^+ > 5$ mEq/L at some point in the trial, and baseline eGFR was lower in patients who experienced hyperkalemia in both the placebo (58.6 vs. 64.8) and spironolactone arms (58.2 vs 67), consistent with model predictions. In the EPHESUS study of eplerenone in severe heart failure[95], serum $K^+ > 5.5$ mEq/L was much more frequent with eplerenone than placebo in those with eGFR < 60 (22% vs. 13.8%), but only slightly greater than placebo in those with eGFR > 60 (10.8% vs. 9.3%), consistent with model predictions.

Consequently, the presented model in previous and this chapter, is an important step toward

being able to quantitatively predict not only the effect of alterations in K^+ homeostasis on plasma K^+, but also on natriuresis, renal function, and blood pressure after getting integrated into the validated Na^+ and water homeostasis model [51, 54].

4. Chapter 4: Specific aim 3, Integration of the potassium-aldosterone homeostasis model into the sodium/water homeostasis model to investigate drug effects on potassium level

4.1. Background

4.1.1. Potassium and sodium regulation

Potassium (K^+) and sodium (Na^+) are ions that responsible for normal body function by regulation of fluid and blood volume which make them essential for human health. Most metabolic processes are affected by both of these ions. They play important roles in regulation of osmotic pressure and water distribution, maintenance of proper pH, regulation of the normal function of the cardiovascular system and muscular tissues, electron transport reactions, and activities for enzymes [23]. In order to maintain fluid and electrolyte homeostasis, water, K^+, and Na^+ are in constant movement between the intracellular and extracellular body compartments, and are highly regulated by the kidney.

In the kidney, both Na^+ and K^+ are freely filtered across the glomerulus. In the proximal nephron, Na^+ is actively reabsorbed, producing a concentration gradient that also drives water reabsorption (**Fig 4.1**).

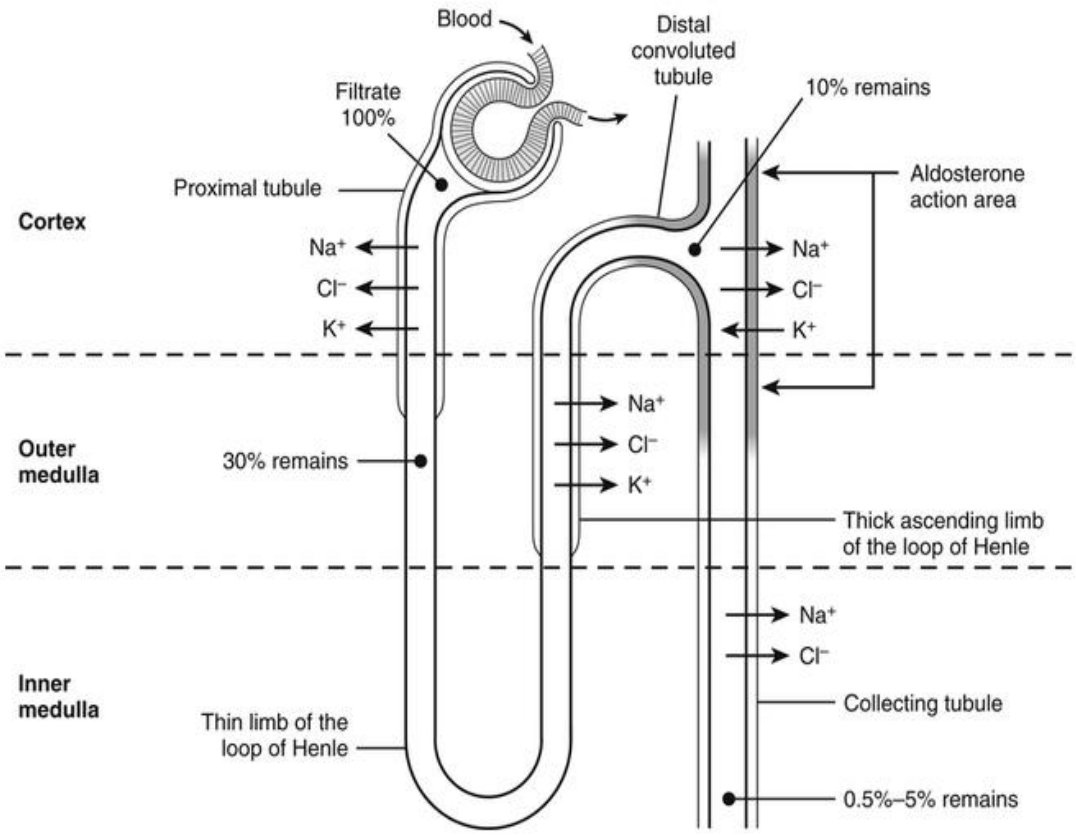

Fig4.1. The nephron structure, Na^+ and K^+ regulation. electrolytes enter afferent arteriole with blood, get filtered, and excrete from the nephron after removal or addition through the nephron[96].

K^+ is then reabsorbed passively along with water and propotionally to Na^+. However, in the distal nephron, Na^+ and K^+ exchange occurs in opposite direction: there is net Na^+ reabsorption and net K^+ secretion through epithelial and principal cells of the distal nephron.On the basolateral side of the cell, K^+ is pumped from the blood into the cell, in exchange for Na^+, which is pumped from the cell back to the blood, at a 2:3 ratio, through the Na^+/K^+ ATPase pump (**Fig 4.2B**) . On the luminal side, the movement of K^+ out of the cell occurs through ROMK by passive forces (permeability and electrochemical gradients to the K^+) while Na^+ moves into the cell through the ENaC channels due to the concentration gradient which is caused by Na^+/K^+ ATPase pump (**Fig 4.2 B**). The Na^+/K^+ ATPase pump's most vital responsibilities is preventing cells from swelling

or shriveling. It must be noted that if the Na^+ is not pumped out of the cell, water builds up within it, causing the cell to swell and eventually burst[23].

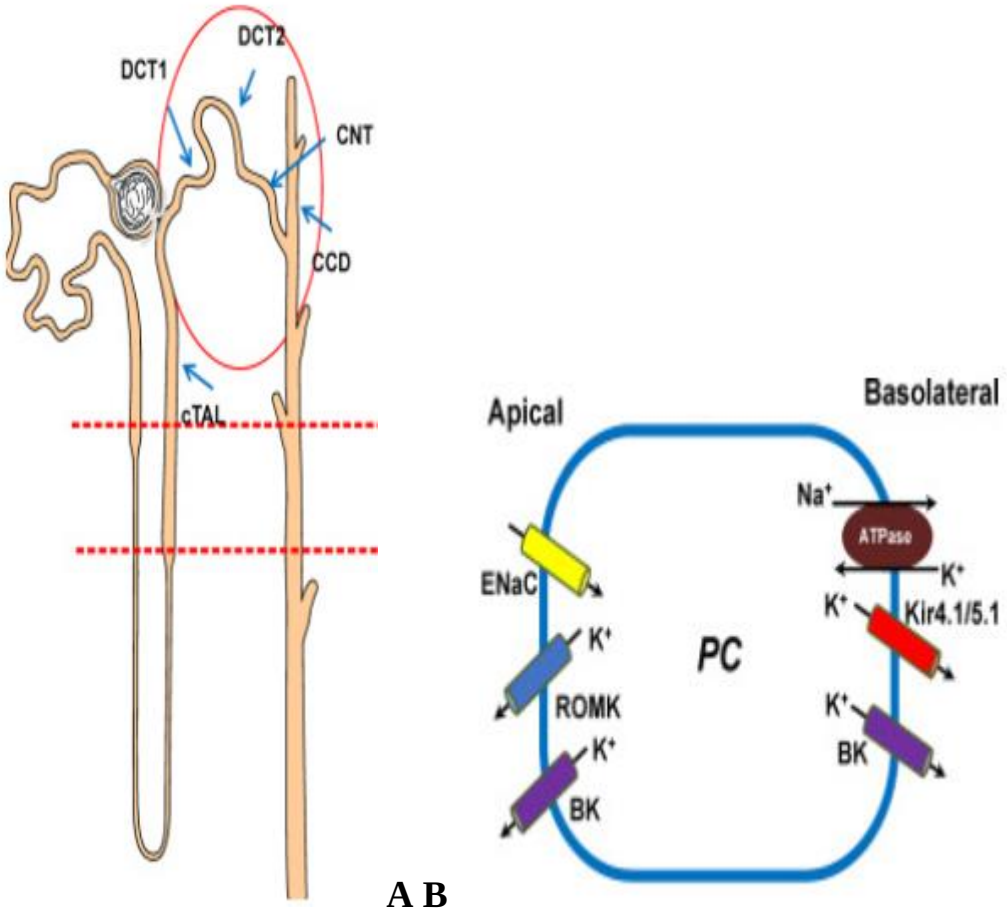

Fig 4.2. (A) Principal cells (PC) are located in distal convoluted tubule segments DCT1 and DCT2, connecting tubule (CNT), and cortical collecting duct (CCD). (B) The process of moving Na^+ and K^+ ions across the principal cell membrane. The extracellular fluid has a higher concentration of Na^+ and lower concentration of K^+ than the principal cells. The extra positive charges in the intracellular fluid are decreasing by moving two K^+ ions to the cell and moving out three Na^+ ions to the extracellular fluid.Figure taken from[16].

4.2. Objective

The complexity of Na^+ and K^+ regulation, their interaction, the effect of hormonal factors such as ALDO, renin, pharmacology, and pathophysiology make it very challenging to investigate

the integrated effects of these factors on Na^+ and K^+ alterations. To deal with these challenges, mathematical models can use the available experimental data and physiology knowledge to help in better understanding these interactions.

In Aim II, a nephron-based model of potassium regulation was developed and validated in order to interpret the consequences of pharmacology and pathophysiology on plasma K^+ levels and to provide information of drug safety (e.g. preventing hyperkalemia) in monotherapy by MR antagonist spironolactone[51, 58].

Previously, a model of renal function and systemic hemodynamics was developed and validated to present a comprehensive computational model that simulates the complex interactions between the kidneys and the cardiovascular system[54]. The model was designed to simulate how blood pressure is regulated through the renin-angiotensin-aldosterone system (RAAS) and how blood volume and Na^+ equilibrum affect the model outputs (e.g. blood pressure). This model describes key processes of kidney function that regulate Na^+ and blood pressure. These processes include blood filtration by the kidneys, the reabsorption of Na^+ and water, the release of renin by the kidneys, and the production and breakdown of hormones such as angiotensin II (ANG II). The model is able to predict blood pressure alteration and the dynamic regulation of glomerular filtration rate (GFR)[54, 55]. The model provides a tool for better understanding the complicated interactions that maintain blood pressure homeostasis and kidney function which lead to development of new treatments for hypertension and kidney disease conditions. However, while this model included a crude effect of aldosterone on sodium reabsorption, it did not include mechanisms of potassium regulation and homeostasis, and thus could not be used to evaluate potential effects of therapies on plasma K^+ and related drug safety concerns.

From physiology, it is well established that K^+ and Na^+ regulations are tightly linked together and involve several hormones and mechanisms (e.g. ALDO and renin) working together to maintain a proper balance. An imbalance in either Na^+ or K^+ levels can lead to various health problems, including high blood pressure, muscle weakness, heart arrhythmias, and hyperkalemia. Before the K^+-ALDO model developed in Aim 2, there was no systemic model that explained the integrated mechanisms of renal function, hormonal response (e.g. ALDO), diseases, diets, and therapies impact on K^+ alteration in our body.

Consequently, the first objective of the research presented in this chapter was to combine the K^+- ALDO model developed in Aim 2 and the previously published Na^+/water homeostasis model [54], in order to describe the interaction in Na^+ and K^+ regulations and to allow prediction of the effects of drug therapies and their combinations therapies on both K+ and Na+ homeostasis, and the resulting consequences, including changes in plasma electrolyte concentrations and blood pressure. A quantitative framework that mechanistically combines K^+- ALDO homeostasis and Na^+/ blood pressure homeostasis may assist in better evaluation of both the safety and efficacy of novel therapeutics and drug combinations and also fully understanding the precise mechanism of interactions between K^+ and Na^+. Thus, a second objective of this chapter was to utilize this integrated model to investigate the impact of therapies on K^+ alteration. In addition to mineralocorticoid receptor antagonists (MRA), the effects of Sodium-Glucose Cotransporter inhibitor (SGLT2i) on potassium homeostasis are of particular interest because these drugs have recently been demonstrated to have beneficial effects on cardiovascular and renal outcomes [97] in patients with heart failure and with chronic kidney disease (CKD), and thus they are being increasingly used in the clinic to treat these patients. All of these patients are at increased risk for hyperkalemia. Many of these patients are also on other therapies, including MRA antagonists, that

further increase their risk of hyperkalemia. However, the specific and integrated effects of the SGLT2i on K^+ homeostasis are not well understood. By investigating the effects of these drugs on K^+ homeostasis via the integrated model and evaluating the risk of hyperkalemia, better management in drug safety and efficacy in patients with diabetes and heart disease may be possible.

4.3. Methods

4.3.1.Model Integration

To integrate the K^+-ALDO model into the cardiorenal model, several modifications needed to be applied in both models. **Fig 4.3** shows the schematics of both models. The areas that were changed are numbered in the **Fig 4.3** and described in **Table 4.1**.

Table 4.1. Required changes to integrate K^+-ALDO and cardiorenal model.

Changes	Cases
1. Linking constants in previous models to dynamic variables in integrated model	1. GFR 2. Plasma Na^+ 3. Blood Volume; Interstitial Fluid Volume
2. Changes to tubular K^+ and Na^+ transport	1. Na^+ fluxes across tubular cells 2. Intracellular Na^+ in tubular cells 3. Coupled transport in Na^+/K^+ ATPase
3. Changes to aldosterone regulation	1. Na^+ intake effect is replaced by effect of plasma Na^+ 2. Modified Ang II effect 3. Add osmolality effect

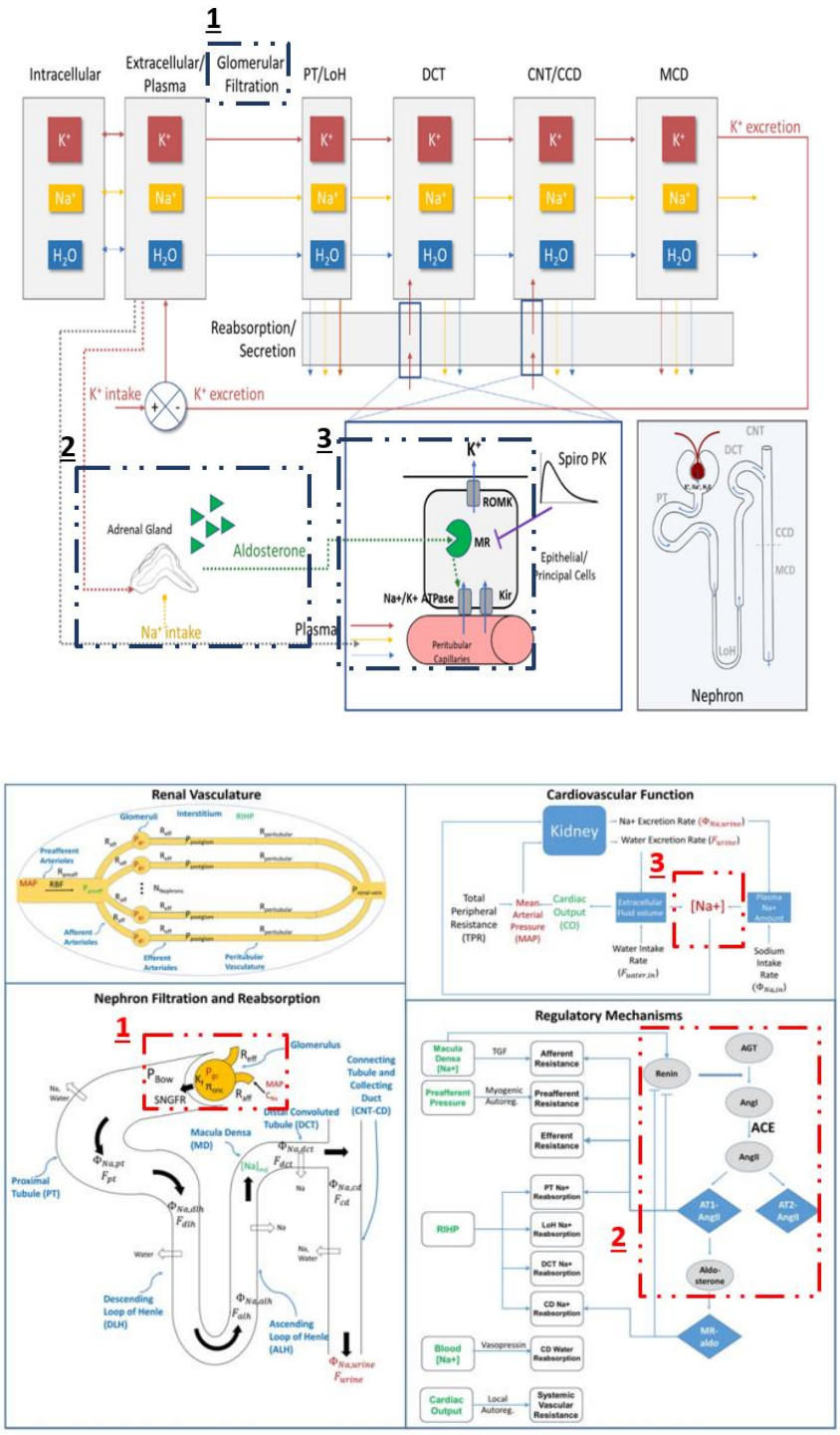

Fig4.3. The numbered sections in the scehematics of K^+-ALDO model (above) were modified by using the highlighted sections that previously were developed in the cardiorenal model (below). The highlighted sections of the cardiorenal model include dynamic variation of GFR and plasma Na^+ and RAAS (renin effect on ALDO). Figures are from[51, 54]

4.3.2. Linking constants in previous models to dynamic variables in integrated model

4.3.2.1. Glomerular filtration rate (GFR)

In the K^+-Aldo homeostasis model (chapter 2, Eqs 1, 2), GFR was a fixed parameter that used to calculate filtered K^+ and Na^+ through the glomerulus. As the highlighted schematic of cardiorenal model, **Fig 4.3** and **Fig 4.4,** shows, GFR is a dynamic variable which depends on the balance of starling forces across the capillary wall, given in Eq 36.

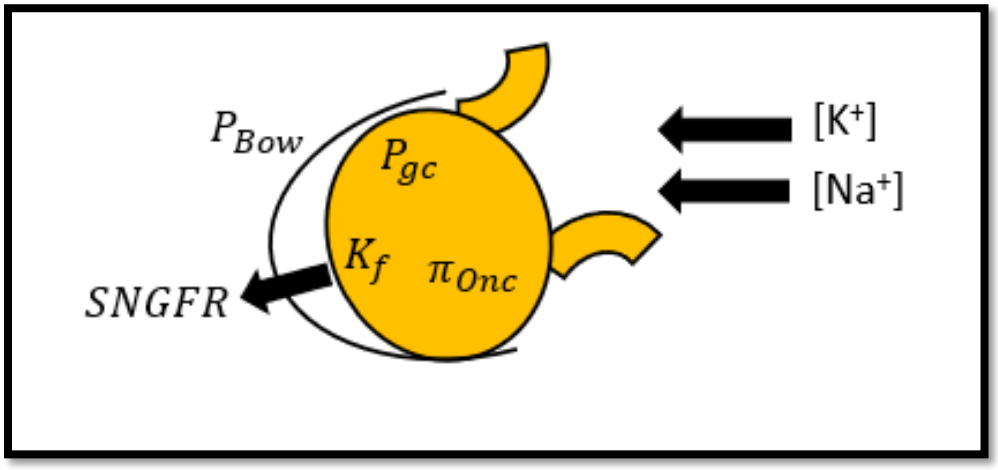

Fig4.4. The single nephron glomerular filtration rate(SNGFR) in the glomerulus is the function of oncotic pressure (π_{Onc}), glomerular capillaries pressure (P_{gc}), Bowman space pressure (P_{Bow}), and filtration coefficient (K_f).

$$GFR = N_{nephrons} K_f (P_{gc} - P_{Bow} - \pi_{go-avg}) \quad (36)$$

K_f is the glomerular membrane ultrafiltration coefficient, and $N_{nephrons}$ stands for number of nephrons. P_{gc} is glomerular hydrostatic capillary pressure, P_{Bow} is the hydrostatic pressure in the Bowman's space, and π_{go-avg} is the average glomerular capillary oncotic pressure. These pressures are all calculated dynamically in the cardiorenal model. P_{gc} is calculated as a function of MAP, renal blood flow, and preglomerular vascular resistances. P_{Bow} is calculated as a function of tubule dimensions flow rates through the tubule. π_{go-avg} is calculated as a function of blood protein

concentration. Modeling of GFR is comprehensively described in the Na^+/water homeostasis model [54].

By integration of two mathematical models of K^+-Aldo and Na^+/Water, we used GFR calculated from Eq 36 from the cardiorenal model which was developed based on the Starling's equation[54], as an input into Eq. 1 and 2 in chapter 2.

4.3.2.2. Plasma Na^+

Previously, in developing of K^+-ALDO model (chapter 2), the plasma Na^+ was considered as a fixed parameter (139 mEq/l). In the integrated model, Na^+ concentration is treated as a dynamic variable (highlighted in **Fig 4.3 below**) and as an input into Eq 2 in chapter 2.

4.3.2.3. Blood volume and interstitial fluid volume

Previously, in K^+-ALDO model, the plasma K^+ was calculated using the total extracellular volume (blood volume plus interstitial fluid volume) fixed to a nominal value (15 L). However, blood volume and interstitial fluid volume in integrated model are dynamic variables that are the function of water intake, urine flow rate, and blood interstitium flux which was developed in the cardiorenal model[54]. These dynamic variables are now used to calculate plasma and interstitial K^+ and Na^+ concentration.

4.3.3. Changes to Na^+ and K^+ tubular fluxes

4.3.3.1. Na^+ fluxes across tubular cells

The schematic of principal/epithelial cells is illustrated in **Fig 4.5**. and previously were highlighted in **Fig 4.3 (above).** To integrate the K^+-ALDO and cardiorenal model, all K^+ and Na^+ fluxes needed to be calculated mechanistically. K^+ movement through the BK channel is negligible in compare of K^+ secretion through the ROMK[50]. Therefore, we assumed all K^+ secretion is

occurring through the ROMK channels. Moreover, the Na^+ movement through the Na^+/H exchanger occurspassively. Therefore, we did not model H and assume the Na^+ moves through the transporter due to the concentration gradient.

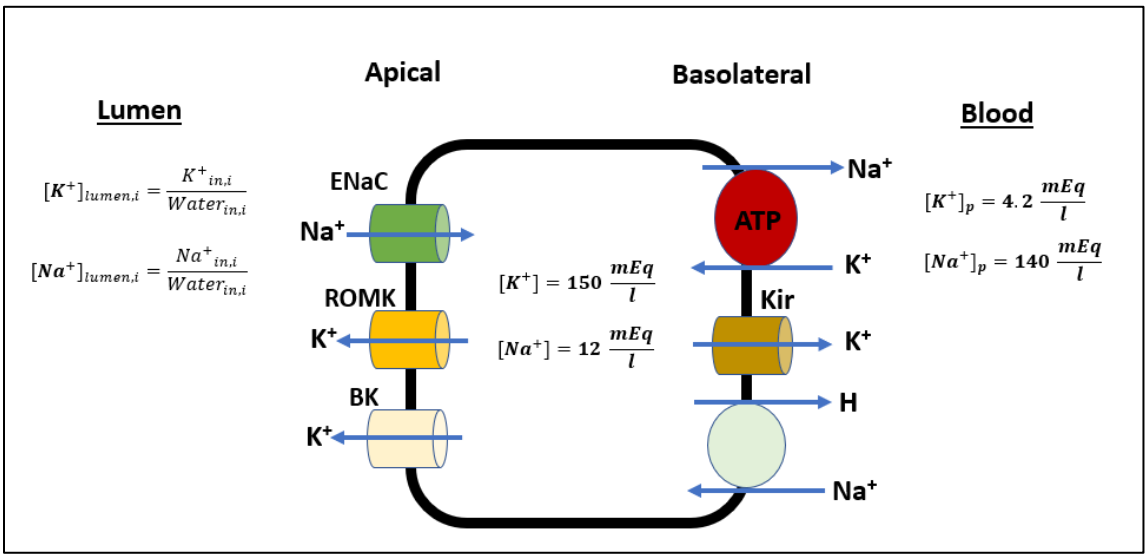

Fig4.5. Fluxes that go through the epithetlial/principal cell transporters determine the K^+ and Na^+ intracellular concentration. Fluxes through the transporters are the function of concentration gradient.

As illustrated in **Fig 4.5**, Na^+ enters the epithelial/principal cell from the luminal side through ENaC channels, enters from the basolateral side through Na^+/H^+ exchangers, and leaves the basolateral side through the Na^+/K ATPase.

Previously, epithelial/principal cell intracellular Na^+ concentration was fixed to a constant. In the integrated model, intracellular Na^+ concentration is determined dynamically based on the fluxes of sodium into the cell passively through ENaC on the apical side ($J(Na^+)_{Enac,i}$) and through Na^+/H^+ on the basolateral side ($J(Na^+)_{passive,basolateral\ i}$), and sodium actively pumped out of the the cell through Na/K^+ ATPase ($J(Na^+)_{active,i}$):

$$\frac{d([Na^+]_{intracellular,i})}{dt} = \frac{1}{SV_i}(J(Na^+)_{Enac,i} + J(Na^+)_{passive,basolateral\,i} - J(Na^+)_{active,i}) \quad (37)$$

SVi is the nephron segment volume to surface area ratio (i=DCT, CNT, and CCD)[48-50].

The following Eq38. and Eq39. are used to calculate fluxes through ENaC and Na^+/H. The passive flux of Na^+ into the cell via ENaC channels on the luminal side, denoted by $J(Na^+)_{ENaC,i}$, depends on the Na^+ concentration difference between the fluid in the lumen and in the cell:

$$J(Na^+)_{Enac,i} = h_{luminal,Na,i}([Na^+]_{luminal,i} - [Na^+]_{intracellular,i}) \quad (38)$$

$h_{luminal,i}$ is the luminal permeability, and essentially represents the density of ENaC channels and the permeability of a single ENaC channel; i is the nephron segment (DCT, CNT, CCD).

The passive Na^+ flux moving from the blood into the cell through the Na^+/H^+ exchanger on the basolateral side, given by $J(Na^+)_{passive,\,basolateral,i}$, depends on the Na^+ concentration difference between the cell and the plasma:

$$J(Na^+)_{passive,basolateral\,i} = h_{basolateral,Na,i}([Na^+]_{Plasma} - [Na^+]_{intracellular,i}) \quad (39)$$

$h_{basolateral,i}$ is the basolateral permeability, and essentially represents the density of Na^+/H^+ channels and the permeability of a single Na^+/H^+ exchanger(for Na^+); i is the nephron segment (DCT, CNT, CCD).

The flux of Na^+ leaving the cell through the Na^+/K^+ ATPase is calculated similar to Eq 12. in chapter 2, but now the intracellular Na^+ calculated dynamically (see below) rather than treated as a constant: This ion movement through the transporter is developed based on the Michaelis-Menten equation, which relates the rate of transport to the concentration of substrate and the maximal transport rate [98].

The flux of potassium and sodium ions through the Na^+/K^+ ATPase is also influenced by the electrochemical gradients across the cell membrane. For each 3 ions of Na^+ that leave the cell, 2 ions of K^+ enter the cell. Concequently the K^+ flux entering the cell (Eq. 12 in Chapter 2) is 2/3

of the Eq 40, and has the opposite sign [50].

$$J(Na^+)_{active,i} = [J(Na^+)_{active}]_{max,i} \left[\frac{[Na^+]_{intracellular,i}}{[Na^+]_{intracellular,i}+K_{Na^+,i}}\right]^3 \left[\frac{[K^+]_{plasma}}{[K^+]_{plasma}+K_{K^+}}\right]^2 \quad (40)$$

$J(Na^+)_{active,max}$ is the maximum Na^+ flux and K_{Na} and K_K are the concentrations of Na^+ and K^+, respectively that produce half the maximum flux and were calculated according to Eq. 13 and 14 in chapter 2.

As shown in **Fig4.5** and can be seen from Eqs 48- 50, at steady state the passive fluxes of Na^+ entering the cell through ENaC and Na^+/H exchanger must be equal to the Na^+ active flux that leaves the cell through the Na^+/K^+ . The steady-state active flux has been determined in previous careful cell-based modeling studies of K^+/Na^+ transport by Alan Weinstein which calculated these fluxes in the rat at steady state for the DCT, CNT, CCD [48-50]. Rat values for active fluxes were used as the initial values and then were adjusted to produce the steady state value for plasma K^+(4.2 mEq/l) and intracellular K^+ (150 mEq/l), in the absence of hormonal feedback. Here, we assumed that the calculated fluxes in the rat studies are proportional to fluxes in humans. From the values calculated in these studies, then, the proportions of sodium entering the cell from the luminal side, $u_{luminalNafrc,i}$ for DCT, CNT, and CCD, were calculated to be 0.71, 0.9, and 0.82 respectively.

$$u_{luminal,Na,i} = \frac{J(Na^+)_{Enac,i}}{J(Na^+)_{Enac,i} + J(Na^+)_{passive,basolateral\,i}} = \frac{J(Na^+)_{Enac,i}}{J(Na^+)_{active,i}} \quad (41)$$

With this assumption, the steady-state values for lumininal permeability through ENaC $h_{luminal,Nai}$ can be calculated by combining Eq. 38 and Eq. 40 and solving for h:

$$h_{luminal,Na,i} = \frac{\mu_{luminalNafrc,i}[J(Na^+)_{active}]_{nom,i}}{[Na^+]_{luminal,i}-[Na^+]_{nomintracellular,i}} \quad (42)$$

Similarly, the steady-state value for basolateral permeability through Na^+/H^+ transporters

is given by:

$$h_{basolateral,Na,i} = \frac{(1-\mu_{luminalNafrc,i})[J(Na^+)_{active}]_{nom,i}}{[Na^+]_{plasma}-[Na^+]_{nomintracellular,i}} \quad (43)$$

Also, $J(Na^+)_{active,nom,i}$ are the nominal fluxes for each segments using baseline values.

4.3.4. Changes to ALDO regulation

4.3.4.1. Na^+ intake effect on ALDO replaced by plasma Na^+ effect

Previously, in Aim 2, control of ALDO was modeled as a function of plasma K^+ and Na^+ intake. The use of Na^+ intake was an approximation – in reality, changes in Na^+ intake lead to changes in plasma Na^+, and this is what is actually sensed by cells of the adrenal gland that secrete ALDO. In the Aldo-K^+ model, this was a necessary simplification because the model did not dynamically track plasma Na^+ concentration. However, because the cardiorenal model is able to track and calculate changes in the plasma Na^+ concentration with changes in Na^+ intake, the integrated K^+-ALDO homeostasis and cardiorenal QSP model can use plasma Na^+ concentration directly to drive ALDO secretion. The ALDO equation was modified by adding a dynamic plasma Na^+ concentration effect. Different forms of this equation (linear, sigmoidal, and exponential) were evaluated during the calibration process (described later), and the following equation structures were found to best fit the experimental data[66, 70, 80].

$$[Na^+]_{effect} = e^{m_{Na,Aldo}([Na_{p,0}]-[Na]_p)} \quad (44)$$

$[Na^+]_{effect}$ describes how plasma Na^+ affects plasma ALDO concentration (see Eq. 49 below). $[Na^+]_p$ is the time-varying plasma Na^+, and $[Na^+]_{p,0}$ is the normal values for plasma Na^+ .concentrations, respectively.

4.3.4.2. Modification of plasma K^+ effect on ALDO

Previously, in K^+-ALDO model, the plasma K^+ effect on ALDO (Eq 25, chapter2) was modeled as an exponential function. In calibrating the integrated model, the plasma K^+ effect on ALDO modeled as a linear function was found to improve the fit to experimental data.

$$[K^+]_{effect} = m_{K,Aldo}([K^+]_p - [K^+]_{p,0}) \quad (45)$$

$[K^+]_{effect}$ describes how plasma K^+ affects plasma ALDO concentration. $[K^+]_p$ is the time-varying plasma K^+, and $[K^+]_{p,0}$ is the normal value for plasma K^+ concentrations, respectively.

4.3.4.3.Update of angiotensin effect on ALDO

The renin-angiotensin-aldosterone system (RAAS) is a feedback system that regulates blood pressure [54, 99]. The RAAS pathway and its effects on sodium regulation are described in detail in the Na^+/water homeostasis model [54]. Also, more information about the submodel and parameters is available in appendix D.

That cardiorenal model already includes an effect of Angiotensin II on ALDO secretion (**Fig 4.3** below): However, in the integrated model, this equation needed to be updated. As described later in the calibration section, the following functional form was found to best describe the available data:

$$[Ang\ II]_{effect} = e^{m_{AT1,aldo}(\text{AT1}-\text{bound}_{\text{AngII}}-\text{AT1}-\text{bound}_{\text{AngII}_0})} \quad (46)$$

$m_{AT1,aldo}$ is the fitting constant for the effect of angiotensin II on plasma ALDO. AT1-$bound_{AngII}$ is the time-varying complex of Angiotensin II bound to the AT1 receptor and AT1-$bound_{AngII,0}$ is the normal value (Appendix D).

4.3.4.4. Addition of osmolality effect on ALDO

ALDO is affected by plasma osmolality [100, 101]. Na^+ and K^+ are the significant cations in the blood [102]. Glucose, in contrary, is a main source of energy for the body and is present in the blood at variable concentrations depending on factors such as diet and insulin levels[103]. Osmolality is estimated as the concentration of glucose, Na^+ and K^+ in the fluid part of blood. The osmolality effect on ALDO is modeled as the following equation.

$$[Osm]_{effect} = e^{m_{osm,aldo}([\mathrm{Osm}]_0 - [\mathrm{Osm}])} \quad (47)$$

$m_{osm,aldo}$ is the slope of osmolality relationship and ALDO. [Osm] is plasma osmolality concentration which is calculated as below.

$$[Osm] = [Glucose^+]_p + 2([Na^+]_p + [K^+]_p) \quad (48)$$

Therefore, the modified equation of ALDO that is utilized in the integrated model is described as below.

$$[Aldo] = [Aldo]_0\big(\big(1 + ([Ang\ II]_{effect} - 1\big) + \big([K^+]_{effect} * [Osm]_{effect} - 1\big) + \big([Na^+]_{effect} - 1\big)\big) \quad (49)$$

$[ALDO]_0$ is the baseline value for the plasma ALDO.

4.3.5. Aldosterone effect on Na^+ and K^+ transporters in nephron epithelial and principal cells

To regulate salt, water and K^+ homeostasis, ALDO increases the activity of the epithelial Na^+ (ENaC) channels and the renal outer medullary K^+ (ROMK) in the distal tubules of the kidney. This stimulation leads to increase in Na^+ reabsorption and from the filtrate back into the blood. This effect is modeled using the following equation:

$$Aldo_{ENaC} = 1 + m_{ENaC,aldo}(Aldo_{MRA} - 1) \quad (50)$$

$m_{ENaC,aldo}$ is a fitting constant for the ALDO effect on ENaC channels and $Aldo_{MRA}$ represents ALDO interactions with the MR receptors. Eq. 50 is multiplied by the defined equations for Na^+ reabsorption in DCT, CNT, and CCD in Na^+/ water homeostasis model [54].

$$J_{ENaC,i} = Aldo_{ENaC} h_{luminal,i}\left([Na^+]_{lumen,i} - [Na^+]_{intracellular,i}\right) \quad (51)$$

$Aldo_{ENaC}$ is the ALDO effect on ENaC channels of nerphron segments, $h_{luminal,i}$ is the luminal permeability, and essentially represents the density of ENaC channels and the permeability of a single ENaC channel; i is the nephron segment (DCT, CNT, CCD).

ALDO also enhances the ROMK channels activity, which are responsible for secreting K^+ ions from the cell back into the filtrate fluid. This effect is achieved by stimulating transporters located on the apical membrane of the cells (**Fig 4.6**)

$$Aldo_{ROMK} = 1 + m_{ROMK,aldo}(Aldo_{MRA} - 1) \quad (52)$$

$m_{ROMK,aldo}$ is the fitting constant of ALDO effect on ROMK channels. Eq. 52 is multiplied by the defined equations for the passive K^+ secretion in DCT, CNT, and CCD in K^+-ALDO homeostasis model (Eq. 7 in chapter 2)[51].

$$J_{L,i} = h_{L,i} * \vartheta_L * Aldo_{ROMK} * \left[\frac{[K^+]_{cell,i}e^{-\vartheta_L} - [K^+]_{lumen,i}}{1-e^{-\vartheta_L}}\right] \quad (53)$$

h_L is the luminal cell membrane permeability to K^+ through ROMK channels, $[K]_{cell}$ and $[K]_{lumen}$ are the intracellular and luminal K^+ concentrations, respectively, and ϑ_L is the normalized membrane potential. $Aldo_{ROMK}$ is the ALDO effect on ROMK channels of nerphron segments.

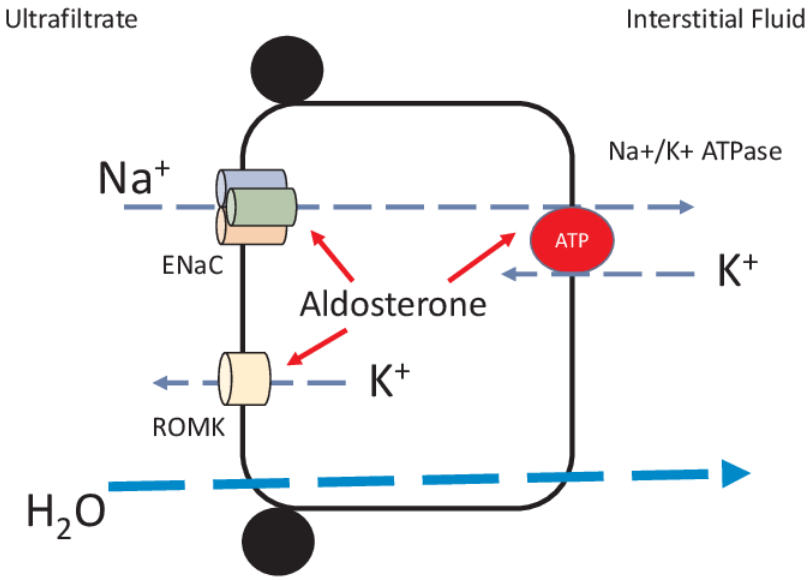

Fig4.6. Aldosterone targets crucial ion transporters to regulate the overall electrolyte balance in the body. Specifically, aldosterone increases the activity of ENaC, Na^+/K^+ ATPase, and ROMK channels, which results in the conservation of Na^+ and the secretion of K^+. This regulation is essential for maintaining proper electrolyte levels in the body. Figure is from [104].

4.3.6. Experimental data

4.3.6.1. Calibration data

4.3.6.2. Calibration of transport and feedback mechanisms

To determine and recalibrate the mechanisms of K^+-ALDO feedback and RAAS feedback in the integrated model, we used the same experimental data used to calibrate the K^+-ALDO homeostasis model in Chapter 2, as well as one additional expeimrental study. The data from Dluhy et al included measurements of plasma K^+ and plasma ALDO during a 2-hour K^+ infusion followed by 3-hour recovery in 4 groups that receive different amounts of K^+ and Na^+ in their diets[70].

Because the integrated model also included effects of Angiotensin II on ALDO, an additional study by Williams et al was used. This study measured plasma ALDO, plasma renin activity, and plasma K^+ concentration in healthy subjects who had received constant amounts of 100 mEq K^+ intake and either 10 mEq or 200 mEq Na^+ intake for 3 consecutive days [66].

4.3.6.2.1. Calibration of Spironolactone response

To recalibrate the pharmacologic effects of MRA in the integrated model, the same study used in Chapter 3 for the K^+-ALDO model was used to calibrate the MR antagonist pharmacologic effect in the integrated model model. This study measured acute response toospironolactone, including urinary Na^+ and K^+ excretion for 2-10 hrs and 12-16 hrs, in healthy subjects that received the different doses of spironolactone (placebo, 25 mg, 50 mg, 100 mg, 200 mg, and 400 mg)[80]. While in Chapter 3, only the the K^+ excretion data could be utilized, in this analysis we are also able to utilize the Na^+ excretion data to constrain the model, since the integrated model should be able to reproduce the Na^+ dynamic behavior.

4.3.6.2.2. Calibration Approach

The integrated model was calibrated by simultaneously fitting the experimental studies described above using the least squares method. The studies were selected due to containing acute data which are perturbed by diets, K^+ infusion and, drug administration in healthy humans. This makes the data eligible to calibrate the developed feedback of K^+, ALDO, RAAS, and the mechanism of MRA action that alters ALDO and indirectly affects Na^+ and K^+ levels.

4.3.6.3. Validation data

Similar to the validation of the K^+/Aldo model described in Chapter 3, the model was validated against the experimental study by Karagiannis et al [83] of spironolactone administration-response in patients with hyperaldostronism. In addition to validating with plasma K^+ data, the model's ability to predict observed blood pressure changes with spironolactone was also tested, since the integrated model is able to simulate mean arterial pressure (MAP).

A virtual patient simulation was utilized to match baseline characteristics in the model, including MAP and GFR, to the clinical data. The virtual patients simulation was conducted using a previously described method [106] by applying disease effects associated with hyptertension such as loss of glomeruli, loss of nephrons, and reduced glomerular permeability.

4.4. Results

4.4.1. Steady-state results

The integrated model is parametarized to produce a state(**Fig 4.7**) in which plasma and intracellular values of electrolytes, ALDO, MAP, and GFR are stable at their normal levels[9].

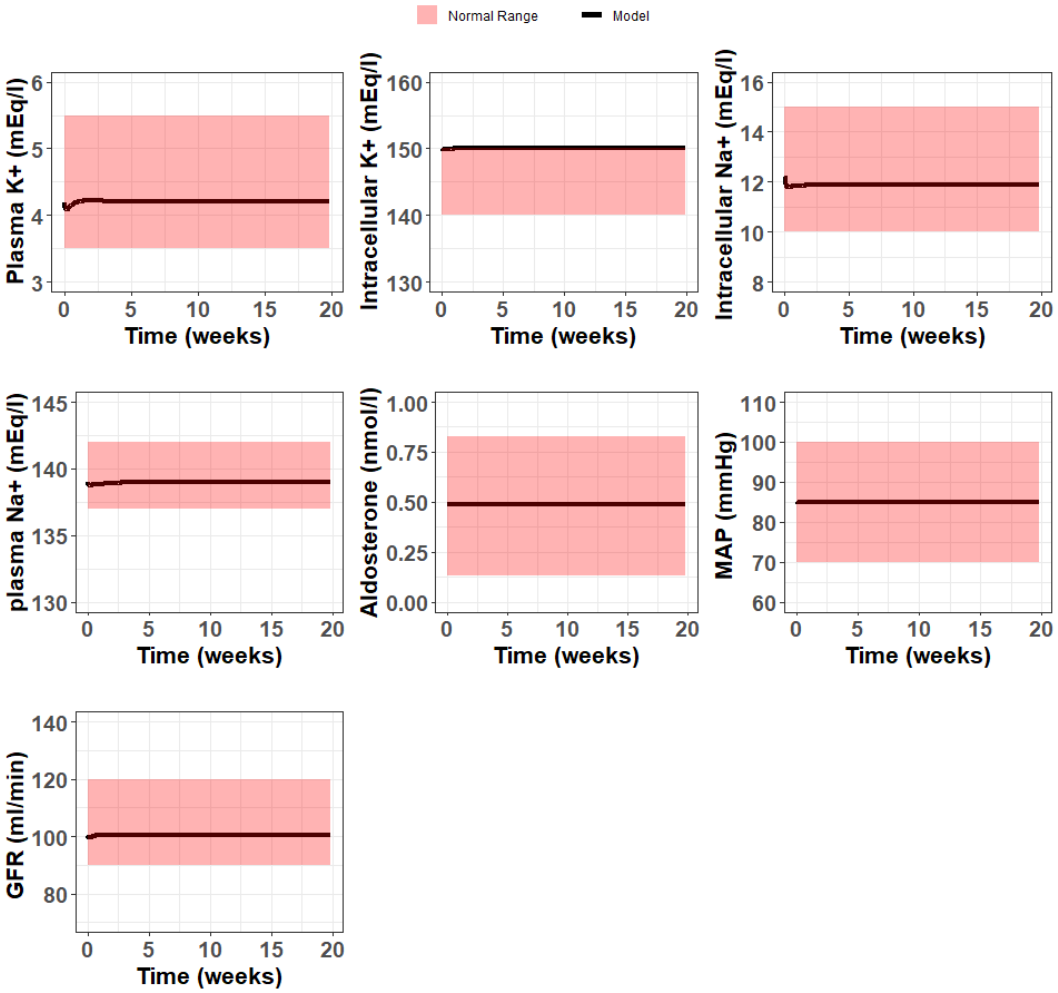

Fig 4.7. Key model variables are stable at steady state and fall within the normal ranges in physiological references [9].

4.4.2. Model calibration

4.4.2.1. Recalibration of aldosterone feedback mechanisms

Estimated parameters for the effects of plasma K^+, plasma Na^+, angiotensin II, and plasma osmolality on ALDO were fit to experimental data from Dluhy et al. [70], and Williams et al. [66]. The estimated parameters values are presented in Table 4.6. As shown in Fig **4.8**, the integrated model still reproduces the responses to diet alteration (K^+ and Na^+ intakes) and K^+ infusion on plasma K^+ and plasma ALDO in Dluhy et al. [70].For high K^+/high Na^+ and low K^+/low Na^+ intakes ,we can see an overestimation and underestimation of plasma K^+, respectively (**Fig 4.8**).

One possible explanation for this observation could be that the model is accurately producing the regulatory effects of ALDO on Na^+ and K^+ levels, but is not capturing the full complexity of the interplay between these ions and other regulatory factors in the renal system.

Since the integrated model accounts for the effects of changes in sodium intake on the RAAS, and effects of RAAS on ALDO, the model should be able reproduce the ALDO and renin alterations in response to diet. **Fig 4.9** presents the model response to changes in sodium intake for 3 consecutive days. After calibration, the model reproduces the observed changes in plasma K^+, plasma ALDO, and renin concentration in Williams et al. [66]. When Na^+ intake is low, renin secretion increases, which in turn increases plasma ALDO. On the other hand, high Na^+ intake decreases renin secretion and increases ALDO. In both cases, though, the system is able compensate to keep plasma K^+ at normal levels.

Table 4.2. Calibrated parameters for the integrated model

Parameter	*Definition*	*Value*	*Unit*	*%SE*
Aldosterone and sodium effect				
$m_{aldo\text{-}RomK}$	Fitting constant for aldosterone effect on RomK transporters potassium permeability	0.8462	-	1.63%
$m_{ENAC,aldo}$	Fitting constant for aldosterone effect on ENaC transporters sodium permeability	0.152	-	2.11%
$m_{K,aldo}$	Slope of plasma potassium effect on plasma aldosterone	1403.83	L/mEq	0.25%
$m_{K\text{-}P,MCD}$	Fitting constant for effect of plasma potassium on MCD K^+ reabsorption	2.06e-7	-	1.9%
$m_{Na,Aldo}$	Fitting constant for plasma sodium effect on plasma aldosterone	0.001	Min/mEq	1452.64%
$Q_{K\text{-}ic}$	Rate constant for interstitial and intracellular potassium exchange	0.191	L/min	6.57%
Pharmacologic effect				
I_{MAX}	Maximum spironolactone MR inhibition	1	-	0.17%
IC50	Spironolactone concentration that provides 50% of maximum inhibition	5.76	mg	2.58 %
Renin angiotensin aldosterone system (RAAS)				
$AT1_{NKCC}$	Slope of angiotensin effect on NKCC transporters on renal epithelial cells	0.001	-	107.4%
$AT1_{NCC}$	Slope of angiotensin effect on NCC transporters on renal epithelial cells	0.3390	-	3.19%
$m_{AT1,aldo}$	Slope of angiotensin effect on aldosterone	0.0434	-	3.08 %
MD_{Renin}	Slope of macula densa effect on renin	6.489	Min/mEq	1.24%
Osmolality effect on aldosterone				
$m_{osm,aldo}$	Fitting constant for osmolality effect on plasma aldosterone	0.3081	KgH_2O/Osm	2.31%

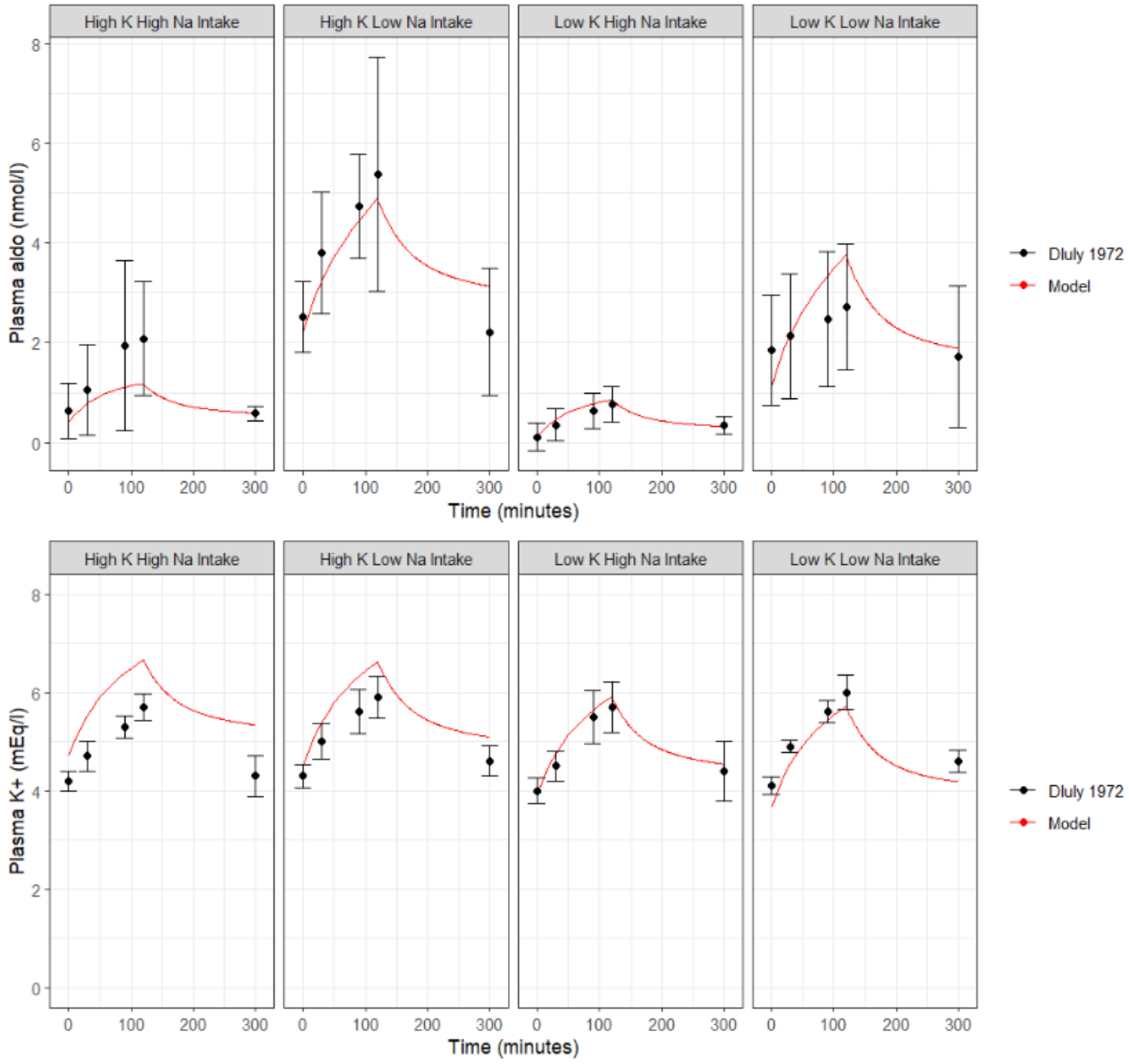

Fig 4.8. The integrated model is fitted to observed aldosterone concentrations (top row) and plasma K^+ (below row) to various K^+ and Na^+ intake rates (40 mEq/day,200mEq/day K^+ and 10 mEq/day, 200 mEq/day Na^+) for healthy humans. The utilized data are mean ±SD from [70]. The model is able to reasonably simulate the diet and 2-hour K^+ infusion(0.62 mEq/min) effects on plasma K^+ and aldosterone concentration followed by the 3-hour recovery time.

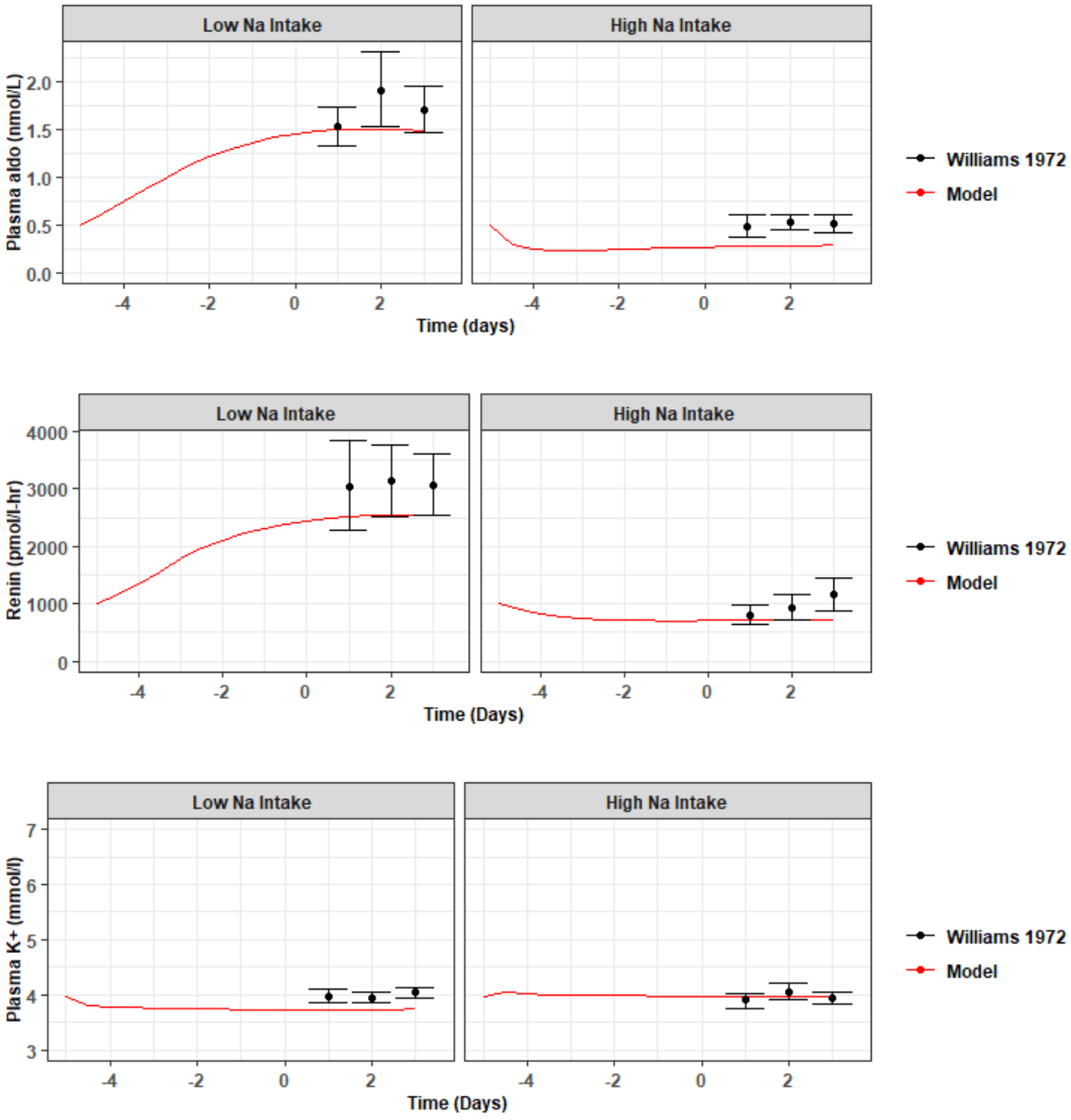

Fig4.9. The integrated model fit to observed plasma aldosterone concentration (top row), plasma renin activity (middle row), and plasma K^+ concentration (bottom row) altered by diet alteration (Na^+ intake 10mEq/day or 200 mEq/day) and 100 mEq/day K^+ intake[66]. The model reasonably reproduced the aldosterone, renin, and K^+ alteration due to the changes in Na^+ intake for 3 consecutive days.

4.4.2.2. Recalibration of the mineralocorticoid antagonists (MRA) pharmacologic effects

Previously, we used acute K^+ excretion data from healthy individuals who were administered different doses of spironolactone, and their urinary K^+ excretion was collected for 2-10 hours and 12-16 hours post-dose[80]. The developed pharmacokinetics (PK) structure of spironolactone that was used in K^+-ALDO model remained unchanged from Aim 2. (Eq 27,32**)**. The pharmacodynamic (PD) structure also remained the same (Eq 33,34) andthe PD parameters were recalibrated using the least square method.

To recalibrate the pharmacodynamic (PD) response for the integrated model, the same data was utilized. In addition, urinary Na^+ excretion data from the same study was included in the recalibration, since the integrated model is also capable of describing Na^+ excretion. As shown in the **Fig 4.10**, the model successfully reproduced the changes in both K^+ and Na^+ excretion after administration of placebo, 25mg, 50mg, 100mg, 200mg, and 400mg doses.

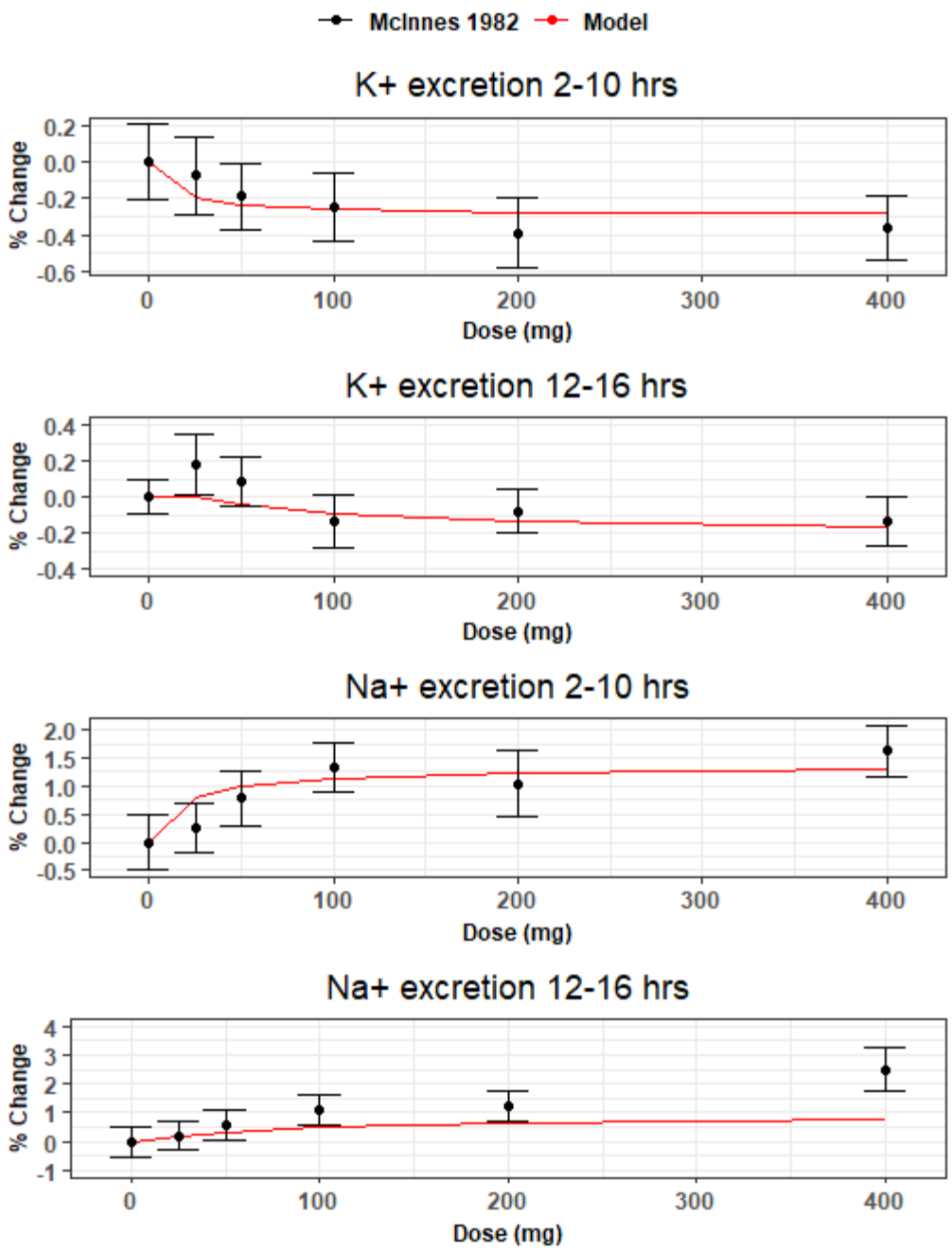

Fig4.10. Integrated model fit to both urinary potassium and sodium excretion dose-response 2-10 hrs and 12-16 hrs following a single dose of spironolactone in n healthy cases from McInnes et al.[80]

4.4.3. Model validation

4.4.3.1. Chronic mineralocorticoid antagonism response in hyperaldosteronism

The same clinical data used to validate K^+-ALDO model were used to validate the integrated model[83]. In **Fig 4.11**, the chronic response to spironolactone predicted by the integrated model was compared to the clinical responses observed in patients with hyperaldosteronism. It was able to reproduce the lower baseline plasma K^+ concentration in subjects with hyperaldosteronism. Furthermore, the model also reproduces the rise in plasma K^+ over time with chronic treatment of increasing doses of spironolactone.

However, unlike the K^+-ALDO model, the integrated model has the added capability of predicting changes in mean arterial pressure (MAP). As illustrated in the **Fig 4.11**, the model reasonably predicted the reduction in MAP with increased administration of spironolactone doses.

This is the power of the integrated K^+/Na^+ homeostasis. It is able to simultaneously model drug-induced changes in plasma potassium concentrations, systemic hemodynamic biomarkers like blood pressure, as well as neurohormonal biomarkers such as renin activity (see **Fig 4.9**).

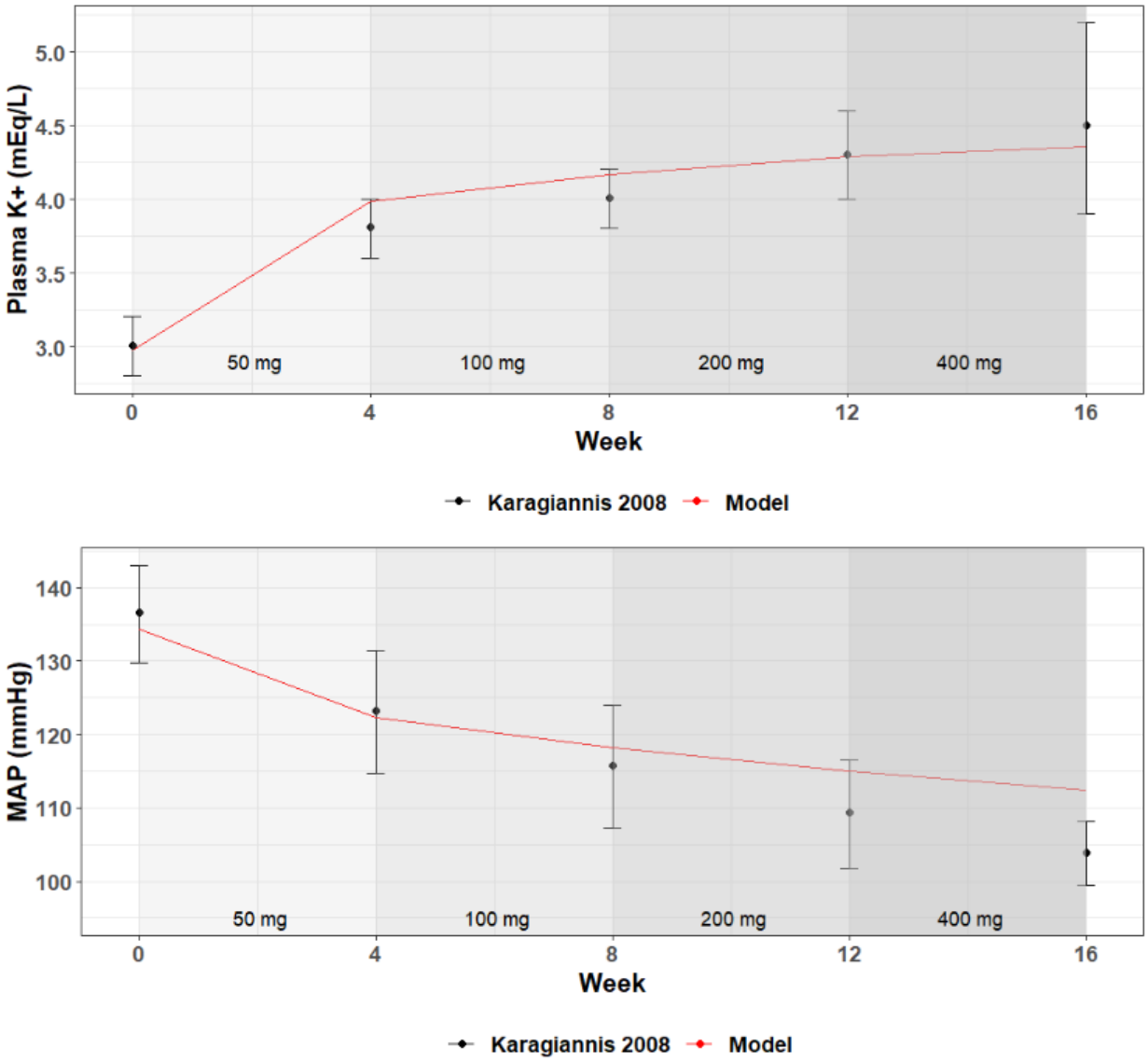

Fig4.11. Model validation: The model predicts the chronic plasma potassium dose-response to elevating doses of spironolactone in hyperaldosteronism cases. The integrated model also reproduces the mean arterial pressure reduction while the spironolactone dose increases. Data are mean ±SD from Karagiannis et al[83].

4.4.4. Model application

The aim of integrating the K^+-ALDO model into the Na^+/water homeostasis model was to provide a comprehensive mathematical framework that can provide a deeper understanding of the complicated interactions between K^+, ALDO, Na^+, and water in the body, especially in response to drug therapies that can have consequences for both efficacy and safety. In Aim 2, the consequences of different spironolactone doses on K^+ concentration were investigated to understand its impact and interaction with alterations in kidney function and/or K^+ intake. However, with the integrated model now calibrated and validated with spironolactone, the next goal was to utilize the model to investigate the K^+ response to SGLT2i in order to mechanistically understand how this new class of therapy may impact K^+ handling and risk of dyskalemia. The effects of SGLT2i were first investigated as a monotherapy under normal conditions, and then under conditions of diabetes and kidney dysfunction. Lastly, the effects of SGLT2i in combination with an MRA antagonist were simulated.

The mechanisms of action of SGLT2i in the integrated model were simulated as previously described in an earlier published mathematical modeling study using the cardiorenal model [106]. This analysis investigated the effects of SGLT2i on sodium and water excretion, but of course was not able to investigate its effects on K^+ handling. This study showed that multiple mechanisms were required to quantitatively describe the renal hemodynamic effects of SGLT2i, including 1) direct inhibition of Na^+ and glucose reabsorption through SGLT2 in the proximal tubule, 2) SGLT2-coupled inhibition of NHE3 Na^+ reabsorption in the proximal tubule, 3) osmotic diuresis due to glucose remaining in the tubule, and 4) pushing of Na^+ into peripheral storage in response to increased free water clearance. Because these mechanisms of SGLT2 and its inhibition were already built into the cardiorenal model in this previous analysis, SGLT2i inhibition could

be simulated simply by setting the inhibtion of SGLT2 transport in the proximal tubule to 80% [106].

4.4.4.1. Investigation of SGLT2i effect on potassium

As described in Chapter 1, Sodium-Glucose Cotransporter 2 (SGLT2) are located in the epithelial cells of the proximal tubule (PT) of the kidney[37]. Glucose in the blood is filtered across the glomerulus into the PT, where it is nearly completely reabsorbed by SGLT2 back into the bloodstream[38]. One consequence of inhibiting SGLT2 on K^+ homeostasis is that glucose remains in the tubule, and since it is osmotically active, it holds water in the tubule as it moves through the distal nephron. Therefore, due to decreased water reabsorption in the distal nephron resulting, concentrations of both K^+ and Na^+ along the tubules may change, and this likely has consequences for potassium reabsorption and secretion, since these processes are driven in part by concetration gradients between the cell and the lumen[36].

A second consequences of SGLT2i, since SGLT2 transporters simultaneously transport both Na^+ ions and glucose molecules, and because function of SGLT2 and NHE3 (the main transporter of Na^+ in the PT) are coupled, inhibition of SGLT2 reduces PT Na^+ reabsorption, resulting in more Na^+ delivered down stream to the distal nephron. This can further alter Na^+ and K^+ concentrations and concentration gradients along the distal nephron. Lastly, because SGLT2 inhibition causes a loss of free water from the body (i.e. water is excreted in excess of sodium), it reduces blood and intertitial fluid volume. Changes in fluid volume will alter plasma interstitial K^+ concentrations separately from any change in K^+ amount. These multiple interrelated effects are difficult to quantitatively predict without a model. In this analysis, by simulating the effect of SGLT2i on potassium transport and homeostasis, the effect(s) of these mechanisms were evaluated.

The total amount of K^+ in the body is the sum of potassium in the blood, interstitial fluid, and intracellular space.

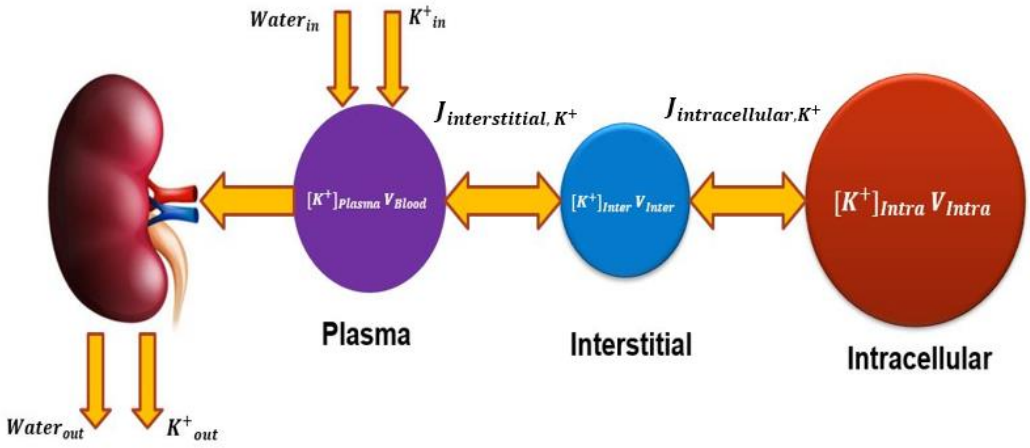

Fig4.12. K^+ movement through the intracellular, interstitial, and plasma volumes, excretion through the renal system. When K^+ concentration elevates in intracellular or plasma, the K^+ moves to environment with lower K^+ concentration.

Changes in total body K^+ over time are determined by the balance between K^+ intake and excretion, and changes in K^+ amount in each compartment are further determined by the fluxes between each compartment, according the following equations:

$$\frac{dK_{intra}}{dt} = J_{intracellular,K^+} \quad (54)$$

$$\frac{dK_P}{dt} = K_{intake} - K_{out} - J_{interstitial,K^+} \quad (55)$$

$$\frac{dK_{inter}}{dt} = J_{interstitial,K^+} - J_{intracellular,K^+} \quad (56)$$

K_{intra}, K_p, and K_{inter} are the K^+ amount in intracellular, plasma, and interstitial environment, respectively. $J_{interacellular,K^+}$ is the flux of K^+ moving between the cells and interstitium, and $J_{interstitial,K^+}$ is the flux moving between the plasma and interstitium. These fluxes are described in detail in section 2.1.2 of chapter2.

Fig 4.14 shows the simulated effect of SGLT2i on plasma K^+ and other dynamic variables over eight weeks of treatment under normal conditions (no diabetes and normal kidney function). As shown in panel A, SGLT2i is predicted to cause a drop in the total amount of K^+ in the blood by the end of 8 weeks. However, as shown in panel B, SGLT2i also causes a drop in blood volume. The net effect is that the final change in plasma K^+ concentration is minimal (panel C). The drop in blood potassium amount is due to a transient increase in K^+ excretion rate (panel G), which eventually returns to baseline (i.e. K^+ balance, where intake equals excretion). Similarly, the drop in blood volume is due to the diuretic effect of SGLT2i (panel H) – water excretion rate transiently increases and then returns to balance over time.

According to Eq. 55 and **Fig 4.12**, the intracellular K^+ alteration depends on the changes in intracellular K^+ flux. The negative value of intracellular flux in plot L and decrease of intracellular K^+ (plot M) shows the K^+ movement from the cell into the blood which explaines the initial increase of plasma K^+ (plot C).

As mentioned in chapter 2, K^+ reabsorption in the PT is proportional to Na^+ and water reabsorption. Therefore, by inhibition of SGLT2 transporters, more K^+ leaves PT, LoH, and MCD (plots D,E and, F in **Fig 4.13**). Any K^+ leaving the MCD is secreted in the urine – thus the spike in MCD K^+ flow rate in panel F indicates increased K^+ excretion. Since K^+_{intake} is constant and excretion increases in the first days, the total K^+ decreases (plot O).

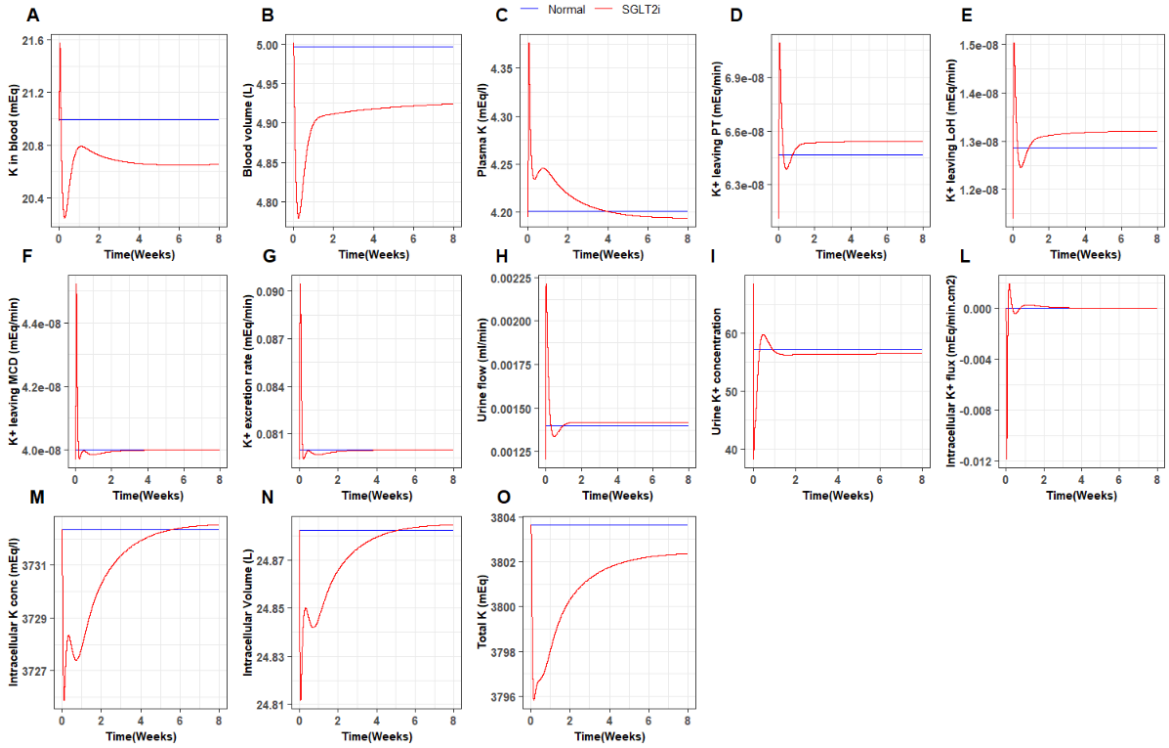

Fig4.13. Simulated changes in variables affected by SGLT2i administration for 8 weeks in under normal conditions. SGLT2i as a diuretic causes blood volume to decrease. In addition, by inhibition of SGLT2, more K^+ is leaving the PT which increases the K^+ excretion and decreases total body potassium. Because both blood volume and blood potassium decrease, the net effect on plasma potassium concentration is minimal.

Fig 4.14 further explores the effect of SGLT2i on plasma K^+, blood volume, and K^+ concetration under normal and diabetic conditions. In both cases, plasma K^+ remains almost equal to normal at 8 weeks without increasing the risk of hypokalemia or hyperkalemia in a diabetic patient([glucose]=8.6 mmol/l).

Although K^+ concentrations are unchanged, total potassium in the blood (Panel A) is reduced in both cases, and more so in the diabetic subject.

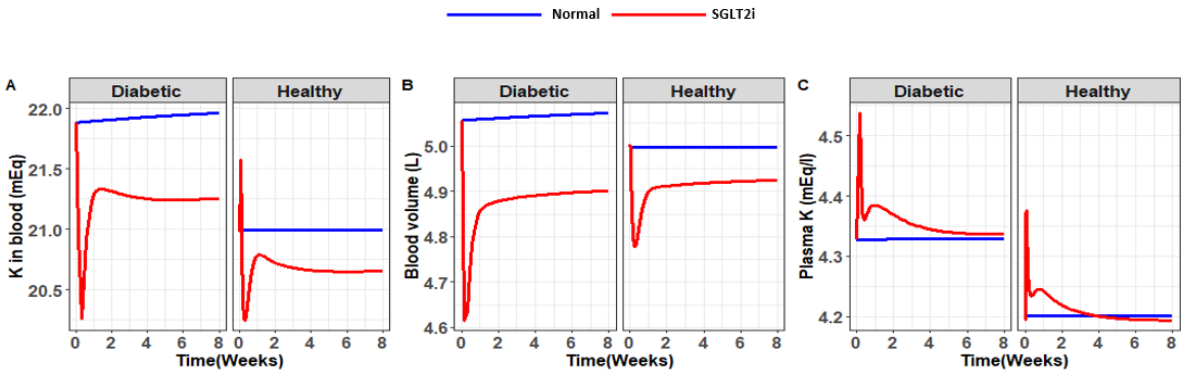

Fig4.14. Simulated changes in blood K^+ amount, blood volume, and plasma K^+ concentration in healthy and diabetic patients treated with SGLT2i for 8 weeks.

The kidney in CKD loses the ability to filter and excrete K^+ sufficiently. Therefore, the hyperkalemia risk tends to increase. As **Fig 4.15** presents, SGLT2i is predicted to decrease the level of total K^+ in both healthy and CKD cases. Note that the simulated baseline K^+ amount in patient with low GFR is higher due to the impaired renal function (GFR=33.13 ml/min, compared to 100 ml/min in healthy subjects) which leads to slightly higher K^+ concentration in patient with CKD. SGLT2i returns the potassium amount to levels similar to healthy subjects. SGLT2i also reduces blood volume, and thus the net effect on plasma K^+ concentration is small, but the model does predict a small decrease in plasma K^+ concentration.

These results are consistent with available clinical data. Several studies have examined the effects of SGLT2i on K^+ levels in patients with CKD. In some studies, SGLT2i therapy was associated with a small decrease in K^+ concentration. In other studies, there were no significant changes or even an increase in K^+ levels[97, 107].

A recent meta-analysis of several clinical trials of SGLT2i, [41, 44], which found that while SGLT2i has no clear effect on average plasma K^+ in populations, SGLT2i administration

appears to decreases the risk of hyperkalemia in diabetic patients and patients with kidney dysfunctions without increasing the hypokalemia risk. Because it lowers total potassium, it may protect against hyperkalemia when other conditions change that tend to favor retension of K^+ for instance, if a subject's renal function declines.

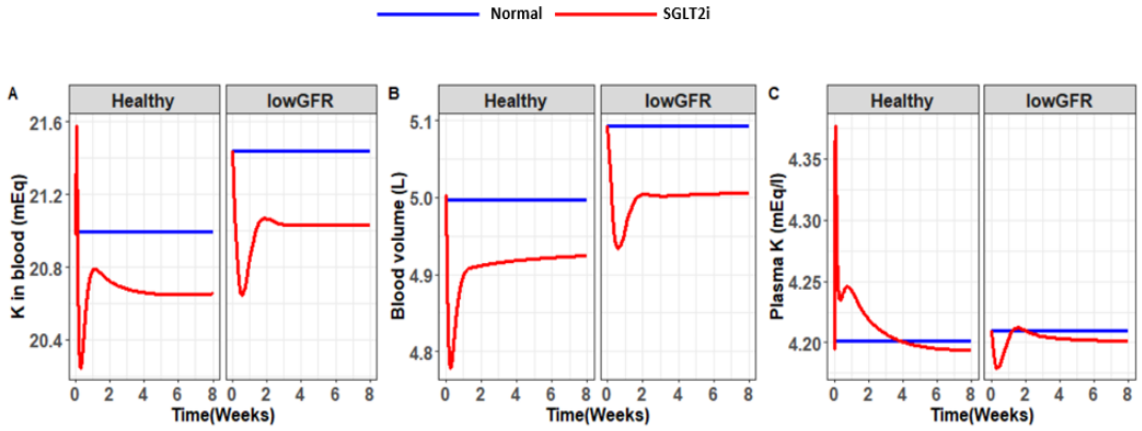

Fig4.15. Simulated changes in blood K^+ amount, blood volume, and plasma K^+ concentration in healthy and patient with chronic kidney dysfunction (CKD) treated with SGLT2i for 8 weeks. The baseline K^+ amount is elevated in CKD patients with renal impairment (A). SGLT2i is predicted to slightly decrease the K^+ concentration in CKD subjects, which is beneficial in avoiding hyperkalemia.

4.4.4.2. Investigation of mineralocorticoid antagonists (MRA) effect on potassium

MRAs are a class of medications used to treat conditions such as hypertension and heart failure. As explained in chapter 3, MRA acts by blocking the actions of ALDO, which regulates Na^+ and K^+ balance in the body. As expected, the plasma K^+ increased in both healthy and diabetic cases due to the increase in K^+ retention and decrease in blood volume (**Fig 4.16**).

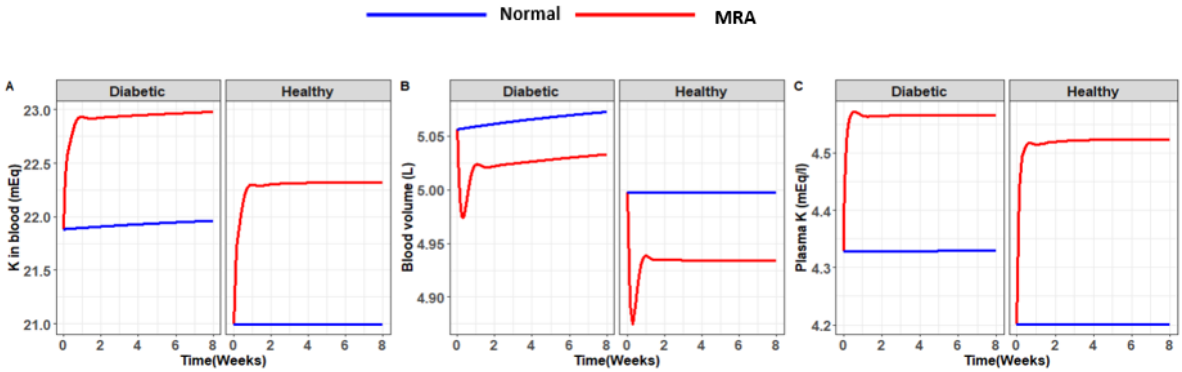

Fig4.16. Simulated changes in blood K^+ amount, blood volume, and plasma K^+ concentration in healthy and diabetic patients (glucose concentration=8.6 mmol/l) treated with MRA for 8 weeks. Simuluated baseline K^+ amount is elevated in diabetic patients, and MRA causes further retention of K^+, increasing K^+ concentration.

Also, CKD renal impairment leads to filtration dysfunction and the K^+ amount increases in the blood (**Fig4.17 A**). Eventually by administration of MRA the plasma K^+ is increasing in both healthy and renal impaired cases.

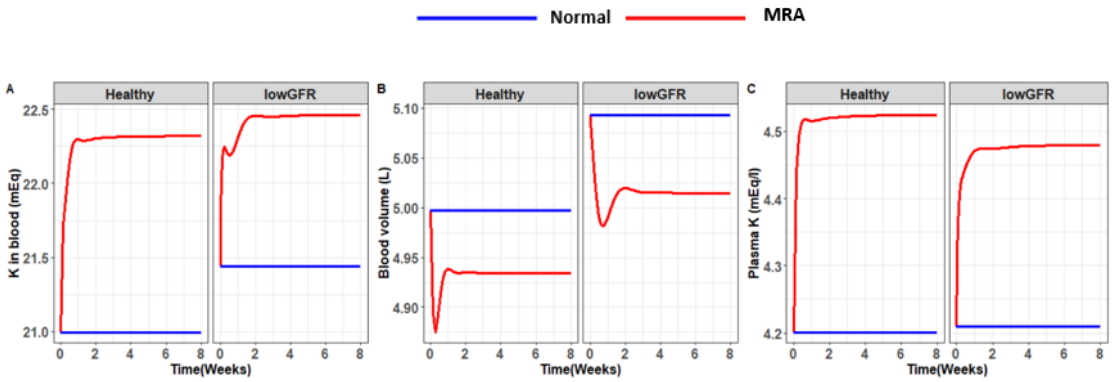

Fig4.17. Simulated changes in blood K^+ amount, blood volume, and plasma K^+ concentration in healthy and patients with CKD (GFR = 33.1 ml/min) treated with MRA for 8 weeks. Simuluated baseline K^+ amount is elevated in diabetic patients, and MRA causes further retention of K+, increasing K^+ concentration.

Fig 4.18 illustrates the effects of SGLT2i and MRA, alone and in combination, (on K^+ levels in healthy cases. MRA alone increases the K^+ amount in the blood (A) and decreases the blood volume (B) which lead to significant increase in plasma K^+(C). When combined with an SGLT2i, the rise in K^+ amount is less than with only MRA administration. However, because blood volume also decreases more with combination of therapies, the plasma K^+ alteration is almost equal to the cases with only MRA administration. Based on the clinical study of esaxerenone administration in combination with SGLT2i, the serum K^+ elevation is lesser than the MRA administration alone. Although, the plasma K^+ levels are almost equal in MRA specifically and in combination with SGLT2i, on the other hand total K^+ amount in combination of therapies is lesser than MRA administration[43].

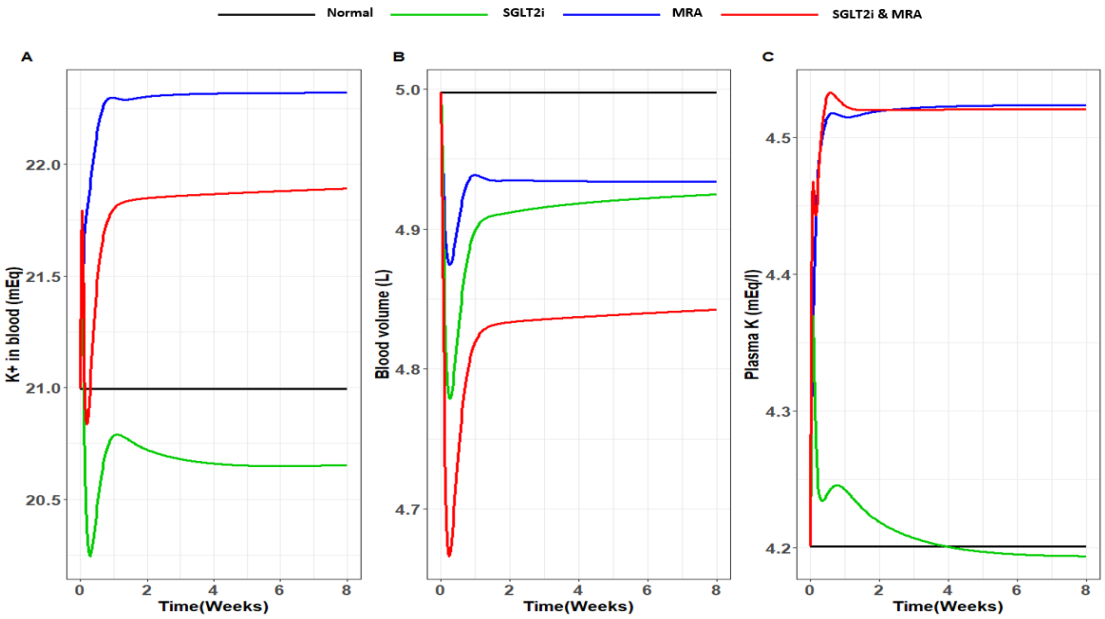

Fig4.18. Simulated changes in K^+ amount, blood volume, and the concentration in healthy cases. The K^+ amount is elevated in cases with MRA administration and combination of MRA and SGLT2i. this feature of the model makes the prediction of clinical consequences (Drug efficacy and safety) in drug development easy.

4.4.4.3. Exploring the effects of combination Therapy (SGLT2i and MRA) on potassium

As the graphs (**Fig 4.19**) show, MRA administration increases the K^+ amount in blood while decreasing blood volume, so that the plasma K^+ concentration increases substantially in both diabetic and healthy cases. On the other hand, SGLT2i administration causes a minimal effect in plasma K^+ in both cases of specific and in combination administration.

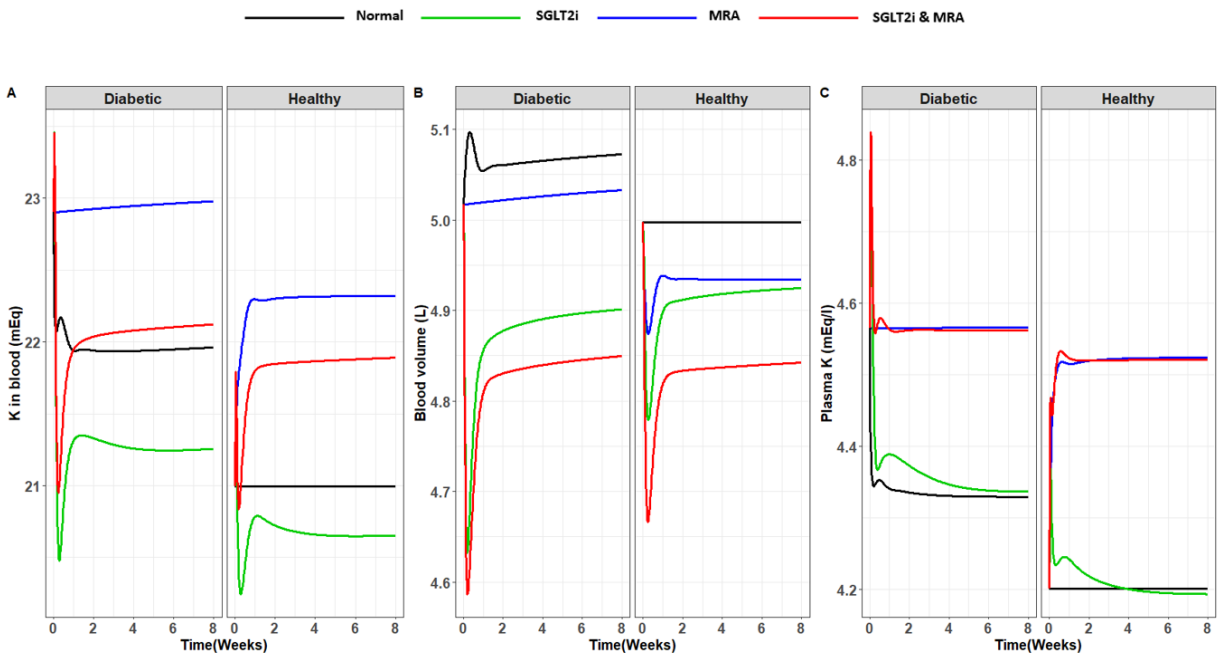

Fig4.19. Alteration of K^+ amount, blood volume, and the concentration are compared in healthy and diabetic cases. The K^+ amount is elevated in cases with MRA administration and combination of MRA and SGLT2i. On the other hand, the SGLT2i decreases the K^+ amount in the blood. However, due to the decrease in blood volume the plasma K^+ is not altered too much despite the instant increase for the first days of administration.

As discussed in chapter 3, in patients with renal impairment (GFR=33 ml/min), MRA administration increases the risk of hyperkalemia which is also consistent with **Fig4.20**. As it shows, SGLT2i administration in combination and specifically, slightly decrease plasma K^+ concentration relative to the MRA alone after 4 weeks of administration. Therefore, combination

of SGLT2i with other drugs may decrease the risk of hyperkalemia in addition to its other demonstrated benefits in protecting again heart and kidney failure.

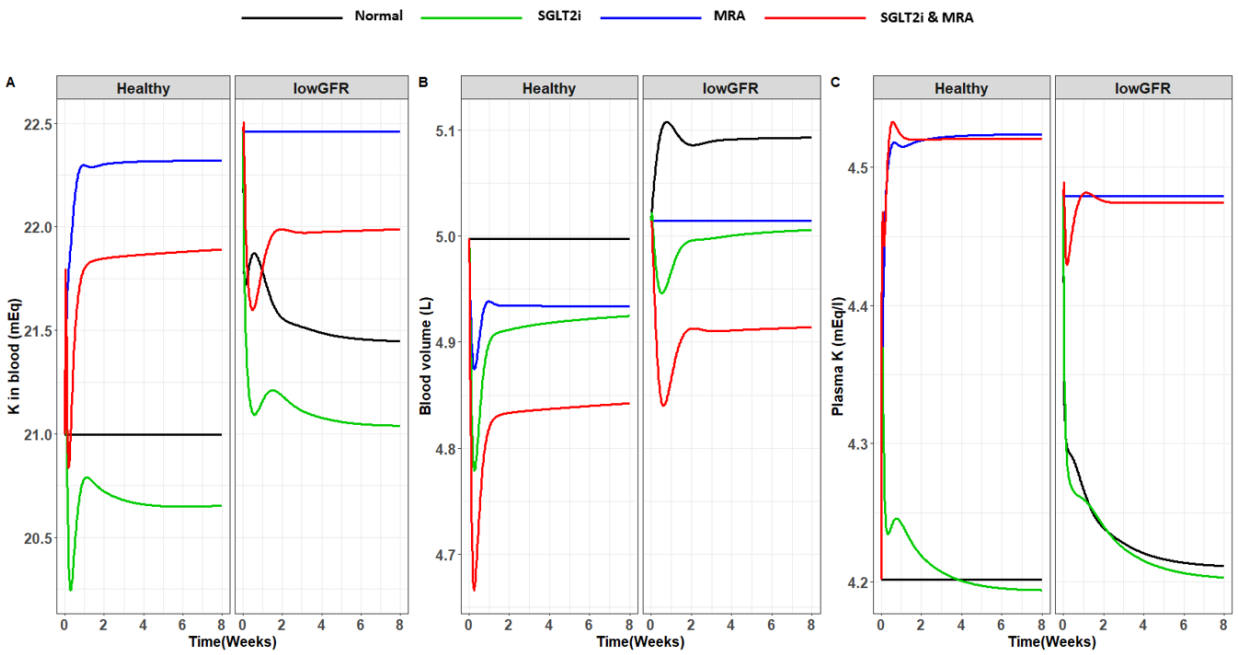

Fig4.20. Alteration of K^+ amount, blood volume, and the concentration are compared in healthy and cases with impaired kidney function (GFR=33 ml/min). The K^+ amount is elevated in cases with MRA administration and combination of MRA and SGLT2i. On the other hand, the SGLT2i decreases the K^+ amount in the blood for both healthy and cases with kidney dysfunction. However, due to the decrease in blood volume the plasma K^+ is not altered too much despite the instant increase for the first days of administration.

Moreover, the integrated model is able to investigate the K^+ alteration in even more complicated cases such as patients who are suffering from both diabetes and kidney impairment. To produce the baseline, we set glucose concentration= 8.6 mmol/l for diabetes and disease effect on nephrons, glomeruli, and decrease in filtration 0.7, 0.7, and 0.4 respectively to set GFR= 33 ml/min for a patient with kidney disease and diabetes. **Fig 4.21.** presents drug administration effect on patients with diabetes and CKD. As the plots presents, plasma K^+ is increasing in patients and

all drug administration increase the plasma K^+ levels in patients.

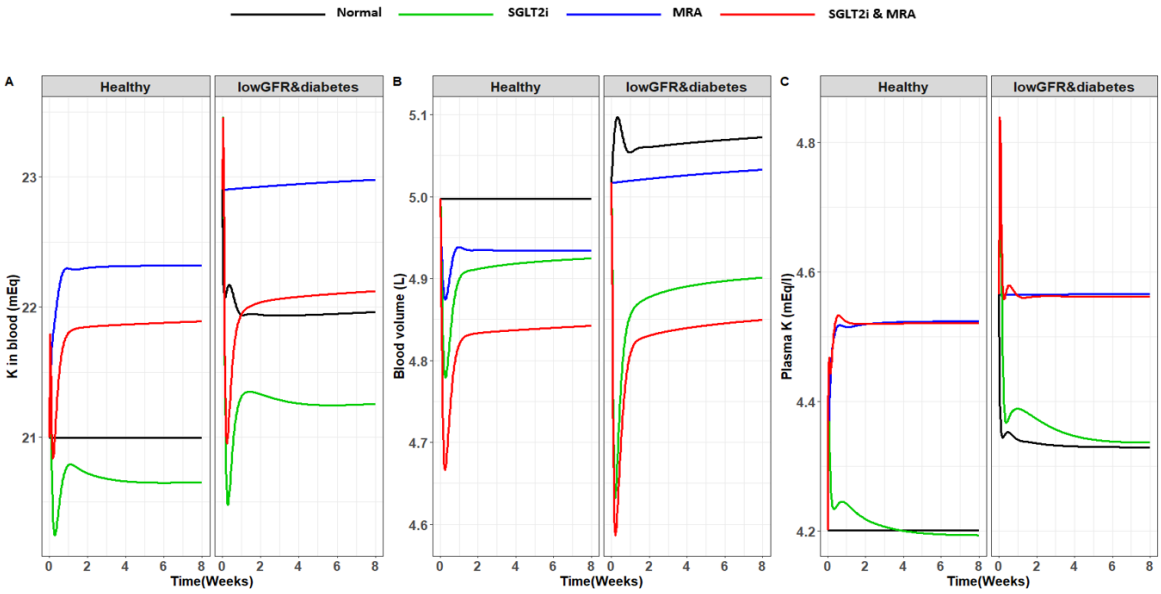

Fig4.21. Alteration of K^+ amount, blood volume, and the concentration are compared in healthy and cases with both impaired kidney function (GFR=33 ml/min) and diabetes (glucose concentration=8.6 mmol/l). The K^+ amount is elevated in cases with MRA administration and combination of MRA and SGLT2i. On the other hand, the SGLT2i decreases the K^+ amount in the blood for both healthy and cases with kidney dysfunction. Combination of drugs case reproduces the highest plasma K^+ after the first days of reduction and this will increase the hyperkalemia risk.

4.5. Discussion and conclusion

The key strength of the integrated model is the ability to predict K^+ alterations in response to various perturbuations, including drug therapies, diabetes, and kidney dysfunction. The model is able to investigate these perturbations alone and in combination. A sobol sensitivity analysis showed the plasma K^+ is most sensitive to the parameters $m_{K\text{-}P,MCD}$ and $m_{K,aldo}$. Moreover, plasma ALDO is most sensitive to $m_{K,aldo}$ and $m_{AT1,aldo}$ (Appendix E). All these parameters were able to be estimated with good precision.

We illustrated how the model can be used to simulate drug combinations by simulating the effect of SGLT2i and MRA on K^+ levels, both when administered as monotherapy and in combination which demonstrates the practical application of the model for simulating drug combinations. Specifically, we used the model to simulate the effect of administering SGLT2i and MRA as monotherapy and in combination on K^+ levels. Through this simulation, we were able to gain insight into the mechanistic explanation behind these drug interactions, which has significant implications for clinical practice. Based on the findings, we can better understand how the model can contribute to the development of more effective and safer drug combinations for various patients populations in the future which can inform decisions about drug dosing and monitoring strategies.

By incorporating patients pathophysiological parameters (glucose concentration= 8.6 mmol/l for diabetes and disease effect on nephrons, glomeruli, and decrease in filtration 0.65, 0.65, and 0.5 respectively to set GFR= 33 ml/min for patients with normal impairment) into the integrated model, we are able to present to evaluate drug therapy effects in patient populations with comorbidities such as CKD or diabetes. **Fig 14.19**, **Fig 14.20**, and **Fig 14.21** present the impacts of various therapies (specific and in combination) in healthy and unhealthy cases with different pathophysiological conditions, **Fig 4.19** evaluates the risk of hyperkalemia in both healthy and diabetic cases when several strategies of drug therapy are available and the model is a reliable tool to choose the best one for a specific patient population. Same evaluations are presented in **Fig 4.20** and **Fig 4.21** for the different populations.

In conclusion, the integrated model is able to produce the various profiles of K^+ alteration due to the changes in lifestyle, diet, drug administration and diseases which informs users about the drug safety (avoid hyperkalemia or hypokalemia) and interpret the efficacy of drug

administration in various patients. Therefore, the integrated model offers several benefits for understanding drug therapies. First, it allows us to better understand the complex interactions between different physiological systems, which can help identify potential drug interactions and side effects. Secondly, the integrated model assists in understanding the mechanistic behavior of physiologic responses to drug administration and selecting strategies to avoid side effects and toxicity. The output also can help in optimal drug dosing in order to reach the desired outcomes that are expected in both drug safety and efficacy protocols.

5. Chapter 5: Conclusion, Limitations and, Future Directions

5.1. Conclusion and discussion

The integrated model describes the kidney role and hormonal responses (e.g. ALDO, RAAS and etc.) in K^+ and Na^+ homeostasis. Prior to research, there was no mathematical model to provide a detailed evaluation on the factors that impact K^+ homeostasis in human body. Therefore, the significance of this research is the development and validation of a K^+-ALDO kidney model that is able to describe the impacts of lifestyle, hormonal responses (e.g. ALDO), disorders (e.g. CKD) and therapies on K^+ alteration.

The K^+-ALDO model alone was able to reproduc the integrated impacts of systemic K^+ balance, renal function, and ALDO dynamic feedback. This feature provides the abilities such as predicting the response to acute perturbations due to the MRA administration or K^+ infusion and predicting the chronic response of K^+ alteration in response to MRA therapies by investigating both renal K^+ handling and systemic K^+ homeostasis. The steady state of the system were developed based on information obtained from physiology references [9]. According to local sensitivity analysis and calibration results, for the K^+-ALDO model, the parameters (**Table 2.2** in chapter 2 and Appendix B) were able to be estimated with good precision. Also, the presented equations in chapter 2 and 3 provided good fits to the data objserved during K^+ infusion and MRA administration in healthy humans[70, 80] and chronic data in hyperaldosteronism patients treated with MRA antagonists[83]. Thus, the

model represents a reasonable implementation to investigate lifestyle, pathophysiologic, and therapeutic factors that affect K^+ alteration. However, K^+ and Na^+ regulations are tightly linked, and the K^+-ALDO model of Aims I and II only empirically accounted for the effect of Na^+ intake on ALDO. It could not account for the their coupled reabsorption in the kidney tubule, and could not mechanistically relate changes in Na^+ intake to change in ALDO through the effect of plasma Na^+ concentration and osmolality[70].

Coupling the K^+-ALDO model with the previously validated Na^+/water homeostasis model [54] brings a more mechanistic explanation of the interrelationship between K^+ and Na^+. Therefore, the integrated model simulates processes of Na^+, water and, K^+ intake, renal filtration, reabsorption, secretion, and excretion, and osmotic exchange between blood and interstitium and regulatory feedback control (e.g. K^+-ALDO and RAAS) of these processes through physiological and neurohormonal mechanisms. The integrated K^+/Na^+ model dynamically calculates GFR, blood pressure, plasma Na^+, K^+, ALDO, and renin concentrations. Moreover, it can calculate the tubular concentrations of Na^+ and K^+ and alterations in tubular flow rates and reabsorptions in different segments of the nephron. In the integrated model, the non-mechanistic effect of Na^+ intake effect on ALDO is replaced by the mechanistic effect of plasma Na^+. In addition, the osmolality and renin effects on ALDO were incorporaeted. After calibrating the integrated model with acute MRA pharmacologic data, and the acute data of K^+, ALDO, and renin concentrations[66, 70, 80] perturbed by K^+ infusion and diets, the integrated model is able to predict MAP, Na^+, and K^+ alteration due to perturbations by drug administration or diseases[83, 105].

As explained in the model application section of the chapter 4, a key feature of the integrated model is to simulate K^+ alteration in patients and healthy cases that are affected by a specific or in combination of drug administration (e.g. SGLT2i and MRA). This model ability can provide valuable information related to drug safety, drug efficacy, and optimal dosing in drug

development. This will help with better understanding the complex interactions between various physiological and feedback systems, which can lead to identification of potential drug interactions and side effects. Secondly, it can provide insight into the optimal dosing of combination therapies to enhance their effect while decreasing adverse effects. Also, it can help identify patient subpopulations that are sensitive to certain drug interactions or side effects.

5.2. Limitations

Several limitations should be noted. One limitation is that our mathematical model was developed based on assumptions and simplifications of complex renal physiology. In the model, we assumed all nephrons are identical. However, in reality the nephrons are different and therefore the reabsorption and secretion amount of Na^+ and K^+ may be heterogenous across nephrons.

Moreover, the individual variability in genetics and lifestyle and their effect on system response is complicated to address. For instance, K^+ and Na^+ intakes were assumed constant in our model. However, it is clear that the diets are quite variable in humans and it may be challenging to show the full impact of diet alteration. Also, the model assumes that the distribution of Na^+ and K^+ within the body is uniform. Cardiac output in this model is a function of MAP and vascular resistances, and complex regulation of cardiac output by the heart was not modeled. Consequently, further modification and details to this model can improve the accuracy and provide more opportunities in cardiorenal drug development progress.

5.3. Future directions

Some additional potential future directions for the integrated model can be considered. This model can be applied to investigate the safety and efficacy of novel drugs that may affect ALDO

or K^+ levels and providing valuable insights for drug development and patient care. For instance, A next step may involve further investigation of a newly developed MRA drug called esaxerenone, which has high potency for MR compared to spironolactone and eplerenone. A published meta-analysis has specifically evaluated the effects of this drug, both alone and in combination with SGLT2i, in patients with diabetic kidney [43, 108]. By identifying the key differences in pharmacokinetics (PK) and pharmacodynamics of esaxerenone and previous MRA and modifying the PK and PD mechanistic components that were developed for MRA in chapter 3, the model can be updated to incorporate drug-specifc information on absorption, distribution, metabolism, and excretion(ADME) as well as the pharmacodynamic effects on MR and other sites of effect. The model could then be applied to predict safety and efficacy endpoints of interest such as blood pressure, GFR, and plasma K^+ level. In addition, the model can be used to explore different dosing regimens and patient populations, which is helpful in prediction of the potential drug combinations and interactions.

As also mentioned in limitations, further model development such as combining the current model with a model of cardiac mechanics can bring more research opportunities such as evaluation of heart failure and related drugs impacts on the electrolyte homeostasis which can bring invaluable information in drug development [109, 110].

Development of a user-friendly interface: To facilitate the use of the integrated model in clinical settings, the Shiny package,which is developed in R[8], can help to develop a user-friendly interface that allows healthcare providers to input patient-specific information and get specific model predictions. This development could help physicians make more accurate decisions by having more clear information about drug safety, efficacy, and dosing for individual cases. This can help to adjust the dose of drugs such as SGLT2i or discontinue the medication altogether to manage problems like K^+ imbalance.

6. **References**

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APPENDICES

APPENDIX A

Developed codes for potassium-aldosterone model

This appendix contains text from the following publication:

Maddah, Erfan, and K. Melissa Hallow. "A quantitative systems pharmacology model of plasma potassium regulation by the kidney and aldosterone." *Journal of Pharmacokinetics and Pharmacodynamics* 49.4 (2022): 471-486.

A-1. Model codes

- contains all model equations

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############################## Potassium Homeostasis Model
 ##########################################

#Authors: Erfan Maddah, KM Hallow, University of Georgia
#November 22, 2021
#############################################################################################
 ##############

ode <- "


################################## Drug Effects
 ############################################

####MRAs

#Emax model
E_MRA_spiro=E_MAX_spiro*canrenone/(canrenone+EC50_spiro);  #E_MAX model
MR=1;
Kon_MRA = Koff_MRA


################################## Systemic Potassium
 ############################################

plasma_K= K/ V_ecf; #(mEq/mL)

intracellular_K_conc = intracellular_K / V_ic;

intracellular_potassium_flux = Q_K_intracellular*(plasma_K - norm_plasma_K - (intracellular_K_conc -
 nom_intracellular_K_conc ));


################################## Aldosterone Regulation
 ############################################

  #Aldosterone, nmol/L
  Aldo= (max(0, norm_Aldo*
       exp(m_K_ALDO*(plasma_K -norm_plasma_K)) +            #effect of plasma potassium
```

```
max(0,(exp(-m_Na_ALDO*(Nain - norm_Na_intake))-1)) +  #effect of sodium intake
      hyperaldo_effect));                              #effect of hyperaldosteronism


  #Effect of aldosterone on tubular potassium secretion
  Aldo_effect_on_K_secretion= MRA_effect*MR*max(0,1 +  Aldo_KSec_scale*(Aldo - norm_Aldo));



################################## PT/LoH Na+ and K+ Reabsorption
############################################

  #Glomerular Filtration of water, potassium, and sodium
  SNGFR = GFR / number_of_nephrons; #(ml/min)
  filtered_K = max(0,SNGFR * plasma_K); #(mEq/min/nephron)
  filtered_Na = SNGFR*(norm_plasma_Na/1000) #(mEq/min/nephron)


##################### PT Sodium Reabsorption ##################
#### Assume sodium balance is always achieved
#### Assume that:
#the PT/LoH adjusts sodium reabsorption so that when sodium load is delivered distally, if 95% is
reabsorbed by CNT/CD,
#then sodium balance is maintained
#However PT/LoH together cannot reabsorb more than 80% of delivered load each or 96% total (1-(1-
0.8)*(1-0.8))
#If Na intake falls too low, the PT/LoH reabsorb 96% and the remaining Na+ is retained by compensation
in the CNT/CD
#### Calculate sodium that must be delivered to the DCT
#### Calculate fraction of filtered sodium reabsorbed prior to DCT
#### Assume K+ reabsorption in PT and LoH is passive and proportional to Na+ reabsorption
#Determine PT/LoH fractional sodium reabsorption based on sodium intake

  max_PT_LoH_reabsorption = filtered_Na*0.96

  Na_out_LoH = max(  Nain/(1-0.95)/number_of_nephrons, filtered_Na -  max_PT_LoH_reabsorption)
#If 95% reabsorbed in DCT/CNT/CD and Na out of CD must equal Na intake

  eta_PT_LoH = (filtered_Na - Na_out_LoH)/(filtered_Na)


##################### PT Sodium Reabsorption ##################

  #Proximal Tubule and LoH K+ reabsorption is proportional to sodium reabsorption
  K_reabsorption_PT_LoH = eta_PT_LoH*filtered_K; #(mEq/min)

  PT_LoH_K_out =filtered_K - K_reabsorption_PT_LoH; #(mEq/min

################################## DCT K+ Secretion
############################################
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```
#Tubular Segment K+ Concentrations

  DCT_luminal_K_conc = max(0,DCT_luminal_K_amount) / DCT_volume
  CNT_luminal_K_conc = max(0,CNT_luminal_K_amount) / CNT_volume
  CCD_luminal_K_conc = max(0,CCD_luminal_K_amount) / CCD_volume
#Membrane Potential Differences
  normalized_luminal_potential_difference = F*(luminal_potential_difference)/(R*T)
  normalized_basolateral_potential_difference = F*(basolateral_potential_difference)/(R*T)

#Potassium and water into DCT
  DCT_K_in = PT_LoH_K_out
  DCT_water_in = SNGFR*(1-eta_PT_LoH)

#DCT Potassium flux from cell to lumen #mEq/min.cm^2 #Goldman equation
  DCT_K_passive_flux_lumenal =
baseline_K_luminal_permeability*normalized_luminal_potential_difference*
    (-DCT_luminal_K_conc + DCT_cell_K_conc*exp(-normalized_luminal_potential_difference))
    /(1-exp(-normalized_luminal_potential_difference));

#DCT passive potassium flux across basolateral membrane #mEq/min.cm^2 #Goldman equation
  DCT_K_passive_flux_basolateral = -
K_basolateral_permeability*normalized_basolateral_potential_difference*
    (-plasma_K + DCT_cell_K_conc*exp(-normalized_basolateral_potential_difference))
    /(1-exp(-normalized_basolateral_potential_difference));

#DCT Active flux the Na+/K+ ATPase across basolateral membrane,  #mEq/min.cm^2

  K_K = (0.1*(1+140 /18.5))/1000  #assume plasma Na+ of 140 mEq/L
  K_Na_DCT = (0.2*(1+DCT_cell_K_conc*1000/8.33))/1000


  J_Na_active_max_eff = J_Na_active_max*max(0,(Aldo_effect_on_K_secretion))
  DCT_K_active_flux_basolateral =
(2/3)*J_Na_active_max_eff*((principal_cell_intracellular_Na/(principal_cell_intracellular_Na +
K_Na_DCT))^3)*((plasma_K/(plasma_K + K_K))^2)


################################## CNT K+ Secretion
#############################################

#CNT Potassium flux from cell to lumen #mEq/min.cm^2 #Goldman equation
  CNT_K_passive_flux_lumenal =
baseline_K_luminal_permeability*normalized_luminal_potential_difference*
    (-CNT_luminal_K_conc + CNT_cell_K_conc*exp(-normalized_luminal_potential_difference))
    /(1-exp(-normalized_luminal_potential_difference)); #mEq/min.cm^2 #Goldman equation

#CNT passive potassium flux across basolateral membrane #mEq/min.cm^2 #Goldman equation
  CNT_K_passive_flux_basolateral = -
K_basolateral_permeability*normalized_basolateral_potential_difference*
    (-plasma_K + CNT_cell_K_conc*exp(-normalized_basolateral_potential_difference))
    /(1-exp(-normalized_basolateral_potential_difference)); #mEq/min.cm^2 #Goldman equation
```

```
#CNT Active flux the Na+/K+ ATPase across basolateral membrane,  #mEq/min.cm^2
  K_Na_CNT = (0.2*(1+CNT_cell_K_conc*1000/8.33))/1000

  CNT_K_active_flux_basolateral =
(2/3)*J_Na_active_max_eff*((principal_cell_intracellular_Na/(principal_cell_intracellular_Na +
K_Na_CNT))^3)*((plasma_K/(plasma_K + K_K))^2)
################################## CCD K+ Secretion
##############################################

#CCD Potassium flux from cell to lumen #mEq/min.cm^2 #Goldman equation
CCD_K_passive_flux_lumenal                                                                    =
baseline_K_luminal_permeability*normalized_luminal_potential_difference*
    (-CCD_luminal_K_conc + CCD_cell_K_conc*exp(-normalized_luminal_potential_difference))
    /(1-exp(-normalized_luminal_potential_difference)); #mEq/min.cm^2 #Goldman equation

#CCD passive potassium flux across basolateral membrane #mEq/min.cm^2 #Goldman equation
CCD_K_passive_flux_basolateral                                          =                  -
K_basolateral_permeability*normalized_basolateral_potential_difference*
    (-plasma_K + CCD_cell_K_conc*exp(-normalized_basolateral_potential_difference))
    /(1-exp(-normalized_basolateral_potential_difference)); #mEq/min.cm^2 #Goldman equation

#CCD Active flux the Na+/K+ ATPase across basolateral membrane,  #mEq/min.cm^2
  K_Na_CCD = (0.2*(1+CCD_cell_K_conc*1000/8.33))/1000
  CCD_K_active_flux_basolateral =
(2/3)*J_Na_active_max_eff*((principal_cell_intracellular_Na/(principal_cell_intracellular_Na +
K_Na_CCD))^3)*((plasma_K/(plasma_K + K_K))^2)

  #Tubular surface areas
  DCT_SA = pi*DCT_diameter*DCT_length
  CNT_SA = pi*CNT_diameter*CNT_length
  CCD_SA = pi*CCD_diameter*CCD_length

  #Tubular potassium secretion rates
  DCT_K_secretion_rate = DCT_K_passive_flux_lumenal*DCT_SA
  CNT_K_secretion_rate = CNT_K_passive_flux_lumenal*CNT_SA*principal_fraction_CNT
  CCD_K_secretion_rate = CCD_K_passive_flux_lumenal*CCD_SA*principal_fraction_CCD

  #Water out of each tubular segment
  DCT_water_out = DCT_water_in #minimal water reabsorption in DCT
  CNT_water_out = DCT_water_out*(1-CNT_water_reabs_fraction) # about 60-70% water reabsorptionin
CNT
  CCD_water_out = DCT_water_in*(1-CCD_water_reabs_fraction) #high water reabsorption in CCD

  #Potassium out of each tubular segment
  DCT_K_out = DCT_luminal_K_conc*DCT_water_in   #no water reabsorbed in DCT
  CNT_K_out = CNT_luminal_K_conc*DCT_water_in      #minimal reabsorption in CNT
  CCD_K_out = CCD_luminal_K_conc*CCD_water_out


################################## MCD K+ Reabsorption
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##############################################

 K_MCD_effect = max(0, exp(m_plasmaK_MCD*( (norm_plasma_K - plasma_K)/norm_plasma_K))-1)

#Na reabsorption through Na+/K+ ATPase required for sodium balanace
  Na_reabsorption_MCD = max(0, (Na_out_LoH*0.5 - Nain/number_of_nephrons)) #sodium reabsorption
required to maintain sodium balance

 #Potassium exchange with sodium


  K_secretion_MCD=max(0,(norm_Na_reabsorption_MCD/number_of_nephrons-
Na_reabsorption_MCD)*m_Na_MCD)


 K_reabsorption_MCD_rate = (K_reabsorption_MCD_rate0 + K_MCD_effect + K_secretion_MCD )


  K_reabsorption_CD = min(K_reabsorption_MCD_rate, CCD_K_out); #(mEq/min), can't reabsorb more
than is filtered

  #Fractional MCD potassium reabsorption
  eta_MCD= max(0, K_reabsorption_MCD_rate/CCD_K_out)


################################  K+ Excretion
##############################################

  #Total potassium excretion is potassium leaving the CD times the number of nephrons
  CD_K_out = number_of_nephrons*(CCD_K_out - K_reabsorption_CD); #(mEq/min)


##########################################################################################
########################

#MRA Pharmacokinetic Depot compartments
  d/dt(spiro_depot) = -Ka_spiro*spiro_depot;

  d/dt(spiro_t1) = Ka_spiro*spiro_depot - Ka_spiro*(spiro_t1)
  d/dt(spiro_t2) = Ka_spiro*(spiro_t1) - Ka_spiro*(spiro_t2)

#Extracellular Potassium Amount
  d/dt(K)= Kin + Kinfusion  - CD_K_out - intracellular_potassium_flux; #(mEq/min)

#Intracellular Potassium amount
d/dt(intracellular_K) = intracellular_potassium_flux;


#Tubule lumen K+ amounts
  d/dt(DCT_luminal_K_amount) = DCT_K_in + DCT_K_secretion_rate - DCT_K_out;
  d/dt(CNT_luminal_K_amount) = DCT_K_out + CNT_K_secretion_rate - CNT_K_out;
```

```
  d/dt(CCD_luminal_K_amount) = CNT_K_out + CCD_K_secretion_rate - CCD_K_out;


#Tubule Cell concentrations
  d/dt(DCT_cell_K_conc) = (-DCT_K_passive_flux_lumenal + DCT_K_passive_flux_basolateral +
DCT_K_active_flux_basolateral)/SV_DCT
  d/dt(CNT_cell_K_conc) = (-CNT_K_passive_flux_lumenal + CNT_K_passive_flux_basolateral +
CNT_K_active_flux_basolateral)/SV_CNT
  d/dt(CCD_cell_K_conc) = (-CCD_K_passive_flux_lumenal + CCD_K_passive_flux_basolateral +
CCD_K_active_flux_basolateral)/SV_CCD

#Cumulative urinary potassium excretion
  d/dt(potassium_excretion_rate)=CD_K_out; #mEq/min

#MRA Indirect response
  d/dt(MRA_effect) = Kon_MRA*(1-E_MRA_spiro) - Koff_MRA*MRA_effect;

###### MRA Pharmacokinetics ######

#Spironolactone Plasma Concentration
  d/dt(spiro_C1) =(Ka_spiro*(spiro_t2)-CL_spiro*(1-Spiro_Fmetabolized)*spiro_C1-
CL_spiro*Spiro_Fmetabolized*spiro_C1 ) / V1_spiro #ug/L/min

#Spironolactone Metabolite Canrenone
  #Plasma concentration
  d/dt(canrenone) = (CL_spiro*Spiro_Fmetabolized*spiro_C1 - CL_canrenone*canrenone -
(Q_canrenone*canrenone - Q_canrenone*canrenone_C2))/V_canrenone
  #Peripheral concentration
  d/dt(canrenone_C2) = (Q_canrenone*canrenone - Q_canrenone*canrenone_C2)/V2_canrenone;

"
```

A-2. Parameters

- contains the baseline parameterization for the model.

```
theta=NULL


#Constants
theta$F= 97 #Faraday constant C/mmol
theta$R= 8.3145 #universal constant for all gases #J/mol.K
theta$T= 310.6 #normal body temperature #K


#Intake Rates
```

theta$Kin= 0.08 #(mEq/min), normal rate of potassium intake for a healthy adult is between 0.073-0.084 mEq/min.#0.08

theta$Nain=100/24/60 #mEq/min, sodium intake rate

theta$Kinfusion = 0 #(mEq/min), potassium infusion rate into extracellular compartment

theta$norm_Na_intake = 100/24/60 #(mEq/min), normal sodium intake rate

#Normal Concentrations

theta$norm_Aldo= 0.49 #nmol/L ##0.13-0.83

theta$norm_plasma_K=0.0042 #meq/ml 0.0035-0.0055 ,0.004-0.006 from paper

theta$nom_intracellular_K_conc = 150 #meq/L

theta$norm_plasma_Na = 140 #mEq/L

theta$principal_cell_intracellular_Na = (8/1000)#mEq/mL

#Compartment volumes

theta$V_ecf = 15000 #(ml)

theta$V_ic = 25000 #mL

#Renal geometry and function

theta$GFR=105 #(ml/min)

theta$number_of_nephrons=2000000

theta$CNT_diameter=2*12*10^-4 #cm

theta$CNT_length= 0.4 #cm #Layton, Anita T., and Harold E. Layton. "A computational model of epithelial solute and water transport along a human nephron." PLoS computational biology 15.2 (2019): e1006108.

theta$DCT_diameter=0.0015 #cm

theta$DCT_length=0.5 #cm

theta$CCD_diameter=0.0025 #cm

theta$CCD_length=0.2 #cm

theta$imcd_length=0.5#cm

theta$imcd_diameter=0.003 #cm

```
theta$SV_CNT=6*10e-4# CNT ratio per volume m^3/m^2
#https://journals.physiology.org/doi/pdf/10.1152/ajprenal.00044.2005

theta$SV_DCT=0.75*10e-3# DCT ratio per volume m^3/m^2

theta$SV_CCD=4*10e-4# CCD ratio per volume m^3/m^2

theta$principal_fraction_CNT=0.6 #fraction of principal cells in CNT

theta$principal_fraction_CCD=0.75 #fraction of principal cells in CCD


theta$DCT_volume = pi*((theta$DCT_diameter/2)^2)*theta$DCT_length

theta$CNT_volume = pi*((theta$CNT_diameter/2)^2)*theta$CNT_length

theta$CCD_volume = pi*((theta$CCD_diameter/2)^2)*theta$CCD_length


theta$DCT_luminal_K_conc0 = 0.0055  #mEq/ml

theta$CNT_luminal_K_conc0 = 0.0072 #mEq/ml

theta$CCD_luminal_K_conc0 = 0.032 #mEq/ml




filtered_Na = theta$GFR*(140/1000) #(mEq/min)

theta$Na_out_loH0 = theta$Nain/(1-0.95)  #If 95% reabsorbed in DCT/CNT/CD and Na out of CD must equal Na intake

eta_PT_LoH = (filtered_Na - theta$Na_out_loH0)/(filtered_Na)



#assumes 50% reabsorption in DCT-CCD, before MCD

theta$norm_Na_reabsorption_MCD = (theta$Na_out_loH0*0.5 - theta$Nain)

theta$norm_K_secretion_MCD = theta$norm_Na_reabsorption_MCD*2/3



filtered_K_load = theta$norm_plasma_K*theta$GFR

theta$potassium_in_MCD0 = filtered_K_load*((1-eta_PT_LoH)+(.12)) #net of reabsorbed and secreted (assumed 8%secretio)
```

#Single-nephron MCD potassium reabsorption rate required for potassium balance (mEq/min)

theta$K_reabsorption_MCD_rate0 = (theta$potassium_in_MCD0 - theta$Kin)/theta$number_of_nephrons #excretion must equal intake for K+ balance

#fractional MCD potassium reasorption

theta$eta_MCD0 = theta$K_reabsorption_MCD_rate0 / (theta$potassium_in_MCD0/theta$number_of_nephrons)

theta$slope_plasmaK_MCD = theta$K_reabsorption_MCD_rate0 / (theta$CCD_luminal_K_conc0 - theta$norm_plasma_K) #reabsorption per unit potassium gradient

theta$CNT_water_reabs_fraction = 0.7

theta$CCD_water_reabs_fraction = 0.75

#Potassium Secretion Parameters

theta$baseline_K_luminal_permeability = 2.4935e-5 #cm/s

theta$K_basolateral_permeability=3.43e-5#5e-7##8.396e-4 #cm/s

theta$J_Na_active_max = 14.66e-5 #mmol/min/cm2

theta$luminal_potential_difference= -18.4 #mV #Weinstien 2001 p.F1078

theta$basolateral_potential_difference= -78.2 #mV #Weinstien 2001 p.F1078

#Fitting Constants

theta$m_K_ALDO= 951.2 #Slope of plasma K+ effect on plasma aldosterone, mL/mEq

theta$m_Na_ALDO=15.569 #Slope of Na intake effect on plasma aldosterone, min/mEq

theta$Aldo_KSec_scale= 103.5 #L/nmol

theta$m_plasmaK_MCD = 8.83e-7 #unitless

theta$m_Na_MCD = 0.69775 #min/mEq

theta$Q_K_intracellular = 465.87 #L/min

#Disease effectsplasma_K

theta$hyperaldo_effect = 0

#Drug effects

theta$E_MAX_spiro= 0.9978#0.9465#0.99#0.9988 #MRA Imax

theta$EC50_spiro = 1.8296#6.44#2.48#20 #ug/L or ng/ml

theta$Koff_MRA = 3.4035 #0.0128

#Spironolactone pharmacokinetics

theta$Ka_spiro = 0.01524458

theta$V1_spiro = 7.15696

theta$CL_spiro = 8.07626

theta$CL_canrenone = 0.222487

theta$V_canrenone = 70.47

theta$V2_canrenone = 8.021

theta$Q_canrenone = 0.110275

theta$Spiro_Fmetabolized = 0.19311

theta$Spiro_bioavailability = 0.91097

A-3. Run file

- loads and compiles model

- runs to equilibrium

- NOTE: Always run this file first and check that it is producing a stable baseline before proceeding further.

############################## Potassium Homeostasis Model Base Run File##############################

#Authors: Erfan Maddah, KM Hallow, University of Georgia

#November 22, 2021

##

```
#Load Packages
library(RxODE)
library(tidyverse)
library(gridExtra)
library(ggpubr)

#Load and compile model file.
source("modelfile_pktransit.R")
erfan83<-RxODE(model=ode)

#Load Parameters
source("calcNomParams.R")
theta=data.frame(theta)

#Define initial condition
inits1<- c(spiro_depot = 0,
          spiro_t1 = 0,
          spiro_t2 = 0,
          K= theta$norm_plasma_K*theta$V_ecf,
          intracellular_K = theta$nom_intracellular_K_conc*theta$V_ic,
          DCT_luminal_K_amount = theta$DCT_luminal_K_conc0*theta$DCT_volume, #5e-6,
          CNT_luminal_K_amount =  theta$CNT_luminal_K_conc0*theta$CNT_volume, #5e-6,
          CCD_luminal_K_amount =  theta$CCD_luminal_K_conc0*theta$CCD_volume, #5e-6,
          DCT_cell_K_conc = 0.15, #mEq/ml
          CNT_cell_K_conc = 0.15, #mEq/ml
          CCD_cell_K_conc = 0.15, #mEq/ml
          potassium_excretion_rate=0,
          MRA_effect=1, #theta$norm_Aldo,
          spiro_C1 = 0,
          canrenone = 0,
          canrenone_C2 = 0) #mEq,#mmol/ml


times=seq(0,60*24*28,1)
```

```
ev=eventTable(time.units = 'minutes')
ev$add.sampling(times)



############ Test #################
#Turn off aldosterone feedbacks
#Are aldosterone and plasma concentrations at their setpoints?
#If not, adjust permeability0

theta$m_K_ALDO = 0
theta$m_Na_ALDO = 0
theta$Aldo_CD_scale = 0
theta$Aldo_Ksec_scale = 0
theta$m_plasmaK_MCD = 0
#
# # #Adjust
theta$baseline_K_luminal_permeability = 2.4935e-5 #cm/s
theta$K_basolateral_permeability=3.43e-5#5e-7##8.396e-4 #cm/s
theta$J_Na_active_max = 14.66e-5 #mmol/min/cm2


h <-data.frame(erfan83$run(theta, ev, inits1))


head(h$DCT_K_active_flux_basolateral + h$DCT_K_passive_flux_basolateral -
h$DCT_K_passive_flux_lumenal)


head(h$CNT_K_active_flux_basolateral + h$CNT_K_passive_flux_basolateral -
h$CNT_K_passive_flux_lumenal)


head(h$CCD_K_active_flux_basolateral + h$CCD_K_passive_flux_basolateral -
h$CCD_K_passive_flux_lumenal)


head(theta$Kin - h$CD_K_out)


p1 = ggplot(h)+geom_path(mapping = aes(x=time,y=plasma_K))+xlab("Time(minutes)") +
  #ylab("plasma potassium concentration")# +
ylim(c(theta$norm_plasma_K-0.00005, theta$norm_plasma_K+0.00005))
```

```
p2 = ggplot(h)+geom_path(mapping = aes(x=time, y= DCT_cell_K_conc))+xlab("Time(minutes)")+
  ylim(c(.149, .151))

grid.arrange(p1,p2,nrow=1)



########## Update any adusted parameters in calcNomParams.R file.#########
#Then reload parameters and rerun
source("calcNomParams.R")
theta=data.frame(theta)

h <-data.frame(erfan83$run(theta, ev, inits1))
p1 = ggplot(h)+geom_path(mapping = aes(x=time,y=plasma_K))+xlab("Time(minutes)")+
  ylab("plasma potassium concentration") +
  ylim(c(theta$norm_plasma_K-0.00005, theta$norm_plasma_K+0.00005))

p2 = ggplot(h)+geom_path(mapping = aes(x=time,y=Aldo))+xlab("Time(minutes)")+
  ylab("plasma aldo concentration") + ylim(c(theta$norm_Aldo-0.005, theta$norm_Aldo + 0.005))

grid.arrange(p1,p2,nrow=1)

#Are plasma potassium and aldosterone concentrations flat and at their nominal values? If not, something
#is wrong and must be corrected before proceeding

#Store baseline steady state parameters and initial conditions for future use
theta_orig = theta
inits_orig = h[dim(h)[1], names(h) %in% names(inits1)]
```

A-4. Model calibration: potassium infusion

-This code simulates Dluhy 1972 and generates Figure 2.2.

```
############################ Potassium Homeostasis Model
########################################

#Authors: Erfan Maddah, KM Hallow, University of Georgia
#November 22, 2021



#This file simulates and plots the following study:



#Dluhy, R. G., et al. (1972). "Studies of the control of plasma aldosterone concentration in
#normal man: II. Effect of dietary potassium and acute potassium infusion."
#The Journal of Clinical Investigation 51(8): 1950-1957.

#Before running this file, run the file "runToEquilibrium.R"

##############################################################################################
##############

#Load base parameters and initial conditions
theta = theta_orig
inits = inits_orig

# simulating the study

simDluly = function(theta) {

  obj = 0

  allCaseResults = NULL

  cases = unique(Normalpotassiumaldo$case)

  ############################ Simulate Study Design ####################
```

```
#simulate each Na/K intake case

for (i in 1:4) {

 thiscase = cases[i]
 thisdata = Normalpotassiumaldo[Normalpotassiumaldo$case == thiscase,]

 inits = inits_orig

 #Set sodium and potassium intake
 theta$Kin = thisdata$KIntake[1]/24/60
 theta$Nain = thisdata$NaIntake[1]/24/60
 theta$Kinfusion=0 #mEq/min
   #Run for 3 days on specified diet
 times=seq(0,24*60*3,1)
 ev=eventTable(time.units = 'minutes')
 ev$add.sampling(times)
 baseline <-data.frame(erfan83$run(theta, ev, inits))

 #Get new starting point
 inits = as.list(baseline[dim(baseline)[1], names(baseline) %in% names(inits1)])

 #Turn on infusion and simulate for two hours
 theta$Kinfusion = 0.62 #mEq/min

 times=seq(0, 2*60,1)
 ev=eventTable(time.units = 'minutes')
 ev$add.sampling(times)
 infusion <-data.frame(erfan83$run(theta, ev, inits))

 #Get new starting point
 inits2 = as.list(infusion[dim(infusion)[1], names(infusion) %in% names(inits1)])

 #Turn off infusion and simulation for 3 hours
 theta$Kinfusion = 0
```

```
  times=seq(1, 3*60,1)
  ev=eventTable(time.units = 'minutes')
  ev$add.sampling(times)
  infusion_off <-data.frame(erfan83$run(theta, ev, inits2))

  infusion_off$time = infusion_off$time + 120

  infusion = rbind(infusion, infusion_off)

  #save this case name
  infusion$case = cases[i]
  allCaseResults = rbind(allCaseResults,infusion )
    ########## Calculate Contribution to Objective function ##########
  K_scale = 1e6  #Scaling factor for potassium, to account for differences in units

  #Get simulation data matching experimental observation times
  sim_at_data_times = infusion[infusion$time %in% thisdata$time, ]

  #Calculate resisuals and  weighted sum of the squares
  residuals_K = sim_at_data_times$plasma_K - thisdata$Potassium/1000
  obj_K = K_scale*(sum(thisdata$weights*(residuals_K)^2))

  residuals_Aldo = sim_at_data_times$Aldo - thisdata$Aldo/36.044
  obj_aldo = (sum(thisdata$weights*(residuals_Aldo)^2))

  #sum up objective function values
  obj = obj + obj_K + obj_aldo
 }

 print(paste("OBJ:",obj))
 return(list(obj = obj, allCaseResults = allCaseResults))


}
```

```
#Load datasets digitized from study
Normalpotassiumaldo = read.csv("Dluly1972.csv")  #Dluly 1972

################################ Publication Figure 2 ############################
theta = theta_orig
inits = inits_orig

out = simDluly(theta)
allCaseResults = out$allCaseResults




h1 =  ggplot() +
  #geom_path(data = Normalpotassiumaldo, mapping = aes(x=time, y = Aldo/36.044)) +
  geom_path(data = allCaseResults, mapping = aes(x=time, y = Aldo,colour="Model" )) +
  geom_point(data = Normalpotassiumaldo, mapping = aes(x=time, y = Aldo/36.044,colour="Dluhy
1972")) +
  geom_errorbar(data = Normalpotassiumaldo, mapping = aes(x=time, ymin =(Aldo -AldoSD)/36.044,
ymax = (Aldo + AldoSD)/36.044))+
  facet_grid(rows = ~case)+xlab("Time (minutes)") +
  ylab("Plasma aldo (nmol/l)")+

  #ggtitle("Response of plasma levels of aldosterone and potassium in normal subjects") +
  expand_limits(y=0) +                        # Expand y range
  scale_y_continuous() + scale_x_continuous()+        # Set tick every 4
  theme_bw()+scale_colour_manual("",
                         breaks = c("Dluhy 1972", "Model"),
                         values = c("Black", "Red"))



h1
pdf("Figure2A.pdf", width = 8, height = 2.5)
grid.arrange(h1, nrow = 1)
dev.off()
```

```
h2 =  ggplot()+
  #geom_path(data = Normalpotassiumaldo, mapping = aes(x=time, y = Aldo/36.44)) +
  geom_path(data = allCaseResults, mapping = aes(x=time, y = plasma_K*1000,colour="Model")) +
  geom_point(data = Normalpotassiumaldo, mapping = aes(x=time, y = Potassium,colour="Dluhy 1972"))
+
  geom_errorbar(data = Normalpotassiumaldo, mapping = aes(x=time, ymin =Potassium -KSD, ymax =
Potassium + KSD))+
  facet_grid(rows = ~case)+xlab("Time (minutes)") +
  ylab("Plasma potassium (mEq/l)")+expand_limits(y=0) +
  scale_y_continuous() + scale_x_continuous()+
  theme_bw()+scale_colour_manual("",
                         breaks = c("Dluhy 1972", "Model"),
                         values = c("Black", "Red"))+ylim(2,8)

h2
pdf("Figure2B.pdf", width = 8, height = 2.5)
grid.arrange(h2, nrow = 1)
dev.off()



pdf("Figure2.pdf", width = 8, height = 5)
grid.arrange(h1, h2, nrow = 2)
dev.off()

allCaseResults$Kin = 200/24/60
allCaseResults$Kin[allCaseResults$case == "Low K Low Na Intake" | allCaseResults$case == "Low K
High Na Intake"] = 40/24/60
allCaseResults$Kin[allCaseResults$time < 120 & allCaseResults$time>0 ] =
allCaseResults$Kin[allCaseResults$time < 120 & allCaseResults$time>0 ] + 0.62



p1 = ggplot(allCaseResults)+ geom_path(aes(x=time, y = Kin, color = "Intake"), linetype = "dashed") +
  geom_path(aes(x=time, y = filtered_K*2e6, color = "Filtration")) +
```

```
  geom_path(aes(x=time, y =  2e6*(DCT_K_secretion_rate+ CNT_K_secretion_rate +
CCD_K_secretion_rate), color = "Tubular \nSecretion"))+
  geom_path(aes(x=time, y = (2e6*K_reabsorption_PT_LoH + 2e6*K_reabsorption_CD), color =
"Tubular \nReabsorption"))+
  geom_path(aes(x=time, y = CD_K_out, color = "Excretion" )) +
  facet_wrap(~case, nrow = 1) +
  theme_bw() +
  ylab("mEq/min") + xlab("Time (minutes)")  +
  scale_color_discrete(breaks = c("Intake", "Filtration", "Excretion", "Tubular \nReabsorption", "Tubular
\nSecretion"))+
  theme(legend.title = element_blank())

p2 = ggplot(allCaseResults)  + geom_path(aes(x=time, y = Kin, color = "Intake"), linetype = "dashed") +
  geom_path(aes(x=time, y = intracellular_potassium_flux, color = "Intracellular Flux"))+
  geom_path(aes(x=time, y = CD_K_out, color = "Excretion" )) +
  facet_wrap(~case, nrow = 1) +
  theme_bw()+
  ylab("mEq/min") + xlab("Time (minutes)") +
  scale_color_discrete(breaks = c("Intake", "Excretion", "Intracellular Flux")) +
  theme(legend.title = element_blank())

hh1 = ggarrange(h1, h2, common.legend = TRUE, nrow = 2, legend = "bottom")

hh1 = annotate_figure(hh1, fig.lab = "A", fig.lab.face = "bold")

hh2 = ggarrange(p1,p2,  nrow = 2, legend = "bottom")
hh2 = annotate_figure(hh2, fig.lab = "B", fig.lab.face = "bold")

ggarrange(hh1, hh2, nrow = 2) %>% ggexport(filename = "Figure2combined.pdf", width = 8, height = 9)
```

A-5. Model calibration: MRA pharmacokinetics parameters calibration

- This code simulates the pharmacokinetics of canrenone – Figure 3.3 .

```
############################## Potassium Homeostasis Model
#########################################
```

```
#Authors: KM Hallow, University of Georgia
#Feb 16, 2022

#This file simulates and plots the following studies:

#Gardiner, P., et al., Spironolactone metabolism: steady-state serum levels of the sulfur-containing metabolites.
#J Clin Pharmacol, 1989. 29(4): p. 342-7.

#Ravis, W.R., et al., Pharmacokinetics of eplerenone after single and multiple dosing in subjects with and without
#renal impairment. J Clin Pharmacol, 2005. 45(7): p. 810-21.

#Before running this file, run the file "runToEquilibrium.R"

################################################################################################

#Load PK data
pkdat = read.csv("Gardiner1989_spiro_PK.csv")

#reset parameters and intial conditions
theta = theta_orig
inits = inits_orig

#Create dosing and sampling table
ev = eventTable()
ev$add.sampling(seq(0,1440,by=1))
```

```
ev$add.dosing(dose = 100*1000, nbr.doses = 1, dosing.interval = 24, dosing.to = 1)


#Simulate
pk <-data.frame(erfan83$run(theta, ev, inits))
pk$time = pk$time


dat=pkdat[pkdat$Drug == "canrenone" ,]



hC=ggplot() + geom_point(data=dat, aes(x=Time_min, y = Conc_ng_ml,colour="Gardiner 1989")) +
geom_path(data=pk, aes(x=time, y = canrenone,color="Model"))+
  geom_errorbar(data = dat, mapping = aes(x=Time_min, ymin = Conc_ng_ml - SD, ymax = Conc_ng_ml
+ SD))+
  xlab("Time (minutes)")+
  ylab("Conc. (ng/ml)") +
  ggtitle("Canrenone")+
  theme_bw()+
  theme(plot.title = element_text(hjust = 0.5), legend.position = c(0.8,0.7))  +
  scale_x_continuous(breaks = seq(0, 1440, by = 240)) +
  scale_colour_manual("",
                      breaks = c("Gardiner 1989", "Model"),
                      values = c("Black", "Red"))

hC = annotate_figure(hC, fig.lab = "A", fig.lab.face = "bold")



pdf("PKfig.pdf", width = 5, height = 5)
grid.arrange(hC)
dev.off()
```

A-6. Model calibration: MRA pharmacodynamics parameters calibration

```
- This code simulates McInnes1982 and generates Figures 3.4 and 3.5.

############################## Potassium Homeostasis Model
########################################

#Authors: E Maddah,KM Hallow, University of Georgia

#November 22, 2021



#This file simulates the following study:

#McInnes, G., et al. (1982). "Spironolactone dose-response relationships in healthy subjects."

#British journal of clinical pharmacology 13(4): 513-518.



#Before running this file, run the file "runToEquilibrium.R"

##############################################################################################
##############

#Load base parameters and initial conditions

theta = theta_orig

inits = inits_orig

# simulating the  study

simMcInnes = function(theta) {

  Spiro_bioavailability = 0.75

  obj = 0
```

```
allCaseResults1=NULL
allCaseResults = NULL
timecoureResults = NULL

############################## Simulate Study Design ####################

#Doses to simulate
Dose<-c(0,25,50,100,200,400)

#Event Tables
times=seq(0,60*2,1)
evF=eventTable(time.units = 'minutes')
evF$add.sampling(times)

times=seq(0,60*24,1)
ev16=eventTable(time.units = 'minutes')
ev16$add.sampling(times)

cases = unique(potassiumexcretionSPR$Dose)

#### Simulate fludricortisone (same for all doses)

#set fludrocortisone
theta$D_FLU=1      #mg

#Run for 2 hours
Flud_ON <-data.frame(erfan83$run(theta, evF, inits))

#Get new starting point
inits = as.list(Flud_ON[dim(Flud_ON)[1], names(Flud_ON) %in% names(inits)])
```

```
  #Simulate placebo
  plac <-data.frame(erfan83$run(theta, ev16, inits))



  #simulate each Dose


  for (i in 1:6) {#6) {#length(cases))
    thiscase = cases[i]
    thisdata = potassiumexcretionSPR[potassiumexcretionSPR$Dose == thiscase,]


    #Set sprinolactone dose
    ev16=eventTable(time.units = 'minutes')
    ev16$add.sampling(times)
    ev16$add.dosing(dose = thisdata$Dose*1000*Spiro_bioavailability, nbr.doses = 1, dosing.interval =
24, dosing.to = 1)



    #Run for 16 hours
    SPR_ON <-data.frame(erfan83$run(theta, ev16, inits))


    #Calculate cumulative K+ excretion during each measurement period
    SPR_ON$K2_10 = SPR_ON$potassium_excretion_rate[SPR_ON$time == 10*60]-
SPR_ON$potassium_excretion_rate[SPR_ON$time == 2*60]
    SPR_ON$K12_16 = SPR_ON$potassium_excretion_rate[SPR_ON$time == 16*60]-
SPR_ON$potassium_excretion_rate[SPR_ON$time == 12*60]


    #Calculate cumulative K+ excretion on placebo during each measurement period
    plac$K2_10 = plac$potassium_excretion_rate[plac$time == 10*60]-
plac$potassium_excretion_rate[plac$time == 2*60]
    plac$K12_16 = plac$potassium_excretion_rate[plac$time == 16*60]-
plac$potassium_excretion_rate[plac$time == 12*60]


    #Calculate change from placebo
```

```
    SPR_ON$K2_10_deltaPlac = SPR_ON$K2_10 - plac$K2_10
    SPR_ON$K12_16_deltaPlac = SPR_ON$K12_16 - plac$K12_16

    #Calculate percent change from placebo
    SPR_ON$K2_10_pctdeltaPlac = SPR_ON$K2_10_deltaPlac/plac$K2_10
    SPR_ON$K12_16_pctdeltaPlac = SPR_ON$K12_16_deltaPlac/plac$K12_16

    #save this case name
    SPR_ON$Dose = Dose[i]
    allCaseResults = rbind(allCaseResults,SPR_ON[SPR_ON$time == max(SPR_ON$time),] )
    timecoureResults = rbind(timecoureResults, SPR_ON)

  }
  return(list(allCaseResults = allCaseResults, timecoureResults = timecoureResults))

}

#Load datasets digitized from  study
potassiumexcretionSPR = read.csv("McInnes1982.csv") #McInnes 1982

############################################################

theta = theta_orig
inits = inits_orig

out = simMcInnes(theta)
allCaseResults = out$allCaseResults


################## Absolute Change from Placebo
```

```
G1=ggplot()+geom_path(data = allCaseResults, mapping = aes(x=Dose ,y =
K2_10_pctdeltaPlac,colour="Model" ))+

  geom_point(data = potassiumexcretionSPR, mapping = aes(x=Dose, y =
pctChange210,colour="McInnes 1982"))+

  geom_errorbar(data = potassiumexcretionSPR, mapping = aes(x=Dose, ymin =(pctChange210  -
SDpct_210), ymax = (pctChange210  + SDpct_210),colour="McInnes 1982")) +

  ylab("Potassium Excretion (% Change)") +

  scale_colour_manual("",

                      breaks = c("McInnes 1982", "Model"),

                      values = c("Black", "Red"))+

  xlab("Dose (mg)") +


  ggtitle("2-10hrs") +

  #ylim(c(-12,6)) +                    # Expand y range

  scale_y_continuous() + scale_x_continuous()+        # Set tick every 4

  theme_bw() +

  theme(plot.title = element_text(hjust=0.5)) #, legend.position = c(0.8,0.8))


G2=ggplot()+geom_path(data = allCaseResults, mapping = aes(x=Dose ,y =
K12_16_pctdeltaPlac,colour="Model" ))+

  geom_point(data = potassiumexcretionSPR, mapping = aes(x=Dose, y =
pctChange1216,colour="McInnes 1982"))+

  geom_errorbar(data = potassiumexcretionSPR, mapping = aes(x=Dose, ymin =(pctChange1216 -
SDpct_1216), ymax = (pctChange1216 + SDpct_1216),colour="McInnes 1982")) +

  ylab("Potassium Excretion (% Change)") + scale_colour_manual("", breaks = c("McInnes 1982",
"Model"),values = c("Black", "Red"))+

  xlab("Dose (mg)") +


  ggtitle("12-16hrs") +

  ylim(c(-0.4,0.4)) +        # Set tick every 4

  theme_bw() +

  theme(plot.title = element_text(hjust=0.5))#, legend.position = c(0.8,0.8))
```

```
#G1 = annotate_figure(G1, fig.lab = "A", fig.lab.face = "bold")

#G2 = annotate_figure(G2, fig.lab = "B", fig.lab.face = "bold")


ggarrange(G1,G2, nrow = 2, common.legend = TRUE, legend = "top") %>% ggexport(filename =
"FigureMcInnes.pdf", width = 5, height = 7)



############# Plot timecourse ##########3

tc = out$timecoureResults


h1 = ggplot(tc, aes(x=time/60, y = MRA_effect, color= factor(Dose))) + geom_path() +

  theme_bw() +

  theme(plot.title = element_text(hjust = 0.5)) +

  labs(color = "Dose") +

  ggtitle("Pharmacodynamic Effect") + ylab("normalized value")  + xlab("Hour") +
scale_x_continuous(breaks = seq(0, 24, by = 4))


h2 =ggplot(tc, aes(x=time/60, y = CD_K_out*60, color= factor(Dose))) + geom_path() +

  theme_bw() +

  theme( plot.title = element_text(hjust = 0.5)) +

  labs(color = "Dose") +

  ggtitle(expression("K"^"+"*" Excretion")) + ylab("mEq/hr")+ xlab("Hour")+
scale_x_continuous(breaks = seq(0, 24, by = 4))


h3 =ggplot(tc, aes(x=time/60, y = plasma_K*1000, color= factor(Dose))) + geom_path() +

  theme_bw() +

  theme(plot.title = element_text(hjust = 0.5)) +

  labs(color = "Dose") +

  ggtitle(expression("Plasma K"^"+")) + ylab("mEq/L") + xlab("Hour")+   scale_x_continuous(breaks =
seq(0, 24, by = 4))


h4 =ggplot(tc, aes(x=time/60, y = Aldo, color= factor(Dose))) + geom_path() +

  theme_bw() +
```

```
  theme( plot.title = element_text(hjust = 0.5)) +
  labs(color = "Dose") +
  ggtitle("Plasma Aldosterone") + ylab("nmol/L") + xlab("Hour")+   scale_x_continuous(breaks = seq(0, 24, by = 4))


h5 = ggplot(tc, aes(x=time/60, y = Aldo_effect_on_K_secretion/MRA_effect, color= factor(Dose))) + geom_path() +
  theme_bw() +
  theme( plot.title = element_text(hjust = 0.5)) +
  labs(color = "Dose") +
  ggtitle(expression("Aldo Effect on K"^"+"*" Secretion")) + ylab("normalized value")+ xlab("Hour")+ scale_x_continuous(breaks = seq(0, 24, by = 4))



h7 = ggplot(tc, aes(x=time/60, y = (DCT_K_secretion_rate + CNT_K_secretion_rate + CCD_K_secretion_rate)*60*2e6, color= factor(Dose))) + geom_path() +
  theme_bw() +
  theme(plot.title = element_text(hjust = 0.5)) +
  labs(color = "Dose") + ggtitle(expression("Tubular K"^"+"*" Secretion"))+
  ylab("mEq/hr") + xlab("Hour")+   scale_x_continuous(breaks = seq(0, 24, by = 4))


ggarrange(h1,h2,h3,h4,h5,h7, common.legend = TRUE,legend = "bottom") %>%
  ggexport(filename = "SpiroPDMcInnes.pdf", width = 10, height = 8)
```

A-7. Model validation: Chronic spironolactone administration in patients with hyperaldosteronism

- This code simulates Karagiannis 2008 and generates Figure 3.6 from the manuscript.

```
############################## Potassium Homeostasis Model
########################################


#Authors: Erfan Maddah, KM Hallow, University of Georgia
#November 22, 2021
```

```
#This file simulates:

#Karagiannis, A., et al. (2008). "Spironolactone versus eplerenone for the treatment of idiopathic
#hyperaldosteronism." Expert opinion on pharmacotherapy 9(4): 509-515.

#Before running this file, run the file "runToEquilibrium.R"

#

################################################################################################
##############



# Load Study data
dat = read.csv("Karagiannis2008.csv")

######################################### Simulate Spironolactone Arm
############################



##################### Make Hyperaldosteronism ################
theta = theta_orig
inits = inits_orig

#Increase aldosterone concentration
theta$hyperaldo_effect =0.55 #0.4 #1

#Time
times=seq(0,28*60*24,1)
ev=eventTable(time.units = 'minutes')
ev$add.sampling(times)
```

```
#Simulate to new baseline
h <-data.frame(erfan83$run(theta, ev, inits))
inits = as.list(h[dim(h)[1], names(h) %in% names(inits)])

#Store new starting parameters
theta_start = theta

### Simulate Spironolactone to week 4
times=seq(0, 4*7*24*60,1)
ev=eventTable(time.units = 'minutes')
ev$add.sampling(times)
ev$add.dosing(dose = 25*1000, nbr.doses = 4*7*2, dosing.interval = 12*60, dosing.to = 1)


SPR4W <-data.frame(erfan83$run(theta, ev, inits))
SPR4W=data.frame(SPR4W)
#Define new starting point
inits = as.list(SPR4W[dim(SPR4W)[1], names(SPR4W) %in% names(inits)])

### Increase Dose, Simulate Spironolactone to week 8
ev=eventTable(time.units = 'minutes')
ev$add.sampling(times)
ev$add.dosing(dose = 50*1000, nbr.doses = 4*7*2, dosing.interval = 12*60, dosing.to = 1)


SPR8W <-data.frame(erfan83$run(theta, ev, inits))
SPR8W=data.frame(SPR8W)

#Define new starting point
inits = as.list(SPR8W[dim(SPR8W)[1], names(SPR8W) %in% names(inits)])
```

```
### Increase Dose, Simulate Spironolactone to week 12
ev=eventTable(time.units = 'minutes')
ev$add.sampling(times)
ev$add.dosing(dose = 100*1000, nbr.doses = 4*7*2, dosing.interval = 12*60, dosing.to = 1)

SPR12W <-data.frame(erfan83$run(theta, ev, inits))
SPR12W=data.frame(SPR12W)

#Define new starting point
inits = as.list(SPR12W[dim(SPR12W)[1], names(SPR12W) %in% names(inits)])

### Increase Dose, Simulate Spironolactone to week 16

ev=eventTable(time.units = 'minutes')
ev$add.sampling(times)
ev$add.dosing(dose = 200*1000, nbr.doses = 4*7*2, dosing.interval = 12*60, dosing.to = 1)

SPR16W <-data.frame(erfan83$run(theta, ev, inits))
SPR16W=data.frame(SPR16W)

#make a new data frame includes the data and model response
modelsprS=c(h$plasma_K[40320],SPR4W$plasma_K[40320],SPR8W$plasma_K[40320],SPR12W$plas
ma_K[40320],SPR16W$plasma_K[40320])

Data_modelS = cbind(dat[dat$Treat == "Spironolactone",],modelsprS*1000)

#plot
N=ggplot(Data_modelS, aes(x=Time, y=serum_potassium, colour="Karagiannis 2008")) +
  geom_errorbar(aes(ymin=PSD1, ymax=PSD2), width=.3) +
  geom_line(aes(y=modelsprS*1000,x=Time,colour="Model")) +
  geom_point(aes(x=Time, y=serum_potassium, colour="Karagiannis 2008")) +
  scale_colour_manual("",
```

```
                breaks = c("Karagiannis 2008", "Model"),
                values = c("Black", "Red"))+
  xlab("Week") +
 ylab("Plasma potassium (mEq/L)") +

 #ggtitle("Chronic changes of serum potassium after spironolactone administration") +
 #expand_limits(y=0) +                    # Expand y range
 scale_y_continuous() + scale_x_continuous(breaks=0:20*4)+        # Set tick every 4
 theme_bw() +theme(text=element_text(size=14 ))+
 annotate("rect", xmin = 0, xmax = 4, ymin = -Inf, ymax = Inf,
             alpha = 0.15, fill = "grey") +
 annotate("rect", xmin = 4, xmax = 8, ymin = -Inf, ymax = Inf,
         alpha = 0.3, fill = "grey") +
 annotate("rect", xmin = 8, xmax = 12, ymin = -Inf, ymax = Inf,
         alpha = 0.45, fill = "grey")+
 annotate("rect", xmin = 12, xmax = 16, ymin = -Inf, ymax = Inf,
         alpha = 0.6, fill = "grey")+
 annotate("text", x = 2, y = 2.9,
         label = " 50 mg\nb.i.d.") +
 annotate("text", x = 6, y = 2.9,
         label = " 100 mg\nb.i.d.")+
 annotate("text", x = 10, y = 2.9,
         label = " 200 mg\nb.i.d.")+
 annotate("text", x = 14, y = 2.9,
         label = " 400 mg\nb.i.d.")+
 theme(legend.position = "bottom", plot.title = element_text(hjust = 0.5))



 #theme(legend.justification=c(1,0),
      #legend.position=c(1,0))              # Position legend in bottom right
N
```

```
N %>% ggexport(filename = "FigureKaragiannis.pdf", width = 5, height = 5)
```

APPENDIX B

Local Sensitivity Analysis

This appendix contains text from the following publication:

Maddah, Erfan, and K. Melissa Hallow.

"A quantitative systems pharmacology model of plasma potassium regulation by the kidney and aldosterone." *Journal of Pharmacokinetics and Pharmacodynamics* 49.4 (2022): 471-486.

B-1. Local sensitivity (analysis 1)

Perturbation: Potassium infusion

Output Response: Plasma Aldosterone, Plasma potassium concentrations

Procedure:

- Parameters varied individually.
- When necessary, dependent baseline parameters and initial conditions recalculated using new parameter value.
- Change in potassium and change in aldosterone calculated at end of 1-hour infusion of 0.1 mEq/min potassium
- Sensitivity Indices calculated:
    - Change in plasma potassium / % change in parameter value
    - Change in plasma aldosterone / % change in parameter value

Results:

Table B.1. Plasma potassium sensitivity (analysis 1)

Parameter	Sensitivity Index
$m_{K,Aldo}$	0.046965194
m_{aldo-K}	0.046090814
Q_{K-ic}	0.000871876
$m_{K-P,MCD}$	7.88E-08
$m_{Na,MCD}$	5.27E-11
$m_{Na,Aldo}$	4.62E-11

Table B.2. Plasma aldosterone sensitivity (analysis 1)

Parameter	Sensitivity Index
m_{aldo-K}	0.232907
$m_{K,Aldo}$	0.017519
Q_{K-ic}	0.00421
$m_{K-P,MCD}$	3.21E-07
$m_{Na,MCD}$	2.10E-10
$m_{Na,Aldo}$	1.84E-10

B-2. Local sensitivity (analysis 2)

Perturbation: Sodium infusion

Output Response: Plasma Aldosterone, Plasma potassium

Procedure:

- Parameters varied individually.
- When necessary, dependent baseline parameters and initial conditions recalculated using new parameter value.
- Change in potassium and change in aldosterone calculated at end of 1-hour infusion of 0.3 mEq/min sodium
- Sensitivity Indices calculated:
    - Change in plasma potassium / % change in parameter value
    - Change in plasma aldosterone / % change in parameter value

Results:

Table B.3. Plasma potassium sensitivity (analysis 2)

Parameter	Sensitivity Index
$\mathbf{m_{K,Aldo}}$	0.046965194
$\mathbf{m_{aldo\text{-}K}}$	0.046090814
$\mathbf{Q_{K\text{-}ic}}$	0.000871876
$\mathbf{m_{K\text{-}P,MCD}}$	7.88E-08
$\mathbf{m_{Na,MCD}}$	5.27E-11
$\mathbf{m_{Na,Aldo}}$	4.62E-11

Table B.4. Plasma aldosterone sensitivity (analysis 2)

Parameter	Sensitivity Index
$\mathbf{m_{aldo\text{-}K}}$	0.232907
$\mathbf{m_{K,Aldo}}$	0.017519
$\mathbf{Q_{K\text{-}ic}}$	0.00421
$\mathbf{m_{K\text{-}P,MCD}}$	3.21E-07
$\mathbf{m_{Na,MCD}}$	2.10E-10
$\mathbf{m_{Na,Aldo}}$	1.84E-10

C-1. Model codes (Integrated model)

```
############################### Define model structure ##########################
ode <- "

  #Disease effects on nephrons
        number_of_functional_glomeruli = baseline_nephrons*(1 -
disease_effect_losing_glomeruli);

  # Assume that other nephrons are lost due to tubular effects, which do not affect the glomerulus
        number_of_functional_tubules = baseline_nephrons*(1-disease_effect_on_nephrons);



############################### Endothelin Kinetics ##################################
#Concentration = Amount / Volume,  pmol/L
BigET = BigET_amt/V_bigET
ET1_total_peri = ET1_total_peri_amt/V_peri
ET1_total_cent = ET1_total_cent_amt/V_cent
ET1_total_peri_labeled =ET1_total_peri_labeled_amt/V_peri
ET1_total_cent_labeled = ET1_total_cent_labeled_amt/V_cent

if (ETA_ramp == 1) {
ETA_inhibitor_effect = min(ETA_inhibition, ETA_inhibition*time/ETA_inhib_slope)
ETA_inhibitor_effect_peri = min(ETA_inhibition_peri,
ETA_inhibition_peri*time/ETA_inhib_slope)

} else {
  if (ETA_ramp ==-1 ) { #turn off drug
     ETA_inhibitor_effect = ETA_inhibition*exp(-time*log(2)/ETA_halflife)
```

```
    ETA_inhibitor_effect_peri = ETA_inhibition_peri*exp(-time*log(2)/ETA_halflife)
  }   else {
    ETA_inhibitor_effect = ETA_inhibition
    ETA_inhibitor_effect_peri = ETA_inhibition_peri
  }
}


if (ETB_ramp == 1) {
ETB_inhibitor_effect = min(ETB_inhibition, ETB_inhibition*time/ETB_inhib_slope)
ETB_inhibitor_effect_peri = min(ETB_inhibition_peri,
ETB_inhibition_peri*time/ETB_inhib_slope)

} else {
  if ( ETB_ramp == -1) {  #turn off drug over time
    ETB_inhibitor_effect = ETB_inhibition*exp(-time*log(2)/ETB_halflife)
    ETB_inhibitor_effect_peri = ETB_inhibition_peri*exp(-time*log(2)/ETB_halflife)
  }   else {
    ETB_inhibitor_effect = ETB_inhibition
    ETB_inhibitor_effect_peri = ETB_inhibition_peri
  }
}


#Assume constant receptor concentrations, pmol/L
ETA_total_cent = ETA_total_cent0*(1-ETA_inhibitor_effect)
ETA_total_peri = ETA_total_peri0*(1-ETA_inhibitor_effect_peri)
ETB_total_cent = ETB_total_cent0*(1-ETB_inhibitor_effect)
ETB_total_peri = ETB_total_peri0*(1-ETB_inhibitor_effect_peri)
```

#Receptor internalization rate

#Qint = Kint*V*[Rtot], pmol/min

QintB_peri = Qint_peri*(ETB_internalization_fraction)*(1-ETB_inhibitor_effect_peri)

QintA_peri = Qint_peri*(1-ETB_internalization_fraction)*(1-ETA_inhibitor_effect_peri)

QintB_cent = Qint_cent*(ETB_internalization_fraction)*(1-ETB_inhibitor_effect)

QintA_cent = Qint_cent*(1-ETB_internalization_fraction)*(1-ETA_inhibitor_effect)

#Track radiolabeled and non-labeled entities, pmol/L

ET1_total_cent_combined = ET1_total_cent + ET1_total_cent_labeled

ET1_total_peri_combined = ET1_total_peri + ET1_total_peri_labeled

ET1_cent_fraction_labeled = ET1_total_cent_labeled/ET1_total_cent_combined

ET1_peri_fraction_labeled = ET1_total_peri_labeled/ET1_total_peri_combined

#Calculate Free ET1 from total ET1 and total receptor concentrations, under quasiequilibrium assumption (Mager 2005)

#pmol/L

ET1_peri_combined =(1/2)*(ET1_total_peri_combined - (ETA_total_peri + ETB_total_peri) - Kd_ET1 +

sqrt((ET1_total_peri_combined - (ETA_total_peri + ETB_total_peri) - Kd_ET1)^2 + 4*Kd_ET1*ET1_total_peri_combined))

ET1_cent_combined =(1/2)*(ET1_total_cent_combined - (ETA_total_cent + ETB_total_cent) - Kd_ET1 +

sqrt((ET1_total_cent_combined - (ETA_total_cent + ETB_total_cent) - Kd_ET1)^2 + 4*Kd_ET1*ET1_total_cent_combined))

#Separate unlabeled and radiolabeled portions

ET1_cent = ET1_cent_combined*(1-ET1_cent_fraction_labeled)

ET1_cent_labeled = ET1_cent_combined*ET1_cent_fraction_labeled

ET1_peri = ET1_peri_combined*(1-ET1_peri_fraction_labeled)

ET1_peri_labeled = ET1_peri_combined*ET1_peri_fraction_labeled

#Receptor-Bound Fraction

#fraction

ETA_cent_bound_fraction_combined = ET1_cent_combined/(Kd_ET1 + ET1_cent_combined)

ETA_peri_bound_fraction_combined = ET1_peri_combined/(Kd_ET1 + ET1_peri_combined)

ETB_cent_bound_fraction_combined = ET1_cent_combined/(Kd_ET1 + ET1_cent_combined)

ETB_peri_bound_fraction_combined = ET1_peri_combined/(Kd_ET1 + ET1_peri_combined

#Ligand-receptor complex, pmol/L

ET1_ETA_peri_combined = ETA_total_peri*ETA_peri_bound_fraction_combined

ET1_ETB_peri_combined = ETB_total_peri*ETB_peri_bound_fraction_combined

ET1_ETA_cent_combined = ETA_total_cent*ETA_cent_bound_fraction_combined

ET1_ETB_cent_combined = ETB_total_cent*ETB_cent_bound_fraction_combined

#Separate unlabeled and radiolabeled portions

ET1_ETA_cent = ET1_ETA_cent_combined*(1-ET1_cent_fraction_labeled)

ET1_ETA_cent_labeled = ET1_ETA_cent_combined*ET1_cent_fraction_labeled

ET1_ETB_cent = ET1_ETB_cent_combined*(1-ET1_cent_fraction_labeled)

ET1_ETB_cent_labeled = ET1_ETB_cent_combined*ET1_cent_fraction_labeled

ET1_ETA_peri = ET1_ETA_peri_combined*(1-ET1_peri_fraction_labeled)

ET1_ETA_peri_labeled = ET1_ETA_peri_combined*ET1_peri_fraction_labeled

ET1_ETB_peri = ET1_ETB_peri_combined*(1-ET1_peri_fraction_labeled)

ET1_ETB_peri_labeled = ET1_ETB_peri_combined*ET1_peri_fraction_labeled

#---------- ET1 production from Big-ET

#pmol/min

ET1_production_from_BIGET = E_ECE_BigET * BigET * ECE_conc * V_peri

#---------- ET1 distribution between compartments

#pmol/min

ET1_distribution = - Q_ET1_pc*ET1_peri + Q_ET1_cp*ET1_cent

ET1_distribution_labeled = - Q_ET1_pc*ET1_peri_labeled + Q_ET1_cp*ET1_cent_labeled

#---------- ET1 clearance by receptor internalization

#pmol/min

ET1_ETB_internalization_peri = QintB_peri*(1-ETB_inhibition)*ETB_peri_bound_fraction_combined*(1-ET1_peri_fraction_labeled)

ET1_ETB_internalization_peri_labeled = QintB_peri*ETB_peri_bound_fraction_combined*ET1_peri_fraction_labeled

ET1_ETA_internalization_peri = QintA_peri*ETA_peri_bound_fraction_combined*(1-ET1_peri_fraction_labeled)

ET1_ETA_internalization_peri_labeled = QintA_peri*ETA_peri_bound_fraction_combined*ET1_peri_fraction_labeled

ET1_ETB_internalization_cent = QintB_cent*(1-ETB_inhibition)*ETB_cent_bound_fraction_combined*(1-ET1_cent_fraction_labeled)

ET1_ETB_internalization_cent_labeled = QintB_cent*ETB_cent_bound_fraction_combined*(ET1_cent_fraction_labeled)

ET1_ETA_internalization_cent = QintA_cent*ETA_cent_bound_fraction_combined*(1-ET1_cent_fraction_labeled)

ET1_ETA_internalization_cent_labeled = QintA_cent*ETA_cent_bound_fraction_combined*(ET1_cent_fraction_labeled)

################################## Systemic Potassium
##

plasma_K= K/ ((blood_volume_L)*1000); #(mEq/mL)

interstitial_K_conc = interstitial_K / (interstitial_fluid_volume*1000); #(mEq/mL)

intracellular_K_conc = intracellular_K / (intracellular_fluid_volume*1000);

interstitial_potassium_flux = Q_Na*1000*(plasma_K - interstitial_K_conc)

intracellular_potassium_flux = Q_K_intracellular*1000*(interstitial_K_conc - norm_plasma_K -

(intracellular_K_conc - nom_intracellular_K_conc));

######################Systemic Hemodynamics
#####################################

#Hematocrit

RBC_content = nom_hematocrit*blood_volume_nom;

hematocrit = RBC_content /blood_volume_L;

######Calculation of Systemic Vascular Resistance

#Systemic vascular resistance is a nominal value modulated by AngII and by a regulatory signal for tissue autoregulation necessary to maintain constant organ blood flow

#Baroreceptor effect

baroreceptor_TPR_effect = 1-Kp_baroreceptor*(MAP_delayed - MAP_setpoint);#

###Whole body autoregulation mechanism wherein TPR adjusts to maintain constant organ blood flow (and thus constant cardiac output)

#Modeled as Proportional-Integral controller of TPR, where the input signal is the cardiac output error signal

tissue_autoregulation_signal = max(0.1,1+tissue_autoreg_scale*((Kp_CO/CO_scale_species)*(cardiac_output_delayed - CO_nom)+(Ki_CO/CO_scale_species)*CO_error));

###Effect of the RAAS (AT1-bound AngII) on systemic vascular resistance. For now, the slope is set to zero, i.e. AngII does not directly affect SVR

AT1_svr_int = 1 - AT1_svr_slope*nominal_equilibrium_AT1_bound_AngII;

AT1_bound_AngII_effect_on_SVR = AT1_svr_int + AT1_svr_slope * AT1_bound_AngII;

###Effect of RSNA on systemic vascular resistance

rsna_svr_int = 1 + rsna_svr_scale/2;

rsna_effect_on_svr = rsna_svr_int - rsna_svr_scale/(1+exp((rsna_delayed - 1) / rsna_svr_slope));

Effect of ANP on systemic vascular resistance

ANP_svr_int = 1 + ANP_svr_scale/2;

ANP_effect_on_svr= ANP_svr_int - ANP_svr_scale/(1+exp(-(ANP_delayed - nom_ANP)/ANP_effect_slope));

#ET1_ETA_cent_on_SVR = 1 + ET1_ETA_svr_scale1/(1+exp(-(ET1_ETA_cent - ET1_ETA_cent0)/ET1_ETA_svr_slope)) - ET1_ETA_svr_scale1/2

ET1_ETA_cent_on_SVR = 1 + ET1_ETA_svr_scale1*(ET1_ETA_cent - ET1_ETA_cent0)

ET1_ETB_cent_on_SVR = 1 + ET1_ETB_svr_scale1*(ET1_ETB_cent - ET1_ETB_cent0)

ET1_ETAETB_effect_on_SVR = (ET1_ETA_cent_on_SVR-1) + (ET1_ETB_cent_on_SVR - 1);

systemic_arterial_resistance = nom_systemic_arterial_resistance*tissue_autoregulation_signal*AT1_bound_AngII_effect_on_SVR*baroreceptor_TPR_effect*rsna_effect_on_svr*ANP_effect_on_svr*(1+ET1_ETAETB_effect_on_SVR); #rsna_effect_on_svr*

ET1_ETA_cent_on_venous_capacity = 1 + ET1_ETA_venous_capacity_scale*(ET1_ETA_cent- ET1_ETA_cent0)

ET1_ETA_cent_on_venous_resistance = 1 + ET1_ETA_venous_resistance_scale*(ET1_ETA_cent- ET1_ETA_cent0)

```
venous_resistance = R_venous*R_ET1_ETA_cent_on_venous_resistance;
#*rsna_effect_on_svr;

#Effect of SNA on venous compliance

sna_venous_stiffness_int = 1 - sna_venous_stiffness_scale/2;

sna_effect_on_venous_stiffness = sna_venous_stiffness_int +
sna_venous_stiffness_scale/(1+exp((rsna_delayed - 1) / sna_stiffness_slope));

#account for strain-stiffening behavior

pressure_effect_on_stiffness =
1+venous_pressure_stiffness_scale*max(right_atrial_pressure_delayed -
nom_right_atrial_pressure, 0);

sna_effect_on_venous_compliance =
1/(sna_effect_on_venous_stiffness*pressure_effect_on_stiffness);

###### Determination of Cardiac Output and Mean Arterial Pressure

#Cardiac output is a function of blood volume and resistance to venous return

###Empirical relationship between SVR, venous resistance, and resistance to venous return

resistance_to_venous_return = ((8 * venous_resistance + systemic_arterial_resistance) /
venous_return_scale); # 31);

effective_venous_compliance = venous_compliance* sna_effect_on_venous_compliance

venous_capacity = nom_venous_capacity*R_ET1_ETA_cent_on_venous_capacity;

mean_filling_pressure = nom_mean_filling_pressure + (blood_volume_L/BV_scale_species-
venous_capacity)/(effective_venous_compliance);

#venous_resistance2= 0.8

#central_venous_pressure = mean_filling_pressure - cardiac_output_delayed*
venous_resistance2;

  right_atrial_pressure = nom_right_atrial_pressure + (blood_volume_L/BV_scale_species-
venous_capacity)/(effective_venous_compliance);

venous_return = ((mean_filling_pressure) / resistance_to_venous_return);

rsna_HR_int = 1 -rsna_HR_scale/2;

rsna_effect_on_HR = rsna_HR_int + rsna_HR_scale/(1+exp((1 -
rsna_delayed2)/rsna_HR_slope));

cardiac_output = VR_CO_scale*venous_return*rsna_effect_on_HR;

total_peripheral_resistance = systemic_arterial_resistance + venous_resistance;

mean_arterial_pressure_MAP = (cardiac_output * total_peripheral_resistance) +
mean_filling_pressure ;
```

Calculation of RSNA, ANP, and MR-bound Aldosterone

###Renal sympathetic nerve activity is assumed to be driven by changes in MAP and Right atrial pressure. The dominant effect is MAP, and the effect of RAP is much less, at least in the normal physiologic range.

MAP_rsna_int = 1-MAP_rsna_scale/2;

MAP_effect_on_rsna = MAP_rsna_int + MAP_rsna_scale / (1 + exp((mean_arterial_pressure_MAP - MAP_setpoint) / map_rsna_slope));

RAP_rsna_int = 1-RAP_rsna_scale/2;

R_atrial_pressure_effect_on_rsna = RAP_rsna_int + RAP_rsna_scale / (1 + exp((right_atrial_pressure - RAP_setpoint) / RAP_rsna_slope));

renal_sympathetic_nerve_activity = nom_rsna*MAP_effect_on_rsna * R_atrial_pressure_effect_on_rsna*BB_effect_on_RSNA;

###ANP release is driven by changes in right atrial pressure

#Raine et al NEJM 1986 315(9):533-7

#measured ANP and right atrial pressure in CHF patients with normal and elevated rigth atrial pressure ANP = 13.6 * RAP - 16.7

normalized_atrial_NP_concentration = ((right_atrial_pressure)*rap_anp_slope - rap_anp_intercept)/nom_ANP

ANP_concentration = normalized_atrial_NP_concentration*nom_ANP;

######################### Aldosterone and Renin Secretion

###Aldosterone is secreted in response to AT1-bound AngII and changes in potassium or sodium concentration

#Potassium concentration is treated as a constant for now

#Empirircal relationship for Karaaslan 2005

AngII_effect_on_aldo =exp(AT1_aldo_slope*(AT1_bound_AngII - nominal_equilibrium_AT1_bound_AngII))

#The equation below contains a number of drugs - this doesn't represent drug interactions. Only one of them is tested at a time, the others are set to zero.

#This is only for convenience to use in shiny app or run the code without making modification when testing each drug individually from runCVRsim

#- the drug interactions should be treated more carefully.

ACEI_effect_on_ACE_activity = (1 - pct_target_inhibition_Enalapril_ACEi - pct_target_inhibition_Ramipril_ACEi);

#The same holds for the equation below

ARB_effect_on_AT1_binding = (1 - pct_target_inhibition_Valsatran_ARB_320mg - pct_target_inhibition_Valsatran_ARB_160mg

- pct_target_inhibition_Losartan_ARB_100mg

- pct_target_inhibition_Irbesartan_ARB_150mg

- pct_target_inhibition_Irbesartan_ARB_300mg

- pct_target_inhibition_Candesartan_ARB_4mg

- pct_target_inhibition_Candesartan_ARB_8mg

- pct_target_inhibition_Candesartan_ARB);

DRI_effect_on_PRA = 1 - pct_target_inhibition_Aliskiren_150mg - pct_target_inhibition_Aliskiren_300mg - pct_target_inhibition_Aliskiren_600mg;

###Renin is secreted in response to decreases in AT1-bound AngII, decreases in MD sodium flow, or increases in RSNA

#RSNA effect on renin secretion

rsna_renin_intercept = 1-rsna_renin_slope;

rnsa_effect_on_renin_secretion = rsna_renin_slope * renal_sympathetic_nerve_activity + rsna_renin_intercept;

#Macula Densa Sodium flow effect on renin secretion

#This relationship is known to be non-linear, and md_renin_tau can be calibrated based on data on changes in renin as a functoin of sodium intake

#e.g. Isaksson 2014

md_effect_on_renin_secretion = md_renin_A*exp(-md_renin_tau*(SN_macula_densa_Na_flow_delayed*baseline_nephrons - nom_LoH_Na_outflow));

md_K_effect_on_renin_secretion = md_renin_A*exp(-md_renin_tau_K*(LoH_K_out_delayed*baseline_nephrons - nom_LoH_K_outflow));

#AT1-bound AngII feedback on renin secretion

AT1_bound_AngII_effect_on_PRA = (10 ^ (AT1_PRC_slope * log10(AT1_bound_AngII / nominal_equilibrium_AT1_bound_AngII) + AT1_PRC_yint));

plasma_renin_activity = concentration_to_renin_activity_conversion_plasma* plasma_renin_concentration*DRI_effect_on_PRA;

```
renin_secretion_rate =
(log(2)/renin_half_life)*nominal_equilibrium_PRC*AT1_bound_AngII_effect_on_PRA*md_eff
ect_on_renin_secretion*md_K_effect_on_renin_secretion*rnsa_effect_on_renin_secretion*HCT
Z_effect_on_renin_secretion*renin_hyperactivity;

renin_degradation_rate = log(2)/renin_half_life;

AngI_degradation_rate = log(2)/AngI_half_life;

AngII_degradation_rate = log(2)/AngII_half_life;

AT1_bound_AngII_degradation_rate =  log(2)/AT1_bound_AngII_half_life;

AT2_bound_AngII_degradation_rate = log(2)/AT2_bound_AngII_half_life;

ACE_activity = nominal_ACE_activity*ACEI_effect_on_ACE_activity;

chymase_activity = nominal_chymase_activity;

AT1_receptor_binding_rate =
nominal_AT1_receptor_binding_rate*ARB_effect_on_AT1_binding;

AT2_receptor_binding_rate = nominal_AT2_receptor_binding_rate;

####MRAs

#Emax model

E_MRA_spiro=E_MAX_spiro*canrenone/(canrenone+EC50_spiro);  #E_MAX model

E_MRA_epl=E_MAX_epl*epl_C1/(epl_C1+EC50_epl);  #E_MAX model

E_MRA_esax = E_esax  #0 when no esax present

MR=1;

Kon_MRA = Koff_MRA

######################## Systemic Volume
##########################################


###################### Plasma sodium concentration and vasopressin secretion

###Plasma sodium concentration

Na_concentration = sodium_amount / blood_volume_L;

IF_Na_concentration = IF_sodium_amount/interstitial_fluid_volume;

if ((IF_Na_concentration - ref_Na_concentration) > 0) { #sodium storage is increasing

  sodium_storate_rate = Q_Na_store*((max_stored_sodium -
stored_sodium)/max_stored_sodium)*(IF_Na_concentration - ref_Na_concentration);

} else {
```

```
  sodium_storate_rate = Q_Na_store*(( stored_sodium - (-
max_stored_sodium))/max_stored_sodium)*(IF_Na_concentration - ref_Na_concentration);

}

###Control of vasopressin secretion

#A proportional-integral controller is used to ensure there is no steady state error in sodium
concentration

#Relative gains of the P and I controller must be chosen carefully.

#In order to permit a steady-state error, the integral controller can be removed. But care should
be given then in choosing the proportional gain

Na_water_controller = Na_controller_gain*(Kp_VP*(Na_concentration - ref_Na_concentration
+ 1000*(plasma_K - norm_plasma_K ))+Ki_VP*(Na_concentration_error));

right_atrial_pressure_effect_on_vasopressin = exp(-
right_atrial_pressure_vasopressin_slope*(right_atrial_pressure_delayed -
nom_right_atrial_pressure));

###Vasopressin

#Vasopressin is critical in the model, because it allows water excretion to be decoupled from
sodium excretion in the collecting duct

normalized_vasopressin_concentration = max(0, 1 + Na_water_controller+
(right_atrial_pressure_effect_on_vasopressin-1));

vasopressin_concentration = nominal_vasopressin_conc *
normalized_vasopressin_concentration;

#Effect of vasopressin on water intake

water_oral_intake_rate = Ka_water*water_oral_depot;  #Added to allow administration of fixed
fluid intake when simulating experimental studies. Value is usually zero.

water_intake_vasopressin_int = 1-water_intake_vasopressin_scale/2;

water_intake =
water_intake_species_scale*(nom_water_intake/60/24)*(water_intake_vasopressin_int +
water_intake_vasopressin_scale/(1+exp((normalized_vasopressin_concentration_delayed-
1)/water_intake_vasopressin_slope))) + water_oral_intake_rate;

daily_water_intake = (water_intake * 24 * 60);

###Systemic Starling Forces

IC_sodium_amount = 10*25; #mmol

IC_Na_concentration = IC_sodium_amount/intracellular_fluid_volume;

plasma_protein_concentration = plasma_protein_amount / (blood_volume_L * L_dL);
```

```
ISF_protein_concentration = ISF_protein_amount / (interstitial_fluid_volume * L_dL);

IC_protein_amount = 127*25; #mg

IC_protein_concentration = IC_protein_amount/intracellular_fluid_volume;

#Fit to Guyton 1965

if (interstitial_fluid_volume <= IFV_piecewise_pressure) {

  ISF_pressure = -A_interstitium_low*exp((IFV_piecewise_pressure-
interstitial_fluid_volume)*k_interstitium_low) + ISF_pressure0;

} else {

  intercept_high = A_interstitium_low + A_interstitium_high - ISF_pressure0;

  ISF_pressure = A_interstitium_high*exp(k_interstitium_high*(interstitial_fluid_volume -
IFV_piecewise_pressure)) -intercept_high;

}

Blood_volume_protein_osmotic_pressure = 1.629*plasma_protein_concentration +
0.2935*plasma_protein_concentration^2;

ISF_protein_osmotic_pressure = 1.629*ISF_protein_concentration +
0.2935*ISF_protein_concentration^2;

IC_protein_osmotic_pressure =
1.629*IC_protein_concentration+0.2935*(IC_protein_concentration^2);

Blood_volume_osmotic_pressure = Blood_volume_protein_osmotic_pressure +
Na_concentration*19.3*2;

ISF_osmotic_pressure = IF_Na_concentration*19.3*2 + ISF_protein_osmotic_pressure;

IC_osmotic_pressure = IC_Na_concentration*19.3*2 + IC_protein_osmotic_pressure;

Protein_sodium_filtration_pressure_grad = (mean_filling_pressure  - ISF_pressure -
Blood_volume_osmotic_pressure + ISF_osmotic_pressure);

blood_interstitium_flux =
Sodium_protein_filtration_rate_Kf*(Protein_sodium_filtration_pressure_grad)*0.001;

interstitial_intracellular_flux = Kf_IC*(ISF_osmotic_pressure - IC_osmotic_pressure);

plasma_osmolality = glucose_concentration + 2*(Na_concentration + plasma_K);

################################## Aldosterone Regulation
##############################################


  #Aldosterone, nmol/L

    plasma_K_effect_on_aldo = 1 + m_K_ALDO*(plasma_K -norm_plasma_K)
```

```
  Na_effect_on_aldo = max(0,(exp(-m_Na_ALDO*(Na_concentration -
ref_Na_concentration))))

  plasma_osmolality_effect_on_aldo = exp(m_osm_ALDO*(283.5 - plasma_osmolality))

  Aldo= (max(0, norm_Aldo*(1+(AngII_effect_on_aldo-1) +
(plasma_K_effect_on_aldo*plasma_osmolality_effect_on_aldo-1) + (Na_effect_on_aldo-1))
#effect of sodium intake

        +           #effect of plasma potassium


      hyperaldo_effect));                          #effect of hyperaldosteronism

  #Effect of aldosterone on tubular potassium secretion

  Aldo_MR_normalised_effect = (Aldo / norm_Aldo)*MRA_effect

    Aldo_effect_on_K_secretion= MR*max(0,1 +
Aldo_KSec_scale*((Aldo_MR_normalised_effect-1)*norm_Aldo));

######################## Renal Vasculature ######################################

nominal_renal_oxygen_delivery_rate = nom_hematocrit;

renal_oxygen_delivery_rate = renal_blood_flow_L_min_delayed*hematocrit;

#RBF aurtoregulation

#Autoregulation of peritubular resistance allows RBF to be autoregulated separately from GFR

#This is exploratory for now. By default, this effect is turned off by setting RBF_autoreg_scale
to zero

RBF_autoreg_int = 1 - RBF_autoreg_scale/2;

RBF_autoreg_signal = RBF_autoreg_int +
RBF_autoreg_scale/(1+exp((nom_renal_blood_flow_L_min -
renal_blood_flow_L_min_delayed)/RBF_autoreg_steepness));

#Assume efferent arteriole only contributes to RBF autoregulation when RBF is low

RBF_eff_autoreg_intercept =1 -  RBF_autoreg_scale_eff/(1+exp((RBF_efferent_autoreg_start -
nom_renal_blood_flow_L_min)/RBF_efferent_autoreg_steepness)) ;

RBF_autoreg_signal_efferent = RBF_eff_autoreg_intercept +
RBF_autoreg_scale_eff/(1+exp((RBF_efferent_autoreg_start -
renal_blood_flow_L_min_delayed)/RBF_efferent_autoreg_steepness));


###AT1-bound AngII constricts the preafferent, afferent, and efferent arterioles

AT1_preaff_int = 1 - AT1_preaff_scale/2;
```

```
AT1_effect_on_preaff = AT1_preaff_int + AT1_preaff_scale/(1+exp(-(AT1_bound_AngII - nominal_equilibrium_AT1_bound_AngII)/AT1_effect_slope));

AT1_aff_int = 1 - AT1_aff_scale/2;

AT1_effect_on_aff = AT1_aff_int + AT1_aff_scale/(1+exp(-(AT1_bound_AngII - nominal_equilibrium_AT1_bound_AngII)/AT1_effect_slope));

AT1_eff_int = 1 - AT1_eff_scale/2;

AT1_effect_on_eff = AT1_eff_int + AT1_eff_scale/(1+exp(-(AT1_bound_AngII - nominal_equilibrium_AT1_bound_AngII)/AT1_effect_slope));

### RSNA constricts the preafferent vasculature

rsna_preaff_int = 1 - rsna_preaff_scale/2;

rsna_effect_on_preaff = rsna_preaff_int + rsna_preaff_scale/(1+exp(-(renal_sympathetic_nerve_activity - nom_rsna)/rsna_preaff_slope));

### ANP may dilate the preafferent, afferent, and/or efferent arterioles

ANP_preaff_int = 1 + ANP_preaff_scale/2;

ANP_effect_on_preaff = ANP_preaff_int - ANP_preaff_scale/(1+exp(-(ANP_concentration - nom_ANP)/ANP_effect_slope));

ANP_aff_int = 1 + ANP_aff_scale/2;

ANP_effect_on_aff = ANP_aff_int - ANP_aff_scale/(1+exp(-(ANP_concentration - nom_ANP)/ANP_effect_slope));

ANP_eff_int = 1 + ANP_eff_scale/2;

ANP_effect_on_eff= ANP_eff_int - ANP_eff_scale/(1+exp(-(ANP_concentration - nom_ANP)/ANP_effect_slope));

################################### Endothelin Renal Vascular Effects ###################################

### Endothelin effects on preafferent, afferent, and efferent

ET1_ETA_effect_on_preaff = 1 + ET1_ETA_preaff_scale1*(ET1_ETA_cent - ET1_ETA_cent0)

ET1_ETA_effect_on_aff = 1 + ET1_ETA_aff_scale1*(ET1_ETA_cent0/ET1_ETA_peri0)*(ET1_ETA_peri - ET1_ETA_peri0)

ET1_ETB_effect_on_aff = 1 + ET1_ETB_aff_scale*(ET1_ETB_cent0/ET1_ETB_peri0)*(ET1_ETB_peri - ET1_ETB_peri0)

ET1_ETAETB_effect_on_aff = (R_ET1_ETA_effect_on_aff-1)+(R_ET1_ETB_effect_on_aff-1);
```

ET1_ETA_effect_on_eff = 1 +
ET1_ETA_eff_scale1*(ET1_ETA_cent0/ET1_ETA_peri0)*(ET1_ETA_peri - ET1_ETA_peri0)

ET1_ETB_effect_on_eff = 1 +
ET1_ETB_eff_scale*(ET1_ETB_cent0/ET1_ETB_peri0)*(ET1_ETB_peri - ET1_ETB_peri0)

ET1_ETAETB_effect_on_eff = (R_ET1_ETA_effect_on_eff-1) + (R_ET1_ETB_effect_on_eff - 1);

peritubular arterials ----------

######Preafferent Resistance

#The resistance of the arcuate, interlobular arterioles, and other vasculature prior the afferent arterioles is represented by a single resistance - the preafferent arteriole resistance

#The preafferent arterioles respond myogenically to changes in pressure, and also responds to AT1-bound AngII, RSNA, and ANP

#The dilation/constriction of the arterioles is limited, and thus the total combined effect of all regulators must saturate

preaff_arteriole_signal_multiplier =
R_ET1_ETA_effect_on_preaff*AT1_effect_on_preaff*rsna_effect_on_preaff*ANP_effect_on_preaff*(preafferent_pressure_autoreg_signal)*RBF_autoreg_signal*CCB_effect_on_preafferent_resistance;

preaff_adjust_int = 1-preaff_signal_nonlin_scale/2;

preaff_arteriole_adjusted_signal_multiplier = preaff_adjust_int +
preaff_signal_nonlin_scale/(1+exp((1-
preaff_arteriole_signal_multiplier)/preaff_signal_nonlin_slope));

preafferent_arteriole_resistance =
nom_preafferent_arteriole_resistance*preaff_arteriole_adjusted_signal_multiplier;

Afferent Arteriole Resistance

#The afferent arteriole responses the tubuloglomerular feedback (calculated later), as well as to AT1-bound AngII and ANP.

#It may respond myogenically as well. Some studies suggest the upstream portion responds myogenically while the distal portion responds to TGF. Thus, one could consider the

#myogenically responsive portion as part of the preafferent resistance.

#The dilation/constriction of the arterioles is limited, and thus the total combined effect of all regulators must saturate

nom_afferent_arteriole_resistance =
L_m3*viscosity_length_constant/(nom_afferent_diameter^4);

afferent_arteriole_signal_multiplier = (AT1_effect_on_aff +
ET1_ETAETB_effect_on_aff)*tubulo_glomerular_feedback_effect *

```
ANP_effect_on_aff*glomerular_pressure_autoreg_signal*RBF_autoreg_signal*CCB_effect_on
_afferent_resistance;

aff_adjust_int = 1-aff_signal_nonlin_scale/2;

afferent_arteriole_adjusted_signal_multiplier = aff_adjust_int +
aff_signal_nonlin_scale/(1+exp((1-
afferent_arteriole_signal_multiplier)/aff_signal_nonlin_slope));

afferent_arteriole_resistance =
nom_afferent_arteriole_resistance*afferent_arteriole_adjusted_signal_multiplier;

###### Efferent Arteriole Resistance

#The efferent arteriole responses to AT1-bound AngII and ANP.

#The dilation/constriction of the arterioles is limited, and thus the total combined effect of all
regulators must saturate

nom_efferent_arteriole_resistance =
L_m3*viscosity_length_constant/(nom_efferent_diameter^4);

efferent_arteriole_signal_multiplier = (AT1_effect_on_eff + ET1_ETAETB_effect_on_eff) *
ANP_effect_on_eff *CCB_effect_on_efferent_resistance*RBF_autoreg_signal_efferent;

eff_adjust_int = 1-eff_signal_nonlin_scale/2;

efferent_arteriole_adjusted_signal_multiplier = eff_adjust_int +
eff_signal_nonlin_scale/(1+exp((1-
efferent_arteriole_signal_multiplier)/eff_signal_nonlin_slope));

efferent_arteriole_resistance =
nom_efferent_arteriole_resistance*efferent_arteriole_adjusted_signal_multiplier;

######Peritubular Resistance

#Autoregulation of peritubular resistance allows RBF to be autoregulated separately from GFR

#This is exploratory for now. By default, this effect is turned off by setting RBF_autoreg_scale
to zero

autoregulated_peritubular_resistance = nom_peritubular_resistance*RBF_autoreg_signal;

###### Renal Vascular Resistance

renal_vascular_resistance = (preafferent_arteriole_resistance + (afferent_arteriole_resistance +
efferent_arteriole_resistance) / number_of_functional_glomeruli +
autoregulated_peritubular_resistance*(baseline_nephrons/number_of_functional_glomeruli));

###Renal blood flow

if (fix_ren_venous_pressure == 0 ) {

renal_venous_pressure = right_atrial_pressure ;
```

```
} else {

renal_venous_pressure = P_venous;

}

renal_blood_flow_L_min = ((mean_arterial_pressure_MAP - renal_venous_pressure) /
renal_vascular_resistance);

renal_blood_flow_ml_hr = renal_blood_flow_L_min * 1000 * 60;

###Renal Vasculature Pressures

preafferent_pressure = mean_arterial_pressure_MAP -
renal_blood_flow_L_min*preafferent_arteriole_resistance;

glomerular_pressure = (mean_arterial_pressure_MAP  - renal_blood_flow_L_min *
(preafferent_arteriole_resistance + afferent_arteriole_resistance /
number_of_functional_glomeruli));

postglomerular_pressure = (mean_arterial_pressure_MAP  - renal_blood_flow_L_min *
(preafferent_arteriole_resistance + (afferent_arteriole_resistance+efferent_arteriole_resistance) /
number_of_functional_glomeruli));

#Autoregulatory signals for preafferent and afferent resistances

preaff_autoreg_int = 1 - preaff_autoreg_scale/2;

preafferent_pressure_autoreg_function =
preaff_autoreg_int+preaff_autoreg_scale/(1+exp((nom_preafferent_pressure -
preafferent_pressure)/myogenic_steepness));

gp_autoreg_int = 1 - gp_autoreg_scale/2;

glomerular_pressure_autoreg_function = gp_autoreg_int +
gp_autoreg_scale/(1+exp((nom_glomerular_pressure -
glomerular_pressure)/myogenic_steepness));

######################### Glomerular Filtration ######################

 # Assume glomerulosclerosis causes a decrease in Kf over time, and also a loss of the renal
vasculature (afferent and efferent arterioles)

  # as glomeruli become completely sclerotic

        #Glomerular hypertrophy resulting in increased surface area and thus increased Kf is
assumed to occur

        #in response to elevated glomerular pressure. A 2 mmHg buffer is built in (i.e.
glomerular pressure must be at least 2 mmHg above normal for hypertrophy to begin

        #The increase in Kf saturates and cannot exceed the fractional increase set by
maximal_glom_surface_area_increase
```

GP_effect_increasing_Kf = (maximal_glom_surface_area_increase - disease_effects_increasing_Kf) * max(glomerular_pressure/(nom_glomerular_pressure+glomerular_pressure_increment) - 1,0) / T_glomerular_pressure_increases_Kf;

temp=glomerular_pressure/(nom_glomerular_pressure+2);

glomerular_hydrostatic_conductance_Kf = nom_Kf*(1+disease_effects_increasing_Kf)*(1-disease_effects_decreasing_Kf);

###Glomerular Fitlration Rate

#Calculation of P_bowmans are described later

net_filtration_pressure = glomerular_pressure - oncotic_pressure_difference - P_bowmans;

SNGFR_nL_min = glomerular_hydrostatic_conductance_Kf * (glomerular_pressure - oncotic_pressure_difference - P_bowmans);

GFR = (SNGFR_nL_min / 1000 / 1000000 * number_of_functional_tubules);

GFR_ml_min = GFR * 1000;

serum_creatinine_concentration = serum_creatinine/(blood_volume_L*10); #mg/dl

creatinine_filtration_rate = GFR_ml_min * dl_ml * serum_creatinine_concentration; #Units: mg/min

filtration_fraction = GFR_ml_min/1000/renal_blood_flow_L_min;

######################## Protein filtering ####################

GPdiff = max(0, glomerular_pressure - (nom_GP_seiving_damage));

GP_effect_on_Seiving = Emax_seiving * GPdiff ^ Gamma_seiving / (GPdiff ^ Gamma_seiving + Km_seiving ^ Gamma_seiving);

IgA_effect_on_Seiving = IgA_on/T_IgA_seiving;

FSGS_effect_on_Seiving = FSGS_on/T_FSGS_seiving;

#Dean and Lazzara 2006 - Seiving coefficient decreases as GFR increases

nom_glomerular_albumin_sieving_coefficient = seiving_inf/(1-(1-seiving_inf)*exp(-c_albumin*SNGFR_nL_min));

glomerular_albumin_sieving_coefficient = nom_glomerular_albumin_sieving_coefficient*(1 + permanent_seiving_damage + GP_effect_on_Seiving + disease_effect_on_seiving);

SN_albumin_filtration_rate = plasma_albumin_concentration*SNGFR_nL_min*1e-6*glomerular_albumin_sieving_coefficient; #mg/min

SN_albumin_excretion_rate = max(0, SN_albumin_filtration_rate - SN_albumin_reabsorptive_capacity)+nom_albumin_excretion_rate;

albumin_excretion_rate = SN_albumin_excretion_rate*number_of_functional_tubules;

```
###Oncotic pressure difference

#Landis Pappenheimer equation used to calculate oncotic pressure at entrance and exit to glomerulus

#Oncotic pressure is approximated as varying linearly along the glomerulus. Oncotic pressure in the Bowman's space is zero

#Thus the average pressure difference is the average of the entrance and exit oncotic pressure

#We do not consider filtration equilibrium

Oncotic_pressure_in = 1.629*plasma_protein_concentration+0.2935*(plasma_protein_concentration^2);

SNRBF_nl_min = 1e6*1000*renal_blood_flow_L_min/number_of_functional_glomeruli;

plasma_protein_concentration_out = (SNRBF_nl_min*plasma_protein_concentration-SN_albumin_filtration_rate)/(SNRBF_nl_min-SNGFR_nL_min);

Oncotic_pressure_out = 1.629*plasma_protein_concentration_out+0.2935*(plasma_protein_concentration_out^2);

oncotic_pressure_avg = (Oncotic_pressure_in+Oncotic_pressure_out)/2;

###################### Plasma sodium concentration and vasopressin secretion

###Plasma sodium concentration

Na_concentration = sodium_amount / blood_volume_L;

IF_Na_concentration = IF_sodium_amount/interstitial_fluid_volume;

sodium_storate_rate = Q_Na_store*((max_stored_sodium - stored_sodium)/max_stored_sodium)*(IF_Na_concentration - ref_Na_concentration);


###################### Tubular Flow and Reabsorption######################

#Length of tubular segments

L_pt_s1 = L_pt_s1_nom*(1+tubular_length_increase);

L_pt_s2 = L_pt_s2_nom*(1+tubular_length_increase);

L_pt_s3 = L_pt_s3_nom*(1+tubular_length_increase);

Dc_pt = Dc_pt_nom*(1+tubular_diameter_increase);

L_pt = L_pt_s1+L_pt_s2 + L_pt_s3;

SN_filtered_Na_load = (SNGFR_nL_min / 1000 / 1000000)*Na_concentration;

filtered_Na_load = SN_filtered_Na_load*number_of_functional_tubules;

######Regulatory effects on reabsorption
```

###Pressure natriuresis effects

pressure_natriuresis_PT_int = 1 - pressure_natriuresis_PT_scale/2;

pressure_natriuresis_PT_effect = max(0.001,pressure_natriuresis_PT_int + pressure_natriuresis_PT_scale / (1 + exp((renal_interstitial_hydrostatic_pressure- RIHP0) / pressure_natriuresis_PT_slope)));

RBF_PT_intercept = 1- RBF_PT_scale / (1 + exp((nom_renal_blood_flow_L_min - rbf_natriuresis_setpoint) / RBF_PT_slope))

RBF_PT_effect = max(0.001, RBF_PT_intercept + RBF_PT_scale / (1 + exp((renal_blood_flow_L_min - rbf_natriuresis_setpoint) / RBF_PT_slope)));

pressure_natriuresis_LoH_int = 1 - pressure_natriuresis_LoH_scale/2;

pressure_natriuresis_LoH_effect = max(0.001,pressure_natriuresis_LoH_int + pressure_natriuresis_LoH_scale / (1 + exp((renal_interstitial_hydrostatic_pressure - RIHP0) / pressure_natriuresis_LoH_slope)));

pressure_natriuresis_DCT_magnitude = max(0,pressure_natriuresis_DCT_scale);

pressure_natriuresis_DCT_int = 1 - pressure_natriuresis_DCT_magnitude/2;

pressure_natriuresis_DCT_effect = max(0.001,pressure_natriuresis_DCT_int + pressure_natriuresis_DCT_magnitude/ (1 + exp((renal_interstitial_hydrostatic_pressure - RIHP0) / pressure_natriuresis_DCT_slope)));

pressure_natriuresis_CD_magnitude = max(0,pressure_natriuresis_CD_scale *(1+disease_effects_decreasing_CD_PN));

pressure_natriuresis_CD_int = 1 - pressure_natriuresis_CD_magnitude/2;

pressure_natriuresis_CD_effect = max(0.001,pressure_natriuresis_CD_int + pressure_natriuresis_CD_magnitude/ (1 + exp((renal_interstitial_hydrostatic_pressure - RIHP0) / pressure_natriuresis_CD_slope)));

RBF_CD_int = 1 - RBF_CD_scale/2;

RBF_CD_effect = max(0.001,RBF_CD_int + RBF_CD_scale / (1 + exp((renal_blood_flow_L_min - nom_renal_blood_flow_L_min) / RBF_CD_slope)));

#Endothelin effects on tubular reabsorption

ET1_ETA_effect_on_PT = 1 + ET1_ETA_PT_scale1*(ET1_ETA_peri - ET1_ETA_peri0)*ET1_ETA_cent0/ET1_ETA_peri0

ET1_ETB_effect_on_CD = 1 + ET1_ETB_CD_scale*(ET1_ETB_peri - ET1_ETB_peri0)

ET1_ETB_effect_on_CD_water = 1 + ET1_ETB_CD_water_scale*(ET1_ETB_peri - ET1_ETB_peri0)

###AT1-bound AngII effect on PT reabsorption

AT1_PT_int = 1 - AT1_PT_scale/2;

AT1_effect_on_PT = AT1_PT_int + AT1_PT_scale/(1+exp(-(AT1_bound_AngII - nominal_equilibrium_AT1_bound_AngII)/AT1_effect_slope));

AT1_NKCC_int = 1 - AT1_NKCC_scale/2;

AT1_effect_on_NKCC = AT1_NKCC_int + AT1_NKCC_scale/(1+exp(-(AT1_bound_AngII - nominal_equilibrium_AT1_bound_AngII)/AT1_effect_slope));

AT1_NCC_int = 1 - AT1_NCC_scale/2;

AT1_effect_on_NCC = AT1_NCC_int + AT1_NCC_scale/(1+exp(-(AT1_bound_AngII - nominal_equilibrium_AT1_bound_AngII)/AT1_effect_slope));

AT1_ENAC_int = 1 - AT1_ENAC_scale/2;

AT1_effect_on_ENAC = AT1_ENAC_int + AT1_ENAC_scale/(1+exp(-(AT1_bound_AngII - nominal_equilibrium_AT1_bound_AngII)/AT1_effect_slope));

RSNA effect on PT and CD sodium reabsorption

#RSNA effect on CD is turned off by default

rsna_PT_int = 1 - rsna_PT_scale/2;

#######NEED To either change rsna_delayed consistently throughout or revert back

rsna_effect_on_PT = rsna_PT_int + rsna_PT_scale/(1+exp((1 - rsna_delayed2)/rsna_PT_slope));

rsna_CD_int = 1 - rsna_CD_scale/2;

rsna_effect_on_CD= rsna_CD_int + rsna_CD_scale/(1+exp((1 - renal_sympathetic_nerve_activity)/rsna_CD_slope));

###Aldosterone effect on distal and collecting duct sodium reabsorption

aldo_DCT_int = 1 - aldo_ROMK_scale/2;

aldo_effect_on_ROMK = max(0, 1+aldo_ROMK_scale*(Aldo_MR_normalised_effect -1))

aldo_ENAC_int = 1 - aldo_ENAC_scale/2;

aldo_effect_on_ENAC= max(0, 1+ aldo_ENAC_scale*(Aldo_MR_normalised_effect -1))

###ANP effect on collecting duct sodium reabsorption

anp_CD_int = 1 + anp_CD_scale/2;

anp_effect_on_CD= anp_CD_int - anp_CD_scale/(1+exp(-(ANP_concentration - nom_ANP)/ANP_effect_slope));

#Assume insulin has effect on NHE3. Use RUGE as surrogate for insulin effect. When RUGE goes up, insulin effect goes down.

SGLT_NHE3_effect = Anhe3*((SGLT2_glucose_reabsorption_delayed - nom_SGLT2_glucose_reabsorption)/nom_SGLT2_glucose_reabsorption); #RUGE_delayed;

```
pt_multiplier = R_ET1_ETA_effect_on_PT*AT1_effect_on_PT * rsna_effect_on_PT
*pressure_natriuresis_PT_effect*(1+SGLT_NHE3_effect)*RBF_PT_effect;

#Calculate the normal flow rate to surface area for the PT

excess_flow_effec_on_pt = 1+excess_flow_pt_scale*min(0,nom_SNGFR/(SNGFR_nL_min-10)
- 1);

e_pt_sodreab_nonSGLT = min(1,nominal_pt_na_reabsorption_nonSGLT *
pt_multiplier*excess_flow_effec_on_pt);# AT1_effect_on_PT * rsna_effect_on_PT
*pressure_natriuresis_PT_effect;

######################################## Proximal Tubule
########################################

###Glucose Filtration and reabsorption in PT

#Assume glucose reabsorption depends only on availability of SGLT1/2

#Assume constant amount of reabsorption per unit length through SGLT2 in convoluted PT

#Assume constant amount of reabsorption per unit length through SGLT1 in straight/recta PT

glucose_conc = glucose_concentration*(1-Emax_SGLT2i_glucose*(1-
SGLT2_inhibition_glucose_effect_delayed));

#Chosen so that UGE becomes non-zero for plasma_glucose concentration ~8.5 mmol/l

glucose_reabs_per_unit_length_s1 =
nom_glucose_reabs_per_unit_length_s1*diabetic_adaptation*SGLT2_inhibition_delayed*(1+R
Tg_compensation);

glucose_reabs_per_unit_length_s2 =
nom_glucose_reabs_per_unit_length_s2*diabetic_adaptation*SGLT2_inhibition_delayed*(1+R
Tg_compensation);

glucose_reabs_per_unit_length_s3 =
nom_glucose_reabs_per_unit_length_s3*diabetic_adaptation*(1+RTg_compensation)*SGLT1_i
nhibition;

SN_filtered_glucose_load = glucose_conc*SNGFR_nL_min / 1000 / 1000000;  #mmol/min

glucose_pt_out_s1 = max(0,SN_filtered_glucose_load-
glucose_reabs_per_unit_length_s1*L_pt_s1); #mmol/min

glucose_pt_out_s2 = max(0,glucose_pt_out_s1-glucose_reabs_per_unit_length_s2*L_pt_s2);
#mmol/min

glucose_pt_out_s3 = max(0,glucose_pt_out_s2-glucose_reabs_per_unit_length_s3*L_pt_s3);
#mmol/min

RUGE = glucose_pt_out_s3*number_of_functional_tubules*180; #RUGE in mg/min
```

```
SGLT2_glucose_reabsorption = SN_filtered_glucose_load - glucose_pt_out_s2;

excess_glucose_increasing_RTg = (maximal_RTg_increase - RTg_compensation) *
max(RUGE,0) / T_glucose_RTg;

###PT Sodium filtration and reabsorption

# Sodium reabsorbed 1:1 with glucose in S1 and S2

# Sodium reabsorbed 2:1 with glucose in S3

# Assume for non-SGLT reabsorption, sodium reabsorbed at a constant RATE along the tubule

# (represents glomerulotubular balance)

SN_filtered_Na_load = (SNGFR_nL_min / 1000 / 1000000)*Na_concentration;    #mmol/min

SGTL2_Na_reabs_mmol_s1 = SN_filtered_glucose_load-glucose_pt_out_s1;
        #mmol/min

SGTL2_Na_reabs_mmol_s2 = glucose_pt_out_s1-glucose_pt_out_s2;
        #mmol/min

SGTL1_Na_reabs_mmol = 2*(glucose_pt_out_s2-glucose_pt_out_s3);
        #mmol/min

total_SGLT2_Na_reabs = SGTL2_Na_reabs_mmol_s1+SGTL2_Na_reabs_mmol_s2;
#+SGTL1_Na_reabs_mmol;  #mmol/min

Na_reabs_per_unit_length = -log(1-e_pt_sodreab_nonSGLT)/(L_pt_s1+L_pt_s2+L_pt_s3);
#non-SGLT2 reabs     #mmol/min

Na_pt_s1_reabs = min(max_s1_Na_reabs, SN_filtered_Na_load*(1-exp(-
Na_reabs_per_unit_length*L_pt_s1)));

Na_pt_out_s1 = SN_filtered_Na_load - Na_pt_s1_reabs - SGTL2_Na_reabs_mmol_s1 ;

Na_pt_s2_reabs = min(max_s2_Na_reabs, Na_pt_out_s1*(1-exp(-
Na_reabs_per_unit_length*L_pt_s2)));

Na_pt_out_s2 = Na_pt_out_s1 - Na_pt_s2_reabs - SGTL2_Na_reabs_mmol_s2;

Na_pt_s3_reabs = min(max_s3_Na_reabs, Na_pt_out_s2*(1-exp(-
Na_reabs_per_unit_length*L_pt_s3)));

Na_pt_out_s3 = Na_pt_out_s2 - Na_pt_s3_reabs - SGTL1_Na_reabs_mmol;

PT_Na_reabs_fraction = 1-Na_pt_out_s3/SN_filtered_Na_load;

#PT Potassium filtration and reabsorption

#Glomerular Filtration of potassium

SN_filtered_K = max(0,(SNGFR_nL_min/1e6) * plasma_K); #mEq/min

K_pt_out_s1 = SN_filtered_K*(Na_pt_out_s1/SN_filtered_Na_load)
```

```
K_pt_out_s2 = K_pt_out_s1*(Na_pt_out_s2/Na_pt_out_s1)

K_pt_out_s3 = K_pt_out_s2*(Na_pt_out_s3/Na_pt_out_s2)

###PT Urea filtration and reabsorption

SN_filtered_urea_load = (SNGFR_nL_min / 1000 / 1000000)*plasma_urea;

urea_out_s1 = SN_filtered_urea_load -
urea_permeability_PT*(SN_filtered_urea_load/(0.5*((SNGFR_nL_min / 1000 /
1000000)+water_out_s1_delayed))-plasma_urea)*water_out_s1_delayed; #For now, assuming
only reabsorbed at the end

urea_out_s2 = urea_out_s1 -
urea_permeability_PT*(urea_out_s1/(0.5*(water_out_s1_delayed+water_out_s2_delayed))-
plasma_urea)*water_out_s2_delayed; #For now, assuming only reabsorbed at the end

urea_out_s3 = urea_out_s2 -
urea_permeability_PT*(urea_out_s2/(0.5*(water_out_s2_delayed+water_out_s3_delayed))-
plasma_urea)*water_out_s3_delayed; #For now, assuming only reabsorbed at the end

urea_reabsorption_fraction = 1-urea_out_s3/SN_filtered_urea_load;

###PT Water Reabsorption

osmoles_out_s1 = 2*(Na_pt_out_s1 + K_pt_out_s1) + glucose_pt_out_s1 + urea_out_s1;

water_out_s1 = (((SNGFR_nL_min / 1000 /
1000000)/(2*SN_filtered_Na_load+SN_filtered_glucose_load+
SN_filtered_urea_load)))*osmoles_out_s1;

osmoles_out_s2 = 2*(Na_pt_out_s2 + K_pt_out_s2) + glucose_pt_out_s2 + urea_out_s2;

water_out_s2 = (water_out_s1/osmoles_out_s1)*osmoles_out_s2;

osmoles_out_s3 = 2*(Na_pt_out_s3 + K_pt_out_s3) + glucose_pt_out_s3 + urea_out_s3;

water_out_s3 = (water_out_s2/osmoles_out_s2)*osmoles_out_s3;

PT_water_reabs_fraction = 1-water_out_s3/(SNGFR_nL_min / 1000 / 1000000);

###Concentrations out of PT

Na_concentration_out_s1 = Na_pt_out_s1/water_out_s1;

Na_concentration_out_s2 = Na_pt_out_s2/water_out_s2;

Na_concentration_out_s3 = Na_pt_out_s3/water_out_s3;

glucose_concentration_out_s1 = glucose_pt_out_s1/water_out_s1;

glucose_concentration_out_s2 = glucose_pt_out_s2/water_out_s2;

glucose_concentration_out_s3 = glucose_pt_out_s3/water_out_s3;

urea_concentration_out_s1 = urea_out_s1/water_out_s1;
```

```
urea_concentration_out_s2 = urea_out_s2/water_out_s2;

urea_concentration_out_s3 = urea_out_s3/water_out_s3;

PT_K_out = K_pt_out_s3  #(mEq/min)

PT_K_out_conc = PT_K_out/water_out_s3/1000 #mEq/ml

osmolality_out_s1 = osmoles_out_s1/water_out_s1;

osmolality_out_s2 = osmoles_out_s2/water_out_s2;

osmolality_out_s3 = osmoles_out_s3/water_out_s3;

creatinine_concentration_out_s3 =
creatinine_filtration_rate/(water_out_s3*number_of_functional_tubules); #mg/L

avg_creatinine_concentration_PT= (serum_creatinine_concentration +
creatinine_concentration_out_s3/10)/2;

#Creatinine secreted actively against a concentration gradient

creatinine_secretion_rate = basal_creatinine_secretion*(1-creatinine_secretion_scale*(-0.5-
1/avg_creatinine_concentration_PT));

creatinine_excretion_rate = creatinine_filtration_rate + creatinine_secretion_rate

#Proximal Tubule and LoH K+ reabsorption is proportional to sodium reabsorption

PT_fractional_Na_reabs = (SN_filtered_Na_load - Na_pt_out_s3)/SN_filtered_Na_load;

PT_Na_outflow = Na_pt_out_s3*number_of_functional_tubules;

#Tubular sodium reabsorption per unit SA as the driver of tubular hypertrophy

PT_Na_reab_perUnitSA = (SN_filtered_Na_load -
Na_pt_out_s3)/(3.14*Dc_pt*(L_pt_s1+L_pt_s2+L_pt_s3));

normalized_PT_reabsorption_density = PT_Na_reab_perUnitSA/PT_Na_reab_perUnitSA_0;

PT_Na_reabs_effect_increasing_tubular_length = 0;#(maximal_tubule_length_increase -
tubular_length_increase) * max(normalized_PT_reabsorption_density - 1,0) /
T_PT_Na_reabs_PT_length;

PT_Na_reabs_effect_increasing_tubular_diameter = 0;#(maximal_tubule_diameter_increase -
tubular_diameter_increase) * max(normalized_PT_reabsorption_density - 1,0) /
T_PT_Na_reabs_PT_diameter;

FELi = 100*(1-PT_Na_reabs_fraction);#

###################################### Loop of Henle
########################################

#####Descending Loop of Henle

water_in_DescLoH = water_out_s3; # L/min
```

Na_in_DescLoH = Na_pt_out_s3;

urea_in_DescLoH = urea_out_s3;

glucose_in_DescLoH = glucose_pt_out_s3;

osmoles_in_DescLoH = osmoles_out_s3;

K_in_DescLoH = PT_K_out

Na_concentration_in_DescLoH = Na_concentration_out_s3;

Urea_concentration_in_DescLoH = urea_concentration_out_s3;

glucose_concentration_in_DescLoH = glucose_concentration_out_s3;

K_concentration_in_DescLoH = PT_K_out_conc

osmolality_in_DescLoH = osmoles_out_s3/water_out_s3;

#No solute reabsorption in descending limb

Na_out_DescLoH = Na_in_DescLoH;

urea_out_DescLoH = urea_in_DescLoH;

glucose_out_DescLoH = glucose_in_DescLoH;

K_out_DescLoH = K_in_DescLoH

osmoles_out_DescLoH = osmoles_in_DescLoH;

#For LoH, baseline osmoles reabsorbed per unit length is calculated from nominal fractional sodium reabsorption (see baseline parameters file)

#The rate of reabsorption per unit length may be flow-dependent, and may be modulated by tubular pressure-natriuresis

deltaLoH_NaFlow = min(max_deltaLoH_reabs,LoH_flow_dependence*(Na_out_DescLoH-nom_Na_in_AscLoH));

#Assume plasma K downregulates NKCC in AscLoH. Can model this mechanistically later ###

plasma_K_effect_on_NKCC = 1 - K_NKCC_scale*(plasma_K - norm_plasma_K)

AscLoH_Reab_Rate =(2*nominal_loh_na_reabsorption*(nom_Na_in_AscLoH + nom_K_in_AscLoH + deltaLoH_NaFlow)*loop_diuretic_effect)/L_lh_des; #osmoles reabsorbed per unit length per minute. factor of 2 because osmoles = 2

effective_AscLoH_Reab_Rate =AscLoH_Reab_Rate*plasma_K_effect_on_NKCC*AT1_effect_on_NKCC*pressure_natriuresis_LoH_effect; #osmoles reabsorbed per unit length per minute. factor of 2 because osmoles = 2*Na

#Min function necesssary to ensure that the LoH does not reabsorb more Na than is delivered to it

osmolality_out_DescLoH = osmolality_in_DescLoH*exp(min(effective_AscLoH_Reab_Rate*L_lh_des,2*(Na_in_DescLoH + K_in_DescLoH))/(water_in_DescLoH*osmolality_in_DescLoH));

water_out_DescLoH = water_in_DescLoH*osmolality_in_DescLoH/osmolality_out_DescLoH;

Na_concentration_out_DescLoH = Na_out_DescLoH/water_out_DescLoH;

glucose_concentration_out_DescLoH = glucose_out_DescLoH/water_out_DescLoH;

urea_concentration_out_DescLoH = urea_out_DescLoH/water_out_DescLoH;

K_concentration_out_DescLoH = K_out_DescLoH/water_out_DescLoH;

#####Ascending Loop of Henle

Na_in_AscLoH = Na_out_DescLoH;

K_in_AscLoH = K_in_DescLoH

urea_in_AscLoH_before_secretion = urea_out_DescLoH;

glucose_in_AscLoH = glucose_out_DescLoH;

osmoles_in_AscLoH_before_secretion = osmoles_out_DescLoH;

water_in_AscLoH = water_out_DescLoH;

#Urea Secretion --> Assume all urea reabsorbed and secreted only at tip of loop

urea_in_AscLoH = urea_in_AscLoH_before_secretion + reabsorbed_urea_cd_delayed;

urea_concentration_in_AscLoH = urea_in_AscLoH/water_out_DescLoH;

osmoles_in_AscLoH = osmoles_in_AscLoH_before_secretion + reabsorbed_urea_cd_delayed;

osmolality_in_AscLoH = osmoles_in_AscLoH/water_in_AscLoH;

#Osmolality descreased due to sodium reabsorption along ascending loop

#min function necessary so that LoH doesn't reabsorb more sodium than is delivered to it

osmolality_out_AscLoH = osmolality_in_AscLoH - min(L_lh_des*effective_AscLoH_Reab_Rate, 2*(Na_in_DescLoH + K_in_DescLoH))*(exp(min(L_lh_des*effective_AscLoH_Reab_Rate, 2*(Na_in_DescLoH + K_in_DescLoH))/(water_in_DescLoH*osmolality_in_DescLoH))/water_in_DescLoH);

osmoles_reabsorbed_AscLoH = (osmolality_in_AscLoH - osmolality_out_AscLoH)*water_in_AscLoH;

Na_reabsorbed_AscLoH = osmoles_reabsorbed_AscLoH*(Na_in_DescLoH/(Na_in_DescLoH + K_in_DescLoH))/2;

K_paracellular_reabsorption = AscLoH_paracellular_K_SA*(plasma_K - AscLoH_luminal_K_avg_conc_delayed)

```
K_reabsorbed_AscLoH = osmoles_reabsorbed_AscLoH*(K_in_DescLoH/(Na_in_DescLoH + K_in_DescLoH))/2 + K_paracellular_reabsorption;

Na_out_AscLoH = max(0,Na_in_AscLoH - Na_reabsorbed_AscLoH);

#Potassium out of the loop of Henle

LoH_K_out = max(0, K_in_AscLoH - K_reabsorbed_AscLoH)

#Water, glucose, and urea are not reabsorbed along the ascending limb

urea_out_AscLoH = urea_in_AscLoH; #urea secretion accounted for above

glucose_out_AscLoH = glucose_in_AscLoH;

water_out_AscLoH = water_in_AscLoH;

osmoles_out_AscLoH = osmolality_out_AscLoH*water_out_AscLoH;

Na_concentration_out_AscLoH = Na_out_AscLoH/water_out_AscLoH;

glucose_concentration_out_AscLoH = glucose_out_AscLoH/water_out_AscLoH;

urea_concentration_out_AscLoH = urea_out_AscLoH/water_out_AscLoH;

K_concentration_out_LoH = LoH_K_out/water_out_AscLoH;


AscLoH_luminal_K_avg_conc = (K_concentration_out_DescLoH + K_concentration_out_LoH)/2/1000

LoH_reabs_fraction = 1-Na_out_AscLoH/Na_in_AscLoH;

SN_macula_densa_Na_flow = Na_out_AscLoH;

MD_Na_concentration = Na_concentration_out_AscLoH;

TGF0_tubulo_glomerular_feedback = 1 - S_tubulo_glomerular_feedback/2;

tubulo_glomerular_feedback_signal = (TGF0_tubulo_glomerular_feedback + S_tubulo_glomerular_feedback / (1 + exp((MD_Na_concentration_setpoint - MD_Na_concentration)/ F_md_scale_tubulo_glomerular_feedback)));

##############################Distal Convoluted Tubule 1 ######################

water_in_DCT = water_out_AscLoH;

Na_in_DCT = Na_out_AscLoH;

urea_in_DCT = urea_out_AscLoH;

glucose_in_DCT = glucose_out_AscLoH;

osmoles_in_DCT = osmoles_out_AscLoH;

Na_concentration_in_DCT = Na_concentration_out_AscLoH;

urea_concentration_in_DCT = urea_concentration_out_AscLoH;
```

```
glucose_concentration_in_DCT = glucose_concentration_out_AscLoH;

osmolality_in_DCT = osmolality_out_AscLoH;

#Assume only sodium reabsorbed along DCT, no water, glucose, or urea reabsorption

urea_out_DCT1 = urea_in_DCT;

glucose_out_DCT1 = glucose_in_DCT;

water_out_DCT1 = water_in_DCT;

urea_concentration_out_DCT1 = urea_out_DCT1/water_out_DCT1;

glucose_concentration_out_DCT1 = glucose_out_DCT1/water_out_DCT1;

#DCT1 Na reabsorption

  #sodium reabsorbtion in distal tubule & collecting duct

  dct1_multiplier = pressure_natriuresis_DCT_effect*AT1_effect_on_NCC
*HCTZ_effect_on_DT_Na_reabs

  #Luminal Na reabsorption in the DCT

  J_NCC_dct1 =
dct1_multiplier*Na_luminal_permeability_dct1*(DCT1_luminal_Na_avg_conc_delayed-
DCT1_cell_Na_conc);

  J_NCC_dct1_effective = min(J_NCC_dct1, Na_in_DCT/(DCT_SA/2))

  dct1_na_reabsorption=J_NCC_dct1_effective*(DCT_SA/2)/Na_in_DCT;

  e_dct1_sodreab = min(1,dct1_na_reabsorption) ;

  #Assume sodium reabsorption at a constant fraction of delivery

  R_dct1 = -log(1-e_dct1_sodreab)/(L_dct/2);

  Na_out_DCT1 = Na_in_DCT*exp(-R_dct1*(L_dct/2));

  Na_concentration_out_DCT1 = Na_out_DCT1/water_out_DCT1;

  DCT1_luminal_Na_avg_conc = (Na_concentration_in_DCT +
Na_concentration_out_DCT1)/2/1000

################################## DCT1 K+ Secretion
##############################################

#Membrane Potential Differences

  normalized_luminal_potential_difference_DCT1 = log((DCT1_luminal_K_avg_conc_delayed
+ 0.05*DCT1_luminal_Na_avg_conc_delayed + 0.45*0.150)/(DCT1_cell_K_conc +
0.05*DCT1_cell_Na_conc  + 0.45*0.01))

  #weighting factors assumed based on differences in permeability
```

normalized_basolateral_potential_difference_DCT1 = log((4*plasma_K + 0.05*Na_concentration/1000 + 0.1*0.1)/(4*DCT1_cell_K_conc + 0.05*DCT1_cell_Na_conc + 0.1*0.01))

#Potassium and water into DCT

DCT_K_in = LoH_K_out

#flow effect on potassium secretion

flow_effect_K_DCT1 = max(0, 1 + m_flow_K*((water_in_DCT - water_in_DCT0)/water_in_DCT0))

#DCT Potassium flux from cell to lumen #mEq/min.cm^2 #Goldman equation

DCT1_K_passive_flux_lumenal = baseline_K_luminal_permeability_DCT*flow_effect_K_DCT1*normalized_luminal_potential_difference_DCT1*

(-DCT1_luminal_K_avg_conc_delayed + DCT1_cell_K_conc*exp(-normalized_luminal_potential_difference_DCT1))

/(1-exp(-normalized_luminal_potential_difference_DCT1));

#DCT passive potassium flux across basolateral membrane #mEq/min.cm^2 #Goldman equation

DCT1_K_passive_flux_basolateral = -K_basolateral_permeability_DCT*normalized_basolateral_potential_difference_DCT1*

(-plasma_K + DCT1_cell_K_conc*exp(-normalized_basolateral_potential_difference_DCT1))

/(1-exp(-normalized_basolateral_potential_difference_DCT1));

#DCT Active flux the Na+/K+ ATPase across basolateral membrane, #mEq/min.cm^2

K_K = (0.1*(1+Na_concentration /18.5))/1000 #assume plasma Na+ of 140 mEq/L

K_Na_DCT1 = (0.2*(1+DCT1_cell_K_conc*1000/8.33))/1000

J_Na_active_max_eff_DCT1 = J_Na_active_max_DCT1

#DCT Passive basolateral sodium transport

DCT1_Na_active_flux_basolateral = J_Na_active_max_eff_DCT1*((DCT1_cell_Na_conc/(DCT1_cell_Na_conc + K_Na_DCT1))^3)*((plasma_K/(plasma_K + K_K))^2)

DCT1_K_active_flux_basolateral = (2/3)*DCT1_Na_active_flux_basolateral

DCT1_Na_passive_flux_basolateral = Na_basolateral_permeability_dct1*(Na_concentration/1000 - DCT1_cell_Na_conc)

```
  #DCT potassium secretion rate

  DCT1_K_secretion_rate = DCT1_K_passive_flux_lumenal*(DCT_SA/2)

  #Potassium out of each tubular segment

  DCT1_K_out = DCT_K_in +  DCT1_K_secretion_rate
#DCT_luminal_K_conc*water_out_DCT1*1000   #no water reabsorbed in DCT

  K_concentration_out_DCT1 = DCT1_K_out/water_out_DCT1

  DCT1_luminal_K_avg_conc = (K_concentration_out_LoH +
K_concentration_out_DCT1)/2/1000

  osmolality_out_DCT1 = 2*(Na_concentration_out_DCT1 +
DCT1_luminal_K_avg_conc*1000)+ glucose_concentration_out_DescLoH +
urea_concentration_in_AscLoH;

  osmoles_out_DCT1 = osmolality_out_DCT1*water_out_DCT1;

  DCT1_Na_reabs_fraction = 1-Na_out_DCT1/Na_in_DCT;

##############################Distal Convoluted Tubule 2 ########################

water_in_DCT2 = water_out_DCT1;

Na_in_DCT2 = Na_out_DCT1;

urea_in_DCT2 = urea_out_DCT1;

glucose_in_DCT2 = glucose_out_DCT1;

osmoles_in_DCT2 = osmoles_out_DCT1;

Na_concentration_in_DCT2 = Na_concentration_out_DCT1;

urea_concentration_in_DCT2 = urea_concentration_out_DCT1;

glucose_concentration_in_DCT2 = glucose_concentration_out_DCT1;

osmolality_in_DCT2 = osmolality_out_DCT1

#Assume only sodium reabsorbed along DCT, no water, glucose, or urea reabsorption

urea_out_DCT2 = urea_in_DCT;

glucose_out_DCT2 = glucose_in_DCT;

water_out_DCT2 = water_in_DCT;

urea_concentration_out_DCT2 = urea_out_DCT2/water_out_DCT2;

glucose_concentration_out_DCT2 = glucose_out_DCT2/water_out_DCT2;

#DCT1 Na reabsorption

  #sodium reabsorbtion in distal tubule & collecting duct
```

```
  dct2_multiplier = pressure_natriuresis_DCT_effect
*AT1_effect_on_ENAC*aldo_effect_on_ENAC_delayed*HCTZ_effect_on_DT_Na_reabs

  #Luminal Na reabsorption in the DCT

  J_NCC_ENAC_dct2 =
dct2_multiplier*Na_luminal_permeability_dct2*(DCT2_luminal_Na_avg_conc_delayed-
DCT2_cell_Na_conc);

  J_NCC_ENAC_dct2_effective = min(J_NCC_ENAC_dct2, Na_in_DCT2/(DCT_SA/2))

  dct2_na_reabsorption=J_NCC_ENAC_dct2_effective*(DCT_SA/2)/Na_in_DCT2;

  e_dct2_sodreab = min(1,dct2_na_reabsorption) ;

  #Assume sodium reabsorption at a constant fraction of delivery

  R_dct2 = -log(1-e_dct2_sodreab)/(L_dct/2);

  Na_out_DCT2 = Na_in_DCT2*exp(-R_dct2*(L_dct/2));

  Na_concentration_out_DCT2 = Na_out_DCT2/water_out_DCT2;

  DCT2_luminal_Na_avg_conc = (Na_concentration_out_DCT1 +
Na_concentration_out_DCT2)/2/1000

################################## DCT2 K+ Secretion
##############################################

#Membrane Potential Differences

  normalized_luminal_potential_difference_DCT2 = log((DCT2_luminal_K_avg_conc_delayed
+ 0.05*DCT2_luminal_Na_avg_conc + 0.45*0.150)/(DCT2_cell_K_conc +
0.05*DCT2_cell_Na_conc  + 0.45*0.01))

  #weighting factors assumed based on differences in permeability

  normalized_basolateral_potential_difference_DCT2 = log((4*plasma_K +
0.05*Na_concentration/1000 + 0.1*0.1)/(4*DCT2_cell_K_conc + 0.05*DCT2_cell_Na_conc  +
0.1*0.01))

#Potassium and water into DCT

  DCT2_K_in = DCT1_K_out


  #flow effect on potassium secretion

flow_effect_K_DCT2 = max(0, 1 + m_flow_K*((water_in_DCT2 -
water_in_DCT20)/water_in_DCT20))

#DCT Potassium flux from cell to lumen #mEq/min.cm^2 #Goldman equation
```

```
  DCT2_K_passive_flux_lumenal =
baseline_K_luminal_permeability_DCT*aldo_effect_on_ROMK*flow_effect_K_DCT2*normal
ized_luminal_potential_difference_DCT2*

    (-DCT2_luminal_K_avg_conc_delayed + DCT2_cell_K_conc*exp(-
normalized_luminal_potential_difference_DCT2))

    /(1-exp(-normalized_luminal_potential_difference_DCT2));

#DCT passive potassium flux across basolateral membrane #mEq/min.cm^2 #Goldman equation

  DCT2_K_passive_flux_basolateral = -
K_basolateral_permeability_DCT*normalized_basolateral_potential_difference_DCT2*

    (-plasma_K + DCT2_cell_K_conc*exp(-normalized_basolateral_potential_difference_DCT2))

    /(1-exp(-normalized_basolateral_potential_difference_DCT2));

  #DCT Active flux the Na+/K+ ATPase across basolateral membrane,  #mEq/min.cm^2

  K_K = (0.1*(1+Na_concentration /18.5))/1000  #assume plasma Na+ of 140 mEq/L

  K_Na_DCT2 = (0.2*(1+DCT2_cell_K_conc*1000/8.33))/1000

  J_Na_active_max_eff_DCT2 = J_Na_active_max_DCT2

  #DCT Passive basolateral sodium transport

  DCT2_Na_active_flux_basolateral =
J_Na_active_max_eff_DCT2*((DCT2_cell_Na_conc/(DCT2_cell_Na_conc +
K_Na_DCT2))^3)*((plasma_K/(plasma_K + K_K))^2)

  DCT2_K_active_flux_basolateral = (2/3)*DCT2_Na_active_flux_basolateral

  DCT2_Na_passive_flux_basolateral =
Na_basolateral_permeability_dct2*(Na_concentration/1000 - DCT2_cell_Na_conc)

  #DCT potassium secretion rate

  DCT2_K_secretion_rate = DCT2_K_passive_flux_lumenal*(DCT_SA/2)

  #Potassium out of each tubular segment

  DCT2_K_out = DCT2_K_in +  DCT2_K_secretion_rate
#DCT_luminal_K_conc*water_out_DCT2*1000   #no water reabsorbed in DCT

  K_concentration_out_DCT2 = DCT2_K_out/water_out_DCT2

  DCT2_luminal_K_avg_conc = (K_concentration_out_DCT1 +
K_concentration_out_DCT2)/2/1000

  osmolality_out_DCT2 = 2*(Na_concentration_out_DCT2 +
DCT2_luminal_K_avg_conc*1000)+ glucose_concentration_out_DescLoH +
urea_concentration_in_AscLoH;

  osmoles_out_DCT2 = osmolality_out_DCT2*water_out_DCT2;
```

```
  DCT2_Na_reabs_fraction = 1-Na_out_DCT2/Na_in_DCT2;

  ##################################################Connecting
Tubule################################

#CNT Na reabsorption

  Na_in_CNT=Na_out_DCT2

  water_out_CNT = water_out_DCT2*(1-CNT_water_reabs_fraction)

  cnt_multiplier =
aldo_effect_on_ENAC_delayed*AT1_effect_on_ENAC*pressure_natriuresis_DCT_effect
*HCTZ_effect_on_DT_Na_reabs

  #Luminal Na reabsorption in the CNT


J_Enac_cnt=cnt_multiplier*Na_luminal_permeability_cnt*(CNT_luminal_Na_avg_conc_delaye
d-CNT_cell_Na_conc);

  J_Enac_cnt_effective = min(J_Enac_cnt, Na_in_CNT/CNT_SA)

  cnt_na_reabsorption=J_Enac_cnt*CNT_SA/Na_in_CNT;

  e_cnt_sodreab = min(1,cnt_na_reabsorption);

  R_cnt = -log(1-e_cnt_sodreab)/L_cnt;

  Na_out_CNT = Na_in_CNT*exp(-R_cnt*L_cnt);

  Na_concentration_out_CNT = Na_out_CNT/water_out_CNT;

  CNT_luminal_Na_avg_conc = (Na_concentration_out_DCT2 +
Na_concentration_out_CNT)/2/1000

################################## CNT K+ Secretion
##############################################

  normalized_luminal_potential_difference_CNT = log((CNT_luminal_K_avg_conc_delayed +
0.05*CNT_luminal_Na_avg_conc + 0.45*0.150)/(CNT_cell_K_conc +
0.05*CNT_cell_Na_conc  + 0.45*0.01))

  #weighting factors assumed based on differences in permeability

  normalized_basolateral_potential_difference_CNT = log((4*plasma_K +
0.05*Na_concentration/1000 + 0.1*0.1)/(4*CNT_cell_K_conc + 0.05*CNT_cell_Na_conc  +
0.1*0.01))

  #flow effect on potassium secretion

flow_effect_K_CNT = max(0, 1 + m_flow_K*((water_out_DCT2 -
water_in_CNT0)/water_in_CNT0))

#CNT Potassium flux from cell to lumen #mEq/min.cm^2 #Goldman equation
```

CNT_K_passive_flux_lumenal = baseline_K_luminal_permeability_CNT*aldo_effect_on_ROMK*flow_effect_K_CNT*normalized_luminal_potential_difference_CNT*

(-CNT_luminal_K_avg_conc_delayed + CNT_cell_K_conc*exp(-normalized_luminal_potential_difference_CNT))

/(1-exp(-normalized_luminal_potential_difference_CNT)); #mEq/min.cm^2 #Goldman equation

#CNT passive potassium flux across basolateral membrane #mEq/min.cm^2 #Goldman equation

CNT_K_passive_flux_basolateral = -K_basolateral_permeability_CNT*normalized_basolateral_potential_difference_CNT*

(-plasma_K + CNT_cell_K_conc*exp(-normalized_basolateral_potential_difference_CNT))

/(1-exp(-normalized_basolateral_potential_difference_CNT)); #mEq/min.cm^2 #Goldman equation

#CNT Active flux the Na+/K+ ATPase across basolateral membrane, #mEq/min.cm^2

K_Na_CNT = (0.2*(1+CNT_cell_K_conc*1000/8.33))/1000

J_Na_active_max_eff_CNT = J_Na_active_max_CNT*max(0,(Aldo_effect_on_K_secretion))

CNT_Na_active_flux_basolateral = J_Na_active_max_eff_CNT*((CNT_cell_Na_conc/(CNT_cell_Na_conc + K_Na_CNT))^3)*((plasma_K/(plasma_K + K_K))^2)

CNT_K_active_flux_basolateral = (2/3)*CNT_Na_active_flux_basolateral

CNT_Na_passive_flux_basolateral = Na_basolateral_permeability_cnt*(Na_concentration/1000 - CNT_cell_Na_conc)

#CNT potassium secretion rate

CNT_K_secretion_rate = CNT_K_passive_flux_lumenal*CNT_SA*principal_fraction_CNT

#Potassium out of each tubular segment

CNT_K_out = DCT2_K_out + CNT_K_secretion_rate #CNT_luminal_K_avg_conc_delayed*water_out_CNT*1000 #minimal reabsorption in CNT

K_concentration_out_CNT = CNT_K_out/water_out_CNT

CNT_luminal_K_avg_conc = (K_concentration_out_DCT2 + K_concentration_out_CNT)/2/1000

glucose_concentration_out_CNT = glucose_concentration_out_DescLoH*(water_out_DCT2/water_out_CNT)

```
  osmolality_out_CNT = 2*(Na_concentration_out_CNT + CNT_luminal_K_avg_conc*1000)+
glucose_concentration_out_CNT + urea_concentration_in_AscLoH;

  osmoles_out_CNT = osmolality_out_CNT*water_out_CNT;

##########################################Cortical
CollectingDuct###############################

water_in_CCD = water_out_CNT;

Na_in_CD = Na_out_CNT;

urea_in_CD = urea_out_DCT2;

glucose_in_CD = glucose_out_DCT2;

osmoles_in_CCD = osmoles_out_CNT;

#Use this to turn off osmotic diuresis effect

osmolality_in_CD = osmoles_in_CCD/water_in_CCD;

Na_concentration_in_CD = Na_concentration_out_CNT;

urea_concentration_in_CD = urea_concentration_out_DCT2;

glucose_concentration_in_CD = glucose_concentration_out_DCT2;

osmotic_diuresis_effect_cd = 1-min(0.5,RUGE *glucose_diuresis_effect_cd);

####Assume sodium reabsorbed, then water follows

#### Then urea reabsorbed at end

#### Then additional water reabsorbed following urea reabsorption

excess_flow_effec_on_cd = min(1,nom_water_in_CD/(water_in_CCD-1.5e-9));

cd_multiplier = max(0,
aldo_effect_on_ENAC_delayed*AT1_effect_on_ENAC*anp_effect_on_CD
*rsna_effect_on_CD*pressure_natriuresis_CD_effect*RBF_CD_effect*R_ET1_ETB_effect_on
_CD);


J_Enac_CCD=cd_multiplier*Na_luminal_permeability_ccd*(CCD_luminal_Na_avg_conc_dela
yed-CCD_cell_Na_conc);

J_Enac_CCD_effective = min(J_Enac_CCD,  Na_in_CD/CCD_SA)

ccd_na_reabsorption=J_Enac_CCD*CCD_SA/Na_in_CD;

e_ccd_sodreab = min(1,ccd_na_reabsorption);

e_mcd_sodreab = min(1,nominal_mcd_na_reabsorption*cd_multiplier);

#Assume sodium reabsorbed at fractional rate eta
```

e_ccd_sodreab_adj = e_ccd_sodreab*excess_flow_effec_on_cd;

R_ccd = -log(1-e_ccd_sodreab_adj)/L_ccd;

Na_reabsorbed_CCD = min(Na_in_CD*(1-exp(-R_ccd*L_ccd)),CD_Na_reabs_threshold);

Na_out_CCD = Na_in_CD-Na_reabsorbed_CCD;

CCD_Na_reabs_fraction = 1-Na_out_CCD/Na_in_CD;

################################ CCD K+ Secretion ##

normalized_luminal_potential_difference_CCD = log((CCD_luminal_K_avg_conc_delayed + 0.05*CCD_luminal_Na_avg_conc_delayed + 0.45*0.1)/(CCD_cell_K_conc + 0.05*CCD_cell_Na_conc + 0.45*0.01))

#weighting factors assumed based on differences in permeability

normalized_basolateral_potential_difference_CCD = log((4*plasma_K + 0.05*Na_concentration/1000 + 0.1*0.1)/(4*CCD_cell_K_conc + 0.05*CCD_cell_Na_conc + 0.1*0.01))

#flow effect on potassium secretion

flow_effect_K_CCD = max(0, 1 + m_flow_K*((water_in_CCD - water_in_CCD0)/water_in_CCD0))

#CCD Potassium flux from cell to lumen #mEq/min.cm^2 #Goldman equation

CCD_K_passive_flux_lumenal = baseline_K_luminal_permeability_CCD*aldo_effect_on_ROMK*flow_effect_K_CCD*normalized_luminal_potential_difference_CCD*

(-CCD_luminal_K_avg_conc_delayed + CCD_cell_K_conc*exp(-normalized_luminal_potential_difference_CCD))

/(1-exp(-normalized_luminal_potential_difference_CCD)); #mEq/min.cm^2 #Goldman equation

#CCD passive potassium flux across basolateral membrane #mEq/min.cm^2 #Goldman equation

CCD_K_passive_flux_basolateral = -K_basolateral_permeability_CCD*normalized_basolateral_potential_difference_CCD*

(-plasma_K + CCD_cell_K_conc*exp(-normalized_basolateral_potential_difference_CCD))

/(1-exp(-normalized_basolateral_potential_difference_CCD)); #mEq/min.cm^2 #Goldman equation

#CCD Active flux the Na+/K+ ATPase across basolateral membrane, #mEq/min.cm^2

K_Na_CCD = (0.2*(1+CCD_cell_K_conc*1000/8.33))/1000

J_Na_active_max_eff_CCD = J_Na_active_max_CCD*max(0,(Aldo_effect_on_K_secretion))

```
  CCD_Na_active_flux_basolateral =
J_Na_active_max_eff_CCD*((CCD_cell_Na_conc/(CCD_cell_Na_conc +
K_Na_CCD))^3)*((plasma_K/(plasma_K + K_K))^2)

 CCD_K_active_flux_basolateral = (2/3)*CCD_Na_active_flux_basolateral

  CCD_Na_passive_flux_basolateral =
Na_basolateral_permeability_ccd*(Na_concentration/1000 - CCD_cell_Na_conc);

  #CCD potassium secretion rates

  CCD_K_secretion_rate = CCD_K_passive_flux_lumenal*CCD_SA*principal_fraction_CCD

  #Potassium out of each tubular segment

  CCD_K_out = CNT_K_out +  CCD_K_secretion_rate
#CCD_luminal_K_avg_conc_delayed*water_out_CCD*1000

  vasopressin_perm_int = 1+vasopressin_perm_scale/2;

  ADH_water_permeability =
max(0,1+vasopressin_perm_scale*(normalized_vasopressin_concentration - 1));

  #Water reabsorption follows gradient but is regulated by ADH

  osmoles_out_CCD = osmoles_in_CCD-2*((Na_out_CNT - Na_out_CCD) + (CNT_K_out -
CCD_K_out));


  interstitial_osmolality_end_of_CCD = osmolality_out_AscLoH + (osmolality_out_DescLoH -
osmolality_out_AscLoH)*(L_ccd/(L_ccd + L_mcd))

  osmolality_out_CCD_before_osmotic_reabsorption = osmoles_out_CCD/water_in_CCD;

  water_reabsorbed_CCD = min(water_in_CCD,
R_ET1_ETB_effect_on_CD_water*nom_water_permeability*ADH_water_permeability*osmoti
c_diuresis_effect_cd*water_in_CCD*(1-
osmolality_out_CCD_before_osmotic_reabsorption/interstitial_osmolality_end_of_CCD));

  water_out_CCD = water_in_CCD-water_reabsorbed_CCD;

  osmolality_out_CCD_after_osmotic_reabsorption = osmoles_out_CCD/water_out_CCD;

  Na_concentration_out_CCD = Na_out_CCD/water_out_CCD;

  CCD_luminal_Na_avg_conc = (Na_concentration_out_CNT +
Na_concentration_out_CCD)/2/1000

  K_concentration_out_CCD = CCD_K_out/water_out_CCD

  CCD_luminal_K_avg_conc = (K_concentration_out_CNT +
K_concentration_out_CCD)/2/1000
```

```
######################Medullary Collecting  Duct################################

osmoles_in_MCD = osmoles_out_CCD

water_in_MCD = water_out_CCD

#Assume sodium reabsorbed at fractional rate eta

J_Enac_MCD=cd_multiplier*Na_luminal_permeability_mcd*(MCD_luminal_Na_avg_conc_del
ayed-MCD_cell_Na_conc);

J_Enac_MCD_effective = min(J_Enac_MCD,  Na_out_CCD/MCD_SA)

mcd_na_reabsorption=J_Enac_MCD*MCD_SA/Na_out_CCD;

e_mcd_sodreab = min(1,mcd_na_reabsorption);

R_mcd = -log(1-e_mcd_sodreab)/L_mcd;

Na_reabsorbed_MCD = min(Na_out_CCD*(1-exp(-R_mcd*L_mcd)),CD_Na_reabs_threshold,
Na_out_CCD);

Na_out_MCD = Na_out_CCD-Na_reabsorbed_MCD;

MCD_Na_reabs_fraction = 1-Na_out_MCD/Na_out_CCD;

################################ MCD K+ Reabsorption
###########################################

  ####come back and make like Na concentration - delayed

  normalized_luminal_potential_difference_MCD = log((MCD_luminal_K_avg_conc_delayed +
0.05*MCD_luminal_Na_avg_conc_delayed + 0.45*0.1)/(MCD_cell_K_conc +
0.05*MCD_cell_Na_conc  + 0.45*0.01))

  #weighting factors assumed based on differences in permeability

  normalized_basolateral_potential_difference_MCD = log((4*plasma_K +
0.05*Na_concentration/1000 + 0.1*0.1)/(4*MCD_cell_K_conc + 0.05*MCD_cell_Na_conc  +
0.1*0.01))

#MCD Potassium flux from cell to lumen #mEq/min.cm^2 #Goldman equation

MCD_K_passive_flux_lumenal =
baseline_K_luminal_permeability_MCD*aldo_effect_on_ROMK*normalized_luminal_potential
_difference_MCD*

    (-MCD_luminal_K_avg_conc_delayed + MCD_cell_K_conc*exp(-
normalized_luminal_potential_difference_MCD))

    /(1-exp(-normalized_luminal_potential_difference_MCD)); #mEq/min.cm^2 #Goldman
equation

#MCD passive potassium flux across basolateral membrane #mEq/min.cm^2 #Goldman
#equation
```

```
MCD_K_passive_flux_basolateral = -
K_basolateral_permeability_MCD*normalized_basolateral_potential_difference_MCD*

   (-plasma_K + MCD_cell_K_conc*exp(-normalized_basolateral_potential_difference_MCD))

   /(1-exp(-normalized_basolateral_potential_difference_MCD)); #mEq/min.cm^2 #Goldman
equation

#MCD Active flux the Na+/K+ ATPase across basolateral membrane,  #mEq/min.cm^2

  K_Na_MCD = (0.2*(1+MCD_cell_K_conc*1000/8.33))/1000

  J_Na_active_max_eff_MCD = J_Na_active_max_MCD*max(0,(Aldo_effect_on_K_secretion))

  MCD_Na_active_flux_basolateral =
J_Na_active_max_eff_MCD*((MCD_cell_Na_conc/(MCD_cell_Na_conc +
K_Na_MCD))^3)*((plasma_K/(plasma_K + K_K))^2)

 MCD_K_active_flux_basolateral = (2/3)*MCD_Na_active_flux_basolateral

  MCD_Na_passive_flux_basolateral =
Na_basolateral_permeability_mcd*(Na_concentration/1000 - MCD_cell_Na_conc);

  #MCD potassium secretion rates

  MCD_K_secretion_rate = MCD_K_passive_flux_lumenal*MCD_SA*principal_fraction_MCD

  MCD_K_transcellular_reabsorption = m_plasmaK_MCD*( (norm_plasma_K -
plasma_K_delayed)/norm_plasma_K)

  MCD_K_out = CCD_K_out +  MCD_K_secretion_rate - MCD_K_transcellular_reabsorption
#CCD_luminal_K_avg_conc_delayed*water_out_CCD*1000

################################  Water Reabsorption
##############################################

  #Total potassium excretion is potassium leaving the CD times the number of nephrons

  CD_K_out = number_of_nephrons*MCD_K_out; #(mEq/min)

  #Water reabsorption follows gradient but is regulated by ADH

  osmoles_out_MCD = osmoles_in_MCD-2*((Na_out_CCD - Na_out_MCD) + (CCD_K_out -
MCD_K_out));

  osmolality_out_MCD_before_osmotic_reabsorption = osmoles_out_MCD/water_in_MCD;

  water_reabsorbed_MCD = min(water_in_MCD,
R_ET1_ETB_effect_on_CD_water*nom_water_permeability*ADH_water_permeability*osmoti
c_diuresis_effect_cd*water_in_MCD*(1-
osmolality_out_MCD_before_osmotic_reabsorption/osmolality_out_DescLoH));

  water_out_MCD = water_in_MCD-water_reabsorbed_MCD;

  osmolality_out_MCD_after_osmotic_reabsorption = osmoles_out_MCD/water_out_MCD;
```

```
  glucose_concentration_after_urea_reabsorption = glucose_in_CD/water_out_MCD;

  Na_concentration_out_MCD = Na_out_MCD/water_out_MCD

  MCD_luminal_Na_avg_conc = (Na_concentration_out_CCD +
Na_concentration_out_MCD)/2/1000

  K_concentration_out_MCD = MCD_K_out/water_out_MCD

  MCD_luminal_K_avg_conc = (K_concentration_out_CCD +
K_concentration_out_MCD)/2/1000

  urine_flow_rate = water_out_MCD*number_of_functional_tubules;

  daily_urine_flow = (urine_flow_rate * 60 * 24);

Na_oral_absorption = Ka_Na*Na_oral_depot; #Allows fixing sodium intake to simulaton
specific experimental designs. Normally set to zero.

Na_intake = Na_intake_rate + Na_oral_absorption;

Na_excretion_via_urine = Na_out_MCD*number_of_functional_tubules;

Na_balance = Na_intake - Na_excretion_via_urine;

water_balance = daily_water_intake - daily_urine_flow;

free_water_clearance = urine_flow_rate*(1-
osmolality_out_MCD_after_osmotic_reabsorption/plasma_osmolality );   #L/min

FENA = Na_excretion_via_urine/filtered_Na_load;

PT_fractional_glucose_reabs = (SN_filtered_glucose_load -
glucose_pt_out_s3)/SN_filtered_glucose_load;

PT_fractional_urea_reabs = ( SN_filtered_urea_load - urea_out_s3)/SN_filtered_urea_load;

PT_fractional_water_reabs = ((SNGFR_nL_min / 1000 / 1000000) -
water_out_s3)/(SNGFR_nL_min / 1000 / 1000000);

LoH_fractional_Na_reabs = (Na_in_DescLoH - Na_out_AscLoH)/Na_in_DescLoH;

LoH_fractional_urea_reabs = (urea_in_DescLoH-urea_out_AscLoH)/urea_in_DescLoH;

LoH_fractional_water_reabs = (water_in_DescLoH - water_out_AscLoH)/water_in_DescLoH;

DCT1_fractional_Na_reabs = (Na_in_DCT - Na_out_DCT1)/Na_in_DCT;

DCT2_fractional_Na_reabs = (Na_in_DCT2 - Na_out_DCT2)/Na_in_DCT2;

CNT_fractionaal_Na_reabs = (Na_out_DCT2 - Na_out_CNT)/Na_out_DCT2;

CCD_fractional_Na_reabs = (Na_in_CD - Na_out_CCD)/Na_in_CD;

MCD_fractional_Na_reabs = (Na_out_CCD - Na_out_MCD)/Na_out_CCD;

CD_fractional_Na_reabs = (Na_in_CD - Na_out_MCD)/Na_in_CD;
```

CD_OM_fractional_water_reabs = (water_in_CCD - water_out_MCD)/water_in_CCD;

######################Renal Interstitial Hydrostatic pressure

######RIHP can be approximated from Starling's equation for the peritubular capillaries

Flow out of the capillary = Kf_peritubular*(Peritubular pressure - RIHP - oncotic pressure difference)

Assume that any fluid reabsorbed reenters the capillary.

Therefore, RIHP = Peritubular Pressure - (oncotic pressure in peritubular capillary - interstitial oncotic pressure) + tubular reabsorption/KF

#Peritubular pressure is assumed to equal postglomerular pressure

#Oncotic pressure at the entrance to the peritubular circulation equals oncotic pressure at the exit of the glomerular

Oncotic_pressure_peritubular_in = Oncotic_pressure_out;

plasma_protein_concentration_peritubular_out = (SNRBF_nl_min)*plasma_protein_concentration/(SNRBF_nl_min-urine_flow_rate*1e6*1000/number_of_functional_tubules);

Oncotic_pressure_peritubular_out = 1.629*plasma_protein_concentration_peritubular_out+0.2935*(plasma_protein_concentration_peritubular_out^2);

oncotic_pressure_peritubular_avg = (Oncotic_pressure_peritubular_in+Oncotic_pressure_peritubular_out)/2;

oncotic_pressure_peritubular_cap_Na = 0;#Na_concentration_peritubular_cap*19.3*2;

oncotic_pressure_peritubular = oncotic_pressure_peritubular_avg+oncotic_pressure_peritubular_cap_Na;

#The amount of fluid reabsorbed is the difference between the amount filtered and the amount excreted

tubular_reabsorption = GFR_ml_min/1000 - urine_flow_rate;

#Renal interstitial-capillary filtration

Renal_plasma_amount= 2.5 * RISF_nom*0.01; # plasma amount in renal interstitium# Plasma protein concentration = 7

RISF_plasma_protein_concentration = Renal_plasma_amount / (RISF*0.01);

interstitial_oncotic_pressure = 1.629*RISF_plasma_protein_concentration+0.2935*(RISF_plasma_protein_concentration^2);

RIHP = RISF/C_RISF;

#As glomeruli are lost, assume the downstream peritubular surface area is also lost.

```
renal_capillary_filtration = nom_peritubular_cap_Kf*(1-
disease_effect_losing_glomeruli)*(RIHP - ((postglomerular_pressure +
renal_venous_pressure)/2) - (interstitial_oncotic_pressure -oncotic_pressure_peritubular));

################## Tubular Pressure ####################

#####See written documentation for derivation of the equations below

#flow rates expressed in m3/min, rather than L/min

mmHg_Nperm2_conv = 133.32;

Pc_pt_s1 = Pc_pt_s1_mmHg*mmHg_Nperm2_conv;

Pc_pt_s2 = Pc_pt_s2_mmHg*mmHg_Nperm2_conv;

Pc_pt_s3 = Pc_pt_s3_mmHg*mmHg_Nperm2_conv;

Pc_lh_des = Pc_lh_des_mmHg*mmHg_Nperm2_conv;

Pc_lh_asc = Pc_lh_asc_mmHg*mmHg_Nperm2_conv;

Pc_dt = Pc_dt_mmHg*mmHg_Nperm2_conv;

Pc_cd = Pc_cd_mmHg*mmHg_Nperm2_conv;

P_interstitial = (RIHP + 0.35)*mmHg_Nperm2_conv;#(renal_interstitial_hydrostatic_pressure-
5)*mmHg_Nperm2_conv;

pi=3.14;

###CD

B1 = (4*tubular_compliance+1)*128*gamma/pi;

mean_cd_water_flow = (water_in_CCD-water_out_MCD)/2;

B2_cd = (Pc_cd^(4*tubular_compliance))/(Dc_cd^4);

P_in_cd = (0^(4*tubular_compliance+1)+B1*B2_cd*(mean_cd_water_flow/1e3)*(L_ccd +
L_mcd))^(1/(4*tubular_compliance+1));

P_in_cd_mmHg = (P_in_cd+P_interstitial)/mmHg_Nperm2_conv;

### DCT

B2_dt = (Pc_dt^(4*tubular_compliance))/(Dc_dt^4);

P_in_dt =
(P_in_cd^(4*tubular_compliance+1)+B1*B2_dt*(water_in_DCT/1e3)*L_dct)^(1/(4*tubular_co
mpliance+1));

P_in_dt_mmHg = (P_in_dt+P_interstitial)/mmHg_Nperm2_conv;

### Asc LoH

B2_lh_asc = (Pc_lh_asc^(4*tubular_compliance))/(Dc_lh^4);
```

```
P_in_lh_asc =
(P_in_dt^(4*tubular_compliance+1)+B1*B2_lh_asc*(water_in_AscLoH/1e3)*L_lh_asc)^(1/(4*
tubular_compliance+1));

P_in_lh_asc_mmHg = (P_in_lh_asc+P_interstitial)/mmHg_Nperm2_conv;

### Desc LoH

A_lh_des = effective_AscLoH_Reab_Rate/(water_in_DescLoH*osmolality_in_DescLoH);

B2_lh_des =
(Pc_lh_des^(4*tubular_compliance))*(water_in_DescLoH/1e3)/((Dc_lh^4)*A_lh_des);

P_in_lh_des = (P_in_lh_asc^(4*tubular_compliance+1)+B1*B2_lh_des*(1-exp(-
A_lh_des*L_lh_des)))^(1/(4*tubular_compliance+1));

P_in_lh_des_mmHg = (P_in_lh_des+P_interstitial)/mmHg_Nperm2_conv;

### PT

#Treat urea as if reabsorbed linearly along whole length of PT

Rurea = (SN_filtered_urea_load - urea_out_s3)/(L_pt_s1+L_pt_s2+L_pt_s3);

urea_in_s2 = SN_filtered_urea_load - Rurea*L_pt_s1;

urea_in_s3 = SN_filtered_urea_load - Rurea*(L_pt_s1+L_pt_s2);

A_na = Na_reabs_per_unit_length;

flow_integral_s3 = 2*(Na_pt_out_s2/A_na)*(1-exp(-A_na*L_pt_s3)) -
(3/2)*glucose_pt_out_s2*L_pt_s3^2 + urea_in_s3*L_pt_s3 - (1/2)*Rurea*(L_pt_s3^2);

flow_integral_s2 = 2*(Na_pt_out_s1/A_na)*(1-exp(-A_na*L_pt_s2)) -
(1/2)*glucose_pt_out_s1*L_pt_s2^2 + urea_in_s2*L_pt_s2 - (1/2)*Rurea*(L_pt_s2^2);

flow_integral_s1 = 2*(SN_filtered_Na_load/A_na)*(1-exp(-A_na*L_pt_s1)) -
(1/2)*SN_filtered_glucose_load*L_pt_s1^2 + SN_filtered_urea_load*L_pt_s1 -
(1/2)*Rurea*(L_pt_s1^2);

#S3 segment

B2_pt_s3 = (Pc_pt_s3^(4*tubular_compliance))/(Dc_pt^4);

B3_pt_s3 = (water_out_s2/1e3)/osmoles_out_s2;

P_in_pt_s3=
(P_in_lh_des^(4*tubular_compliance+1)+B1*B2_pt_s3*B3_pt_s3*flow_integral_s3)^(1/(4*tub
ular_compliance+1));

P_in_pt_s3_mmHg = (P_in_pt_s3+P_interstitial)/mmHg_Nperm2_conv;

B2_pt_s2 = (Pc_pt_s3^(4*tubular_compliance))/(Dc_pt^4);

B3_pt_s2 = (water_out_s1/1e3)/osmoles_out_s1;
```

```
P_in_pt_s2=
(P_in_pt_s3^(4*tubular_compliance+1)+B1*B2_pt_s2*B3_pt_s2*flow_integral_s2)^(1/(4*tubul
ar_compliance+1));

P_in_pt_s2_mmHg = (P_in_pt_s2+P_interstitial)/mmHg_Nperm2_conv;

B2_pt_s1 = (Pc_pt_s1^(4*tubular_compliance))/(Dc_pt^4);

B3_pt_s1 = (SNGFR_nL_min / 1e12)/(2*SN_filtered_Na_load+SN_filtered_glucose_load+
SN_filtered_urea_load);

P_in_pt_s1=
(P_in_pt_s2^(4*tubular_compliance+1)+B1*B2_pt_s1*B3_pt_s1*flow_integral_s1)^(1/(4*tubul
ar_compliance+1));

P_in_pt_s1_mmHg = (P_in_pt_s1+P_interstitial)/mmHg_Nperm2_conv;

ISF = interstitial_fluid_volume;

######################### Renal Oxygenation ##############################

  hemoglobin_concentration = hemoglobin_amount / (blood_volume_L * L_dL);

  #Oxygen concentration in ml O2 / 100 ml (well-established empirical relationship)

  arterial_oxygen_concentration_ml = hemoglobin_concentration*1.36 * SaO2 + 0.0031*PaO2;
#ml O2/ 100 ml

  #Oxygen concentration converted to mols/L    PV = nRT

  #arterial_oxygen_concentration_mols =
(arterial_oxygen_concentration_ml*10)*(1+mean_arterial_pressure_MAP*mmHg_atm)/((R/.00
1)*Temperature) #mol/L

  PT_oxygen_delivery = renal_blood_flow_L_min*(arterial_oxygen_concentration_ml*10); #ml
O2/min

  peritubular_oxygen_conc =
peritubular_oxygen/(PT_capillary_volume*number_of_functional_tubules/baseline_nephrons);
#ml O2/L

  PT_oxygen_out = renal_blood_flow_L_min*peritubular_oxygen_conc;

  oxygen_consumption_rate = number_of_functional_tubules*((SN_filtered_Na_load -
Na_pt_out_s3)/PT_Na_oxygen_ratio +

  (Na_pt_out_s3 - Na_out_AscLoH)/LoH_Na_oxygen_ratio +

  (Na_out_AscLoH - Na_out_MCD)/DCT_CD_Na_oxygen_ratio);

######################### Renal Disease Progression
#########################################

age_sclerosis_effect = 1/T_age_sclerosis;
```

```
pressure_glomerulosis_effect = GPdiff/T_pressure_glomerulosclerosis;

IgA_sclerosis_effect = IgA_on/T_IgA_sclerosis;

FSGS_sclerosis_effect = FSGS_on/T_FSGS_sclerosis;

glomerulosclerosis_factors = pressure_glomerulosis_effect+age_sclerosis_effect +
FSGS_sclerosis_effect + IgA_sclerosis_effect;

FSGS_nephron_loss_effect = FSGS_on/T_FSGS_nephronloss;

age_fibrosis_effect = 1/T_age_fibrosis;

albumin_fibrosis_effect = max(0, SN_albumin_excretion_rate*1.5e6*24*60 -
30)/T_albumin_fibrosis;

PT_overload_fibrosis_effect = max(0,normalized_PT_reabsorption_density-
PT_overload_threshold)/T_PT_overload_fibrosis;

tubulointerstitial_fibrosis_factors = albumin_fibrosis_effect+age_fibrosis_effect +
FSGS_nephron_loss_effect + PT_overload_fibrosis_effect;

#Rate at which Kf (average single nephron permeability and surface area) decreases

glomerulosclerosis_rate = (1-disease_effects_decreasing_Kf)*glomerulosclerosis_factors;

#Rate at which glomerulosclerosis results in full glomerulus loss

glomerulosclerosis_glomeruli_loss_rate = (1-
disease_effect_losing_glomeruli)*beta_gs_gl*glomerulosclerosis_factors;

tubular_fibrosis_tubule_loss_rate   = (1-
disease_effect_on_nephrons)*(tubulointerstitial_fibrosis_factors);

#All fully sclerotic glomeruli are assumed to result in full nephron loss (functionally, if not
histologically) because

#there is no filtrate to be reabsorbed

glomerulosclerosis_tubule_loss_rate = glomerulosclerosis_glomeruli_loss_rate;

#beta_tf_nl - fraction of glomerili that are lost when tubule is lost. Glomerili may remain open
but  atubular

#In this case, blood flows through them but no filtration occurs.

tubular_fibrosis_to_glomeruli_loss_rate = beta_tf_nl*tubular_fibrosis_tubule_loss_rate;

tubule_loss_rate = max(tubular_fibrosis_tubule_loss_rate, glomerulosclerosis_tubule_loss_rate)
+                0.25*min(tubular_fibrosis_tubule_loss_rate, glomerulosclerosis_tubule_loss_rate);

glomeruli_loss_rate =
max(glomerulosclerosis_glomeruli_loss_rate,tubular_fibrosis_to_glomeruli_loss_rate) +


0.25*min(glomerulosclerosis_glomeruli_loss_rate,tubular_fibrosis_to_glomeruli_loss_rate);
```

#Yeshi added for DRI effect on PRA - dose mediated (for TAK-272)

DRI_central_conc = DRI_central/DRI_Vc;

d/dt(DRI_depot) = -DRI_KA*DRI_depot;

d/dt(DRI_central) = DRI_KA*DRI_depot - (Vm1/(Km1*DRI_Vc + DRI_central))*DRI_central + DRI_K21*DRI_periph -(Vm2/(Km2*DRI_Vc + DRI_central))*DRI_central;

d/dt(DRI_periph) = (Vm2/(Km2*DRI_Vc + DRI_central))*DRI_central - DRI_K21*DRI_periph ;

d/dt(DRI_eff) = DRI_KDEL*(DRI_central_conc-DRI_eff);

End - Yeshi addition

d/dt(AngI) = plasma_renin_activity - (AngI) * (chymase_activity + ACE_activity) - (AngI) * AngI_degradation_rate;

d/dt(AngII) = AngI * (chymase_activity + ACE_activity) - AngII * AngII_degradation_rate - AngII*AT1_receptor_binding_rate - AngII* (AT2_receptor_binding_rate);

d/dt(AT1_bound_AngII) = AngII * (AT1_receptor_binding_rate) - AT1_bound_AngII_degradation_rate*AT1_bound_AngII;

d/dt(AT2_bound_AngII) = AngII * (AT2_receptor_binding_rate) - AT2_bound_AngII_degradation_rate*AT2_bound_AngII;

d/dt(plasma_renin_concentration) = renin_secretion_rate - plasma_renin_concentration * renin_degradation_rate;

#Change in interstitial fluid volume over time is determined by the different between water intake and urine outflow

d/dt(blood_volume_L) = water_intake - urine_flow_rate - blood_interstitium_flux;

d/dt(interstitial_fluid_volume) = blood_interstitium_flux - interstitial_intracellular_flux; #Q_water*(IF_Na_concentration - Na_concentration) ;

d/dt(intracellular_fluid_volume) = interstitial_intracellular_flux;

#Change in total body sodium over time is determined by the different between sodium intake and excretion

d/dt(sodium_amount) = Na_intake - Na_excretion_via_urine + Q_Na*(IF_Na_concentration - Na_concentration);

d/dt(IF_sodium_amount) = Q_Na*(Na_concentration - IF_Na_concentration) - sodium_storate_rate;

d/dt(stored_sodium) = sodium_storate_rate;

#These equations serve only to delay the input variable by one timestep. This allows the previous value of the input variable to be used in an equation that appears

#in the code before the input variable was defined

d/dt(tubulo_glomerular_feedback_effect) = C_tgf * (tubulo_glomerular_feedback_signal-tubulo_glomerular_feedback_effect);

d/dt(preafferent_pressure_autoreg_signal) = 500*(preafferent_pressure_autoreg_function - preafferent_pressure_autoreg_signal);

d/dt(glomerular_pressure_autoreg_signal) = 500*(glomerular_pressure_autoreg_function - glomerular_pressure_autoreg_signal);

d/dt(F_out_dt_delay) = 0;#100*(F_out_dt - F_out_dt_delay);

d/dt(cardiac_output_delayed) = C_cardiac_output_delayed*(cardiac_output - cardiac_output_delayed);

d/dt(MAP_delayed) = C_map_delay*(mean_arterial_pressure_MAP - MAP_delayed);

d/dt(MAP_setpoint) = map_reset_rate*(mean_arterial_pressure_MAP - MAP_setpoint);

d/dt(RAP_setpoint) = RAP_reset_rate*(right_atrial_pressure_delayed - RAP_setpoint);

#Error signals for PI controllers of cardiac output and sodium concentration

d/dt(CO_error) = C_co_error*(cardiac_output-CO_nom);

d/dt(Na_concentration_error) = C_Na_error*(Na_concentration - ref_Na_concentration);

d/dt(K_concentration_error) = C_Na_error*(1000*(plasma_K - norm_plasma_K));

#This equation allows a delay between the secretion of vasopression and its effect on water intake and tubular water reabsorption

d/dt(normalized_vasopressin_concentration_delayed)= C_vasopressin_delay*(normalized_vasopressin_concentration - normalized_vasopressin_concentration_delayed);

#TGF resetting. If C_tgf_reset = 0, no TGF resetting occurs. If it is greater than zero, the setpoint will change over time and will eventually

#come to equal the ambient MD sodium flow rate.

d/dt(F0_TGF) = C_tgf_reset*(SN_macula_densa_Na_flow*baseline_nephrons - F0_TGF);

#As above, these equations allow a variable to be used in equations that appear in the code before the variable was first defined.

d/dt(P_bowmans) = C_P_bowmans*(P_in_pt_s1_mmHg - P_bowmans);

d/dt(oncotic_pressure_difference) = C_P_oncotic*(oncotic_pressure_avg - oncotic_pressure_difference);

d/dt(renal_blood_flow_L_min_delayed)=C_rbf*(renal_blood_flow_L_min - renal_blood_flow_L_min_delayed);

d/dt(renal_interstitial_hydrostatic_pressure) = C_rihp*(RIHP - renal_interstitial_hydrostatic_pressure);

d/dt(SN_macula_densa_Na_flow_delayed) = C_md_flow*(SN_macula_densa_Na_flow - SN_macula_densa_Na_flow_delayed);

d/dt(LoH_K_out_delayed) = LoH_K_out - LoH_K_out_delayed;

d/dt(rsna_delayed) = C_rsna*(renal_sympathetic_nerve_activity - rsna_delayed);

d/dt(rsna_delayed2) = C_rsna2*(renal_sympathetic_nerve_activity - rsna_delayed2);

###Disease effects (turned off by default)

#Glomerular hypertrophy

d/dt(disease_effects_increasing_Kf) = GP_effect_increasing_Kf;

#Loss of CD pressure natriuresis response over time

d/dt(disease_effects_decreasing_CD_PN) = CD_PN_loss_rate;

#Tubular hypertrophy

d/dt(tubular_length_increase) = PT_Na_reabs_effect_increasing_tubular_length;

d/dt(tubular_diameter_increase) = PT_Na_reabs_effect_increasing_tubular_diameter;

d/dt(water_out_s1_delayed) = C_pt_water*(water_out_s1 - water_out_s1_delayed);

d/dt(water_out_s2_delayed) = C_pt_water*(water_out_s2 - water_out_s2_delayed);

d/dt(water_out_s3_delayed) = C_pt_water*(water_out_s3 - water_out_s3_delayed);

d/dt(reabsorbed_urea_cd_delayed) = 0;#C_pt_water*(reabsorbed_urea_cd - reabsorbed_urea_cd_delayed);

#Urinary glucose excretion

d/dt(UGE) = RUGE;

#Serum Creatinine

d/dt(serum_creatinine) = creatinine_synthesis_rate - creatinine_excretion_rate;

d/dt(cumNaExcretion) = Na_excretion_via_urine;

d/dt(cumWaterExcretion) = urine_flow_rate;

d/dt(cumCreatinineExcretion) = creatinine_excretion_rate;

d/dt(RTg_compensation) = excess_glucose_increasing_RTg;

d/dt(SGLT2_inhibition_delayed) = C_sglt2_delay*(SGLT2_inhibition - SGLT2_inhibition_delayed);

d/dt(SGLT2_glucose_reabsorption_delayed) = C_ruge*(SGLT2_glucose_reabsorption - SGLT2_glucose_reabsorption_delayed);

```
d/dt(SGLT2_inhibition_glucose_effect_delayed) = C_sglt2i_glucose_delay*(SGLT2_inhibition -
SGLT2_inhibition_glucose_effect_delayed);

d/dt(disease_effect_on_nephrons) = tubule_loss_rate;

d/dt(disease_effects_decreasing_Kf) = glomerulosclerosis_rate;

d/dt(disease_effect_on_seiving) = IgA_effect_on_Seiving + FSGS_effect_on_Seiving;

d/dt(peritubular_oxygen) =  (PT_oxygen_delivery - oxygen_consumption_rate - PT_oxygen_out); #ml O2

d/dt(disease_effect_losing_glomeruli) =  glomeruli_loss_rate;

d/dt(RISF) = tubular_reabsorption - renal_capillary_filtration;

d/dt(right_atrial_pressure_delayed) = C_rap*(right_atrial_pressure - right_atrial_pressure_delayed);

d/dt(ANP_delayed) = C_anp*(ANP_concentration - ANP_delayed);

d/dt(BigET_amt) =

    #Production of bigET

    BigET_prod_rate -

    #Conversion to ET1

    ET1_production_from_BIGET;

d/dt(ET1_total_peri_amt)=

    # production from bigET:

    ET1_production_from_BIGET +

    # ET1 transfer between cent and peri:

     - Q_ET1_pc*ET1_peri + Q_ET1_cp*ET1_cent -


    #Internalization and clearance through receptor binding

    ET1_ETB_internalization_peri - ET1_ETA_internalization_peri

d/dt(ET1_total_cent_amt)=

  ET1_infusion_rate_cent +

   # ET1 transfer between cent and peri:

    Q_ET1_pc*ET1_peri -  Q_ET1_cp*ET1_cent -

      #Internalization and clearance through receptor binding

    ET1_ETB_internalization_cent - ET1_ETA_internalization_cent

#Radiolabeled ET-1
```

d/dt(ET1_total_cent_labeled_amt) =

ET1 transfer between cent and peri:

Q_ET1_pc*ET1_peri_labeled - Q_ET1_cp*ET1_cent_labeled -

#Internalization and clearance through receptor binding

ET1_ETB_internalization_cent_labeled - ET1_ETA_internalization_cent_labeled

d/dt(ET1_total_peri_labeled_amt) =

ET1 transfer between cent and peri:

- Q_ET1_pc*ET1_peri_labeled + Q_ET1_cp*ET1_cent_labeled -

#Internalization and clearance through receptor binding

ET1_ETB_internalization_peri_labeled - ET1_ETA_internalization_peri_labeled

d/dt(Na_oral_depot) = -Ka_Na*Na_oral_depot;

d/dt(water_oral_depot) = -Ka_water*water_oral_depot;

d/dt(R_ET1_ETB_effect_on_aff) = Kin_ET1_ETB*(ET1_ETB_effect_on_aff) - Kout_ET1_ETB*R_ET1_ETB_effect_on_aff

d/dt(R_ET1_ETB_effect_on_eff) = Kin_ET1_ETB*(ET1_ETB_effect_on_eff) - Kout_ET1_ETB*R_ET1_ETB_effect_on_eff

d/dt(R_ET1_ETA_effect_on_aff) = Kin_ET1_ETA*(ET1_ETA_effect_on_aff) - Kout_ET1_ETA*R_ET1_ETA_effect_on_aff

d/dt(R_ET1_ETA_effect_on_preaff) = Kin_ET1_ETA*(ET1_ETA_effect_on_preaff) - Kout_ET1_ETA*R_ET1_ETA_effect_on_preaff

d/dt(R_ET1_ETA_effect_on_eff) = Kin_ET1_ETA*(ET1_ETA_effect_on_eff) - Kout_ET1_ETA*R_ET1_ETA_effect_on_eff

d/dt(R_ET1_ETA_cent_on_SVR) = Kin_ET1_ETA*(ET1_ETA_cent_on_SVR) - Kout_ET1_ETA*R_ET1_ETA_cent_on_SVR;

d/dt(R_ET1_ETA_cent_on_venous_capacity) = Kin_ET1_ETA*(ET1_ETA_cent_on_venous_capacity) - Kout_ET1_ETA*R_ET1_ETA_cent_on_venous_capacity

d/dt(R_ET1_ETA_cent_on_venous_resistance) = Kin_ET1_ETA*(ET1_ETA_cent_on_venous_resistance) - Kout_ET1_ETA*R_ET1_ETA_cent_on_venous_resistance

d/dt(R_ET1_ETA_cent_on_venous_compliance) = 0

d/dt(R_ET1_ETB_effect_on_CD) = Kin_ET1_ETB_peri*(ET1_ETB_effect_on_CD) - Kout_ET1_ETB_peri*R_ET1_ETB_effect_on_CD;

d/dt(R_ET1_ETB_effect_on_CD_water) = Kin_ET1_ETB_peri*(ET1_ETB_effect_on_CD_water) - Kout_ET1_ETB_peri*R_ET1_ETB_effect_on_CD_water;

d/dt(R_ET1_ETA_effect_on_PT) = Kin_ET1_ETA*(ET1_ETA_effect_on_PT) - Kout_ET1_ETA*R_ET1_ETA_effect_on_PT;

#MRA Pharmacokinetic Depot compartments

d/dt(spiro_depot) = -Ka_spiro*spiro_depot;

d/dt(spiro_t1) = Ka_spiro*spiro_depot - Ka_spiro*(spiro_t1)

d/dt(spiro_t2) = Ka_spiro*(spiro_t1) - Ka_spiro*(spiro_t2)

d/dt(epl_depot) = -Ka_epl*epl_depot;

#Extracellular Potassium Amount

d/dt(K)= Kin + Kinfusion - CD_K_out - interstitial_potassium_flux; #(mEq/min)

d/dt(interstitial_K) = interstitial_potassium_flux - intracellular_potassium_flux

#Intracellular Potassium amount

d/dt(intracellular_K) = intracellular_potassium_flux;

#Tubule lumen Na+ amounts

d/dt(DCT1_luminal_Na_avg_conc_delayed)= DCT1_luminal_Na_avg_conc - DCT1_luminal_Na_avg_conc_delayed

d/dt(DCT2_luminal_Na_avg_conc_delayed)= DCT2_luminal_Na_avg_conc - DCT2_luminal_Na_avg_conc_delayed

d/dt(CNT_luminal_Na_avg_conc_delayed)= CNT_luminal_Na_avg_conc - CNT_luminal_Na_avg_conc_delayed

d/dt(CCD_luminal_Na_avg_conc_delayed)= CCD_luminal_Na_avg_conc - CCD_luminal_Na_avg_conc_delayed

d/dt(MCD_luminal_Na_avg_conc_delayed)= MCD_luminal_Na_avg_conc - MCD_luminal_Na_avg_conc_delayed

d/dt(AscLoH_luminal_K_avg_conc_delayed)= AscLoH_luminal_K_avg_conc - AscLoH_luminal_K_avg_conc_delayed

d/dt(DCT1_luminal_K_avg_conc_delayed)= DCT1_luminal_K_avg_conc - DCT1_luminal_K_avg_conc_delayed

d/dt(DCT2_luminal_K_avg_conc_delayed)= DCT2_luminal_K_avg_conc - DCT2_luminal_K_avg_conc_delayed

d/dt(CNT_luminal_K_avg_conc_delayed)= CNT_luminal_K_avg_conc - CNT_luminal_K_avg_conc_delayed

d/dt(CCD_luminal_K_avg_conc_delayed)= CCD_luminal_K_avg_conc - CCD_luminal_K_avg_conc_delayed

d/dt(MCD_luminal_K_avg_conc_delayed)= MCD_luminal_K_avg_conc - MCD_luminal_K_avg_conc_delayed

#Tubule Cell concentrations

d/dt(DCT1_cell_K_conc) = (-DCT1_K_passive_flux_lumenal + DCT1_K_passive_flux_basolateral + DCT1_K_active_flux_basolateral)/SV_DCT/2;

d/dt(DCT2_cell_K_conc) = (-DCT2_K_passive_flux_lumenal + DCT2_K_passive_flux_basolateral + DCT2_K_active_flux_basolateral)/SV_DCT/2;

d/dt(CNT_cell_K_conc) = (-CNT_K_passive_flux_lumenal + CNT_K_passive_flux_basolateral + CNT_K_active_flux_basolateral)/SV_CNT;

d/dt(CCD_cell_K_conc) = (-CCD_K_passive_flux_lumenal + CCD_K_passive_flux_basolateral + CCD_K_active_flux_basolateral)/SV_CCD;

d/dt(MCD_cell_K_conc) = (-MCD_K_passive_flux_lumenal + MCD_K_passive_flux_basolateral + MCD_K_active_flux_basolateral)/SV_MCD;

d/dt(DCT1_cell_Na_conc)= -(1/SV_DCT/2)*DCT1_Na_active_flux_basolateral+ (1/SV_DCT/2)*(J_NCC_dct1_effective + DCT1_Na_passive_flux_basolateral);

d/dt(DCT2_cell_Na_conc)= -(1/SV_DCT/2)*DCT2_Na_active_flux_basolateral+ (1/SV_DCT/2)*(J_NCC_ENAC_dct2_effective + DCT2_Na_passive_flux_basolateral);

d/dt(CNT_cell_Na_conc)= -(1/SV_CNT)*CNT_Na_active_flux_basolateral+ (1/SV_CNT)*(J_Enac_cnt_effective + CNT_Na_passive_flux_basolateral);

d/dt(CCD_cell_Na_conc)= -(1/SV_CCD)*CCD_Na_active_flux_basolateral+ (1/SV_CCD)*(J_Enac_CCD_effective + CCD_Na_passive_flux_basolateral);

d/dt(MCD_cell_Na_conc)= -(1/SV_MCD)*MCD_Na_active_flux_basolateral+ (1/SV_MCD)*(J_Enac_MCD_effective + MCD_Na_passive_flux_basolateral);

d/dt(plasma_K_delayed) = C_MCD_K*(plasma_K - plasma_K_delayed)

d/dt(aldo_effect_on_ENAC_delayed)=C_aldo_on_ENAC*(aldo_effect_on_ENAC-aldo_effect_on_ENAC_delayed)

#Cumulative urinary potassium excretion

d/dt(potassium_excretion_rate)=CD_K_out; #mEq/min

#MRA Indirect response

d/dt(MRA_effect) = Kon_MRA*(1-E_MRA_spiro - E_MRA_epl-E_MRA_esax) - Koff_MRA*MRA_effect;

MRA Pharmacokinetics

#Spironolactone Plasma Concentration

```
  d/dt(spiro_C1) = (Ka_spiro*(spiro_t2) - CL_spiro*(1-Spiro_Fmetabolized)*spiro_C1 - CL_spiro*Spiro_Fmetabolized*spiro_C1 ) / V1_spiro #ug/L/min

#Spironolactone Metabolite Canrenone

  #Plasma concentration

  d/dt(canrenone) = (CL_spiro*Spiro_Fmetabolized*spiro_C1 - CL_canrenone*canrenone - (Q_canrenone*canrenone - Q_canrenone*canrenone_C2))/V_canrenone

  #Peripheral concentration

  d/dt(canrenone_C2) = (Q_canrenone*canrenone - Q_canrenone*canrenone_C2)/V2_canrenone;

#Eplerenone central concentration

d/dt(epl_C1) = Ka_epl*epl_depot/V1_epl - (CL_epl/V1_epl)*epl_C1 -Q_epl*(epl_C1/V1_epl) + Q_epl*(epl_C2)/V2_epl

#Eplerenone peripheral concentration

d/dt(epl_C2) = Q_epl*(epl_C1/V1_epl) - Q_epl*(epl_C2)/V2_epl;

"

aaad
```

C-2. Initial condition code

```
###Initial conditions - do NOT change order!!!

#Order must match order in model file

#labels are not used by RxODE to match init to compartment

inits <- c(DRI_depot = 0,

        DRI_central = 0,

        DRI_periph = 0,

        DRI_eff = 0,

        AngI=theta$nominal_equilibrium_AngI,

        AngII=theta$nominal_equilibrium_AngII,

        AT1_bound_AngII=theta$nominal_equilibrium_AT1_bound_AngII,

        AT2_bound_AngII = theta$nominal_equilibrium_AT2_bound_AngII,

        plasma_renin_concentration= as.numeric(theta$nominal_equilibrium_PRC),

        blood_volume_L = theta$blood_volume_nom,

        interstitial_fluid_volume=theta$IF_nom,
```

```
        intracellular_fluid_volume = theta$IC_nom,
        sodium_amount=
as.numeric(theta$blood_volume_nom)*as.numeric(theta$ref_Na_concentration),
        IF_sodium_amount= as.numeric(theta$IF_nom)*as.numeric(theta$ref_Na_concentration),
        stored_sodium = 0,  #relative number - actual value not known
        tubulo_glomerular_feedback_effect=1,
        preafferent_pressure_autoreg_signal=1,
        glomerular_pressure_autoreg_signal=1,
        F_out_dt_delay=5e-12,
        cardiac_output_delayed=theta$CO_nom,
        MAP_delayed = theta$nominal_map_setpoint,
        MAP_setpoint = theta$nominal_map_setpoint,
        RAP_setpoint = theta$nom_right_atrial_pressure,
        CO_error=0,
        Na_concentration_error = 0,
        K_concentration_error = 0,
        normalized_vasopressin_concentration_delayed = 1,
        F0_TGF=theta$nom_LoH_Na_outflow,
        P_bowmans=theta$Pc_pt_s1_mmHg,
        oncotic_pressure_difference=theta$nom_oncotic_pressure_difference,
        renal_blood_flow_L_min_delayed=theta$nom_renal_blood_flow_L_min,
        renal_interstitial_hydrostatic_pressure =
theta$RIHP0,#theta$nom_postglomerular_pressure,
        SN_macula_densa_Na_flow_delayed =
as.numeric(theta$nom_LoH_Na_outflow)/as.numeric(theta$baseline_nephrons),
        LoH_K_out_delayed =
as.numeric(theta$nom_LoH_K_outflow)/as.numeric(theta$baseline_nephrons),
        rsna_delayed = 1,
        rsna_delayed2 = 1,
        disease_effects_increasing_Kf= 0,
        disease_effects_decreasing_CD_PN = 0,
```

```
        tubular_length_increase=0,
        tubular_diameter_increase=0,
        water_out_s1_delayed=3e-8,
        water_out_s2_delayed=1.9e-8,
        water_out_s3_delayed=1.2e-8,
        reabsorbed_urea_cd_delayed =0,#10e-8,
        UGE = 0,
        serum_creatinine =
as.numeric(theta$equilibrium_serum_creatinine)*as.numeric(theta$blood_volume_nom),
        cumNaExcretion = 0,
        cumWaterExcretion = 0,
        cumCreatinineExcretion = 0,
        RTg_compensation = 0,
        SGLT2_inhibition_delayed = 1,
        SGLT2_glucose_reabsorption_delayed =
as.numeric(theta$nom_SGLT2_glucose_reabsorption),
        SGLT2_inhibition_glucose_effect_delayed = 1,
        disease_effect_on_nephrons = 0,
        disease_effects_decreasing_Kf =0,
        disease_effect_on_seiving = 0,
        peritubular_oxygen = as.numeric(theta$peritubular_oxygen0),
        disease_effect_losing_glomeruli = 0,
        RISF = as.numeric(theta$RISF_nom),
        right_atrial_pressure_delayed = theta$nom_right_atrial_pressure,
        ANP_delayed = theta$nom_ANP,
        BigET_amt = theta$BigET0*theta$V_bigET,
        ET1_total_peri_amt = theta$ET1_total_peri0*theta$V_peri,
        ET1_total_cent_amt = theta$ET1_total_cent0*theta$V_cent,
        ET1_total_cent_labeled_amt = 0,
        ET1_total_peri_labeled_amt = 0,
```

```
Na_oral_depot = 0,
water_oral_depot = 0,
R_ET1_ETB_effect_on_aff = 1,
R_ET1_ETB_effect_on_eff = 1,
R_ET1_ETA_effect_on_aff = 1,
R_ET1_ETA_effect_on_preaff = 1,
R_ET1_ETA_effect_on_eff = 1,
R_ET1_ETA_cent_on_SVR = 1,
R_ET1_ETA_cent_on_venous_capacity = 1,
R_ET1_ETA_cent_on_venous_resistance = 1,
R_ET1_ETA_cent_on_venous_compliance = 1,
R_ET1_ETB_effect_on_CD = 1,
R_ET1_ETB_effect_on_CD_water = 1,
R_ET1_ETA_effect_on_PT = 1,
spiro_depot = 0,
spiro_t1 = 0,
spiro_t2 = 0,
epl_depot = 0,
K= theta$norm_plasma_K*(theta$blood_volume_nom)*1000,
interstitial_K= theta$norm_plasma_K*(theta$IF_nom)*1000,
intracellular_K = theta$nom_intracellular_K_conc*theta$IC_nom*1000
DCT1_luminal_Na_avg_conc_delayed = theta$DCT1_luminal_Na_conc0,
DCT2_luminal_Na_avg_conc_delayed = theta$DCT2_luminal_Na_conc0,
CNT_luminal_Na_avg_conc_delayed= theta$CNT_luminal_Na_conc0,
CCD_luminal_Na_avg_conc_delayed =theta$CCD_luminal_Na_conc0,
MCD_luminal_Na_avg_conc_delayed = theta$MCD_luminal_Na_conc0,
AscLoH_luminal_K_avg_conc_delayed = theta$AscLoH_luminal_K_conc0,
DCT1_luminal_K_avg_conc_delayed = theta$DCT1_luminal_K_conc0,
DCT2_luminal_K_avg_conc_delayed = theta$DCT2_luminal_K_conc0,
CNT_luminal_K_avg_conc_delayed = theta$CNT_luminal_K_conc0,
```

```
        CCD_luminal_K_avg_conc_delayed = theta$CCD_luminal_K_conc0,
        MCD_luminal_K_avg_conc_delayed = theta$MCD_luminal_K_conc0,
        DCT1_cell_K_conc = theta$DCT_cell_K_conc0, #mEq/ml
        DCT2_cell_K_conc = theta$DCT_cell_K_conc0, #mEq/ml
        CNT_cell_K_conc = theta$CNT_cell_K_conc0, #mEq/ml
        CCD_cell_K_conc = theta$CCD_cell_K_conc0, #mEq/ml
        MCD_cell_K_conc = theta$MCD_cell_K_conc0, #mEq/ml
        DCT1_cell_Na_conc= theta$DCT_cell_Na_conc0, #mEq/ml
        DCT2_cell_Na_conc= theta$DCT_cell_Na_conc0, #mEq/ml
        CNT_cell_Na_conc= theta$CNT_cell_Na_conc0, #mEq/ml
        CCD_cell_Na_conc= theta$CCD_cell_Na_conc0, #mEq/ml
        MCD_cell_Na_conc = theta$MCD_cell_Na_conc0, #mEq/ml
        plasma_K_delayed = theta$norm_plasma_K, #mEq/ml
        aldo_effect_on_ENAC_delayed=1,
        potassium_excretion_rate=0,
        MRA_effect=1, #theta$norm_DCT_cell_Na_concAldo,
        spiro_C1 = 0,
        canrenone = 0,
        canrenone_C2 = 0,
        epl_C1 = 0,
        epl_C2 = 0
)
```

C-3. Parameters code

```
calcNomParams_human <- function(){
```

```
#Constants and Unit conversions
nL_mL=1e+06
dl_ml=0.01
L_dL=10
L_mL=1000
L_m3=0.001
g_mg=0.001
ng_mg=1e-06
secs_mins=60
min_hr=60
hr_day=24
min_day=1440
MW_creatinine=113.12
Pi=3.1416
pi=3.14
viscosity_length_constant=1.5e-09
gamma =  1.16667e-5;  #  viscosity of tubular fluid
mmHg_Nperm2_conv = 133.32
glucose_mg_mmol = 0.0056
uric_acid_mg_mmol = 0.006
mmHg_atm = 0.00132
#Scaling parameters - can be used to parameterize model for other species
ECF_scale_species = 1
BV_scale_species=1
water_intake_species_scale = 1
CO_scale_species = 1

##############################################################################
##########
```

```
 #Parameters of normal human physiology based on literature and commmon medical knowledge

##########################################################################################

 ####Systemic parameters
 nominal_map_setpoint=85   #mmHg
 CO_nom= 5                          #L/min
 IF_nom = 12 #L
 IC_nom = 25 #L
 blood_volume_nom = 5               #L
 Na_intake_rate=100/24/60 #.07              #mEq/min  - 100mmol/day or 2300 mg/day
 nom_water_intake = 2.1             #L/day
 ref_Na_concentration=139           #mEq/L
 norm_plasma_K=0.0042 #meq/ml 0.0035-0.0055 ,0.004-0.006 from paper
 plasma_protein_concentration = 7   #g/dl
 plasma_albumin_concentration= 35 #mg/ml
 glucose_concentration = 5.5        #mmol/L
 plasma_urea = 0 #mmol/L
 nom_serum_uric_acid_concentration = 5.5 #mg/dl
 equilibrium_serum_creatinine=0.92 #mg/dl
 creatinine_secretion_scale = 0.75
 potassium_concentration=5  #mEq/L
 R_venous=3.4         #mmHg
 nom_right_atrial_pressure= 3 #mmHg
 P_venous=nom_right_atrial_pressure                 #mmHg
 nom_mean_filling_pressure=7 #mmHg
 nom_venous_capacity = blood_volume_nom
 venous_compliance = 1.534  #Fit to Schmidlin and Molstrom RSE 25.1%
```

VR_CO_scale = 1 #scale between venous return and cardiac output

venous_pressure_stiffness_scale = 0 #Fit to Schmidlin, unidentifiable so set to zero

#Systemic Starling forces

plasma_protein_concentration_nom = 7   #g/dl

plasma_protein_amount = plasma_protein_concentration_nom * blood_volume_nom*L_dL #g

ISF_protein_concentration_nom = 3.44   #g/dl - check interstitial fluid volume if this is changed

ISF_protein_amount = ISF_protein_concentration_nom *IF_nom*L_dL

#ISF_protein_osmotic_pressure = 1.629*ISF_protein_concentration + 0.2935*ISF_protein_concentration^2

Sodium_protein_filtration_rate_Kf = 1000 #6.67

Kf_IC = -1

#Note: if these parameters are changed, check to make sure BL interstitial fluid volume is normal

#Fit to Guyton 1965

IFV_piecewise_pressure = 15 #12#17 #L ISF volume at which ISF pressure starts to rise exponentially

ISF_pressure0 = -8.35 #mmHg   average pressure in ISF stable range

A_interstitium_low = 0.0654 #1.313594 #0.0654,

k_interstitium_low = 1

A_interstitium_high = 1

k_interstitium_high = 0.19

####Renal parameters

nom_renal_blood_flow_L_min = 1 #L/min

baseline_nephrons=2e6

nom_Kf=3.9   #nl/min*mmHg

nom_oncotic_pressure_difference = 27.6   #mmHg

nom_oncotic_pressure_peritubular= 27.6   #mmHg

interstitial_oncotic_pressure = 5   #mmHg

RISF_nom = 0.034 #L #34 # ml #normal renal interstitial fluid volume

RIHP0 = 5

C_RISF = RISF_nom/RIHP0# L/mmHg #units = m3/pa assme 10% of the mass of the kidneys(340 g), RISF = 34 ml = 0.034L = 0.000034 m3, RIHP0 = 9.655273* 133.322

#Renal Vasculature

nom_preafferent_arteriole_resistance= 14 ##15 #mmHg

nom_afferent_diameter=1.65e-5 ###1.5e-05 #mmHg

nom_efferent_diameter=1.1e-05 #mmHg

#Renal Tubules

Dc_pt_nom = 27e-6 #m

Dc_lh = 17e-6 #m

Dc_dt = 17e-6 #m

Dc_cd = 17e-6

L_pt_s1_nom = 0.005

L_pt_s2_nom = 0.005 #m

L_pt_s3_nom =0.004 #m

L_lh_des = 0.01 ###0.003 #m

L_lh_asc = 0.01 ###0.003 #m

L_dct = 0.005 #m

L_cnt=0.001 #m

L_ccd = 0.002

L_mcd = 0.008

tubular_compliance = 0.2

Pc_pt_s1_mmHg = 20.2#20.1#19.4#13.2 #15 #mmHg

Pc_pt_s2_mmHg = 15

Pc_pt_s3_mmHg = 11 #mmHg

Pc_lh_des_mmHg = 8 #mmHg

Pc_lh_asc_mmHg = 7 #mmHg

Pc_dt_mmHg = 6 #mmHg

Pc_cd_mmHg = 5 #mmHg

nominal_pt_na_reabsorption_total=0.7# 0.75 #fraction

nominal_loh_na_reabsorption = 0.8 #0.65 #fraction

nominal_dt_na_reabsorption=0.3 #fraction - assume dt and CNT together reabsorb ~0.5 1*(1-0.3)*(1-0.3)

nominal_cnt_na_reabsorption = 0.3

nominal_ccd_na_reabsorption = 0.65

LoH_flow_dependence = 0.75

max_s2_Na_reabs = 1#3e-6

max_s3_Na_reabs = 1#2e-6

max_deltaLoH_reabs=0.75e-6#2e-6

nom_water_permeability = 0.951

###Renal Glucose reabsorption

nom_glucose_reabs_per_unit_length_s1 =(5.2e-5)/2 #95% of filtered glucose 5.4e-5#2e-4

nom_glucose_reabs_per_unit_length_s2 = (5.2e-5)/2

nom_glucose_reabs_per_unit_length_s3 = 2.8e-5

diabetic_adaptation = 1

maximal_RTg_increase = 0.5

T_glucose_RTg = 10^6.75 #Fit to Dapa, exponent RSE = 1.86%

glucose_diuresis_effect_cd = 0.00706 #Fit to Dapa, RSE = 1.53%

###Renal urea reabsorption

urea_permeability_PT = 0.5

Renal and systemic oxygen

nom_hemoglobin_concentration = 15 #g/dl

hemoglobin_amount= nom_hemoglobin_concentration * blood_volume_nom *L_dL

nom_hematocrit = 0.4; #fraction

SaO2= 0.95 #% of hemoglobin saturated with oxygen

PaO2 = 90 # Units: mmHg Arterial oxygen partial pressure

R = 8.205e-5 #atm-m^3/mol-k

Temperature = 310.15 #K

PT_Na_oxygen_ratio = 1 #ml/mol

LoH_Na_oxygen_ratio = 1/2

DCT_CD_Na_oxygen_ratio = 1/10

hemoglobin_amount= nom_hemoglobin_concentration * blood_volume_nom *L_dL

arterial_oxygen_concentration_ml0 = nom_hemoglobin_concentration*1.36 * SaO2 + 0.0031*PaO2; #ml O2/ 100 ml

PT_capillary_volume = 0.002 #scaled by 40 from rat value in Lee 2017

peritubular_oxygen0 = PT_capillary_volume*arterial_oxygen_concentration_ml0

####Renal albumin seiving

nom_glomerular_albumin_sieving_coefficient = 0.00062 #%

max_albumin_reabsorption_fraction=0.98448 #%

maximum_reabsorption_capacity = 5.5e-7 #mg/min

permanent_seiving_damage = 0

####RAAS Pathway parameters

concentration_to_renin_activity_conversion_plasma = 61

nominal_equilibrium_PRA = 1000 #937   #fmol/ml/hr

nominal_equilibrium_AngI = 7.5 #fmol/ml

nominal_equilibrium_AngII =4.75 #fmol/ml

nominal_renin_half_life = 0.1733 # (hr)

nominal_AngI_half_life = 0.5/60 #(hr)

nominal_AngII_half_life = 0.66/60 #(hr)

nominal_AT1_bound_AngII_half_life = 12/60 #hr

nominal_AT2_bound_AngII_half_life = 12/60 #hr

ACE_chymase_fraction = 0.95   #% of AngI converted by ACE. The rest is converted by chymase

fraction_AT1_bound_AngII = 0.75   #assume AngII preferentially binds to AT1 vs AT2

##

#RAAS inhibition parameters

ACEI_effect_on_ACE_activity = 1.0

ARB_effect_on_AT1_binding = 1.0

Emax_Eplerenone = 0.99

ED50_Eplerenone = 45 # in mg

Emax_Aliskiren = 0.99

ED50_Aliskiren = 20 # in mg

#Aliskiren using pct_inhibition - will be used for testing and/or lack of PK data

pct_target_inhibition_Aliskiren_150mg = 0

pct_target_inhibition_Aliskiren_300mg = 0

pct_target_inhibition_Aliskiren_600mg = 0

#ARBs

pct_target_inhibition_Valsatran_ARB_320mg = 0 #92% for 160 mg dose; 94.7% for 320 mg Dose

pct_target_inhibition_Valsatran_ARB_160mg = 0 #

pct_target_inhibition_Losartan_ARB_100mg = 0 #93.7% for 100 mg dose

pct_target_inhibition_Irbesartan_ARB_150mg = 0 #89% for 150 mg dose

pct_target_inhibition_Irbesartan_ARB_300mg = 0 #

pct_target_inhibition_Candesartan_ARB = 0 #88% for 2 mg dose

pct_target_inhibition_Candesartan_ARB_4mg = 0

pct_target_inhibition_Candesartan_ARB_8mg = 0

#ACE inhibitors

pct_target_inhibition_Enalapril_ACEi = 0 #97.2% for 20 mg dose

pct_target_inhibition_Ramipril_ACEi = 0 #94% for 10 mg dose

hill_DRI = 1

DRI_KDEL=0.1878676/60

Km_DRI=0.0002855224

Vmax_DRI=1

DRI_KA = 0.396652/60

DRI_Vc = 9136.96

DRI_K21 = 0.12057/60

Vm1 = 28162

Km1 = 1.01686

Vm2 = 466200

Km2 = 30

##

#The following parameters are calculated at equilibrium using the parameters above

##

#This pressure is the setpoint that determines the myogenic response of the preafferent vasculature

nom_preafferent_pressure = nominal_map_setpoint - nom_renal_blood_flow_L_min*nom_preafferent_arteriole_resistance;

#This pressure is the setpoint that determines the myogenic response of the afferent vasculature

nom_glomerular_pressure = nom_preafferent_pressure - nom_renal_blood_flow_L_min*(L_m3*viscosity_length_constant/(nom_afferent_diameter^4)/baseline_nephrons);

#This pressure is the setpoint that determines the tubular pressure-natriuresis response

nom_postglomerular_pressure = nom_preafferent_pressure - nom_renal_blood_flow_L_min*(L_m3*viscosity_length_constant*(1/(nom_afferent_diameter^4)+1/(nom_efferent_diameter^4))/baseline_nephrons);

The rate of sodium excretion must equal the rate of sodium intake. Sodium reabsorption rates vary along the tubule, but based on literature

measurements we have a good, and literature data provides estimates for these rates. However, there is a precise

rate of sodium reabsorption required to achieve the equilibrium defined by the parameters above.

Assuming that reabsorption rates are known in all but one segment of the tubule, the exact rate

```
  # of reabsorption of the remaining segment can be calculated. We chose to calculate the CD rate of reabsorpion based on estimates for

  # PT, LoH, and DT reabsorption.

  nom_GFR = nom_Kf*(nom_glomerular_pressure - nom_oncotic_pressure_difference - Pc_pt_s1_mmHg)/nL_mL*baseline_nephrons;

  nom_filtered_sodium_load = (nom_GFR/L_mL)*ref_Na_concentration;

  nom_filtered_glucose_load = (nom_GFR/L_mL)*glucose_concentration  #mmol/min

  #Glucose reabsorbed by SGLT2 in S1 and S2, Na reabsorbed 1:1

  nom_SGLT2_Na_reabsorption = (nom_glucose_reabs_per_unit_length_s1*L_pt_s1_nom + nom_glucose_reabs_per_unit_length_s2*L_pt_s2_nom)*baseline_nephrons

  #Remaining glucose reabsorbed by SGLT1 in S2, Na reabsorbed 2:1

  nom_SGLT1_Na_reabsorption = 2*(nom_filtered_glucose_load - nom_SGLT2_Na_reabsorption)

  nom_SGLT_Na_reabsorption_fraction = (nom_SGLT2_Na_reabsorption + nom_SGLT1_Na_reabsorption)/nom_filtered_sodium_load; #assume all glucose reabsorbed, and sodium reabsorbed 1:1

  adj = 0.0145

  nominal_pt_na_reabsorption_nonSGLT = nominal_pt_na_reabsorption_total - nom_SGLT_Na_reabsorption_fraction + adj  #0.02 is a correction factor, because when SGLT reabsorbtion subtracted from each section, resutling reabsorption fraction will be a little smaller

  nom_PT_Na_outflow = nom_filtered_sodium_load*(1-(nominal_pt_na_reabsorption_nonSGLT - adj) - nom_SGLT_Na_reabsorption_fraction);

  nom_SNGFR = nom_GFR*1e9/1000 / baseline_nephrons; #nL/min

  filtered_K_load = norm_plasma_K*nom_GFR

  nom_PT_K_outflow = filtered_K_load*(1-nominal_pt_na_reabsorption_nonSGLT)

  nom_Na_in_AscLoH = nom_PT_Na_outflow/baseline_nephrons;

  nom_K_in_AscLoH = nom_PT_K_outflow/baseline_nephrons

  nom_AscLoH_Reab_Rate =(2*nominal_loh_na_reabsorption*(nom_Na_in_AscLoH + nom_K_in_AscLoH))/L_lh_des; #osmoles reabsorbed per unit length per minute. factor of 2 because osmoles = 2

  nom_LoH_Na_outflow = nom_PT_Na_outflow*(1-nominal_loh_na_reabsorption);

  nom_DT_Na_outflow = nom_LoH_Na_outflow*(1-nominal_dt_na_reabsorption);
```

```
  nom_CNT_Na_outflow = nom_DT_Na_outflow*(1-nominal_cnt_na_reabsorption);

  nom_CCD_Na_outflow = nom_CNT_Na_outflow*(1-nominal_ccd_na_reabsorption);

  nominal_mcd_na_reabsorption = 1-Na_intake_rate/nom_CCD_Na_outflow;

  nom_RVR = (nominal_map_setpoint - P_venous)/nom_renal_blood_flow_L_min

  nom_peritubular_resistance = nom_RVR - (nom_preafferent_arteriole_resistance +
L_m3*viscosity_length_constant*(1/nom_afferent_diameter^4+1/nom_efferent_diameter^4)/bas
eline_nephrons);

  #Calculate the normal amount of sodium reabsorbed per unit surface area of the PT

  PT_Na_reab_perUnitSA_0 = (nom_filtered_sodium_load/baseline_nephrons)*
nominal_pt_na_reabsorption_nonSGLT/(3.14*Dc_pt_nom*(L_pt_s1_nom+L_pt_s2_nom+L_pt
_s3_nom))

  #Calculate the normal flow rate to surface area for the PT

  PT_SA_per_mm = pi*((Dc_pt_nom*1e6)^2)/4  #um^2 / unit length

  PT_SA_flow_ratio_nom = (nom_SNGFR)/PT_SA_per_mm   #nl/min/um^2

  #Given the values for baseline MAP and CO above, the baseline TPR required to maintain this
MAP and CO can be calculated. Since TPR includes renal vascular resistance, the baseline
systemic (non-renal) resistance

  #can be calculated from this TPR and the values for baseline renal resistances defined above.

  nom_TPR = (nominal_map_setpoint - nom_mean_filling_pressure)/CO_nom

  nom_systemic_arterial_resistance= nom_TPR-R_venous

  nom_resistance_to_venous_return = nom_mean_filling_pressure/CO_nom

  venous_return_scale = (8*R_venous +
nom_systemic_arterial_resistance)/nom_resistance_to_venous_return

  #Calculation of peritubular ultrafiltration coefficient

  tubular_reabsorption = nom_GFR/1000 - nom_water_intake*water_intake_species_scale/60/24
#at SS, water excretion equals water intake

  #Both RIHP and Kf are unknown, so we can either assume RIHP and calculate Kf, or vice
versa. Since RIHP has been measured experimentally,

  #it seems better to assume a normal value for RIHP and calculate Kf

  nom_peritubular_cap_Kf = - tubular_reabsorption/((nom_postglomerular_pressure +
nom_right_atrial_pressure)/2 - RIHP0 - (nom_oncotic_pressure_peritubular -
interstitial_oncotic_pressure))
```

```
  #Creatinine synthesisrate at equilibrium

  basal_creatinine_filtration_rate = equilibrium_serum_creatinine * dl_ml * nom_GFR; #units: mg/min

  basal_creatinine_secretion = 0.1*basal_creatinine_filtration_rate #assume 20% additional creatinine secreted normally

  creatinine_synthesis_rate  = basal_creatinine_filtration_rate + basal_creatinine_secretion

  #### Uric Acid reabsorption

  uric_acid_fractional_reabs_rate = 0.95  #%

  uric_acid_synthesis_rate  = nom_serum_uric_acid_concentration * dl_ml * nom_GFR*(1-uric_acid_fractional_reabs_rate) #Units: mg/min

  nom_SGLT2_glucose_reabsorption = glucose_concentration*nom_GFR/2e6/1000


################################################################################################################################

  #                                Endothelin                                #


################################################################################################################################

    ######################################### Known Parameters ##################################################

  # ------ bigET

  #https://doi.org/10.1016/j.lfs.2012.08.008

  #Big ET-1    4 pg/ml in healthy subjects, 17 pg/ml in hemodialysis patients

  mw_BigET = 4283.0 #g/mol

  BigET0 = 4*1000/mw_BigET #pg/ml converted to pmol/l https://doi.org/10.1016/j.lfs.2012.08.008  # value: 0.9339248

  #https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1218999/

  #ECE - Big ET1 Kcat/Km = 4.6 - 6 /s/uM

  Km = 0.75*1e6 #pM

  Kcat = 3.3*60 #(/min)

  E_ECE_BigET = Kcat/Km  #L/pmol/min
```

```
  # ------ ET-1
  #https://doi.org/10.1016/j.lfs.2012.08.008
  #Plasma ET-1  1.5 +- 0.1 fmol/mL or pmol/L
  mw_ET1 = 2491.9 #g/mol
  ET1_cent0 = 2.5 #1.2 #pmol/L
  #Bacon and Davenport 1996
  #ET-1 is equipotent for ETA and ETB and will therefore
  #activate both
  #Kd = 0.4 #nM
  Kd_ET1 = 400 #pmol/L
  BigET_infusion_rate =0 #pmol/min
  ######################################## Estimated ET-1 Parameters
##########################################
  ECE_conc = 1.54e5 #pmol/L
  ECE_activity = ECE_conc*(E_ECE_BigET*BigET0) #pmol/L value: 13086.94
(pmol/L/min)/(L/min/pmol * pmol/L)
  V_cent = 150 #194.8#196.9  #L
  V_peri = 0.2632 #0.0052045   #L
  V_bigET =1#0.2674# 0.44206  #L
  Q_ET1_pc = 4.98   #L/min
  Q_ET1_cp = 244.3  #to be estimated
  #Fraction of internalization that occurs through ETB vs ETA (1 - all ETB, 0 - all ETA)
  ETB_internalization_fraction = 0.980 #0.85212
  Qint_cent= 10.238*V_cent #461.67 #pmol/min --> Kint*V_cent*Rtot_cent
  KintB = 0.00784 #0.008564 #/min, internalization rate
  ######################################## Calculated Parameters
##################################################
  #Can solve for Qint_peri and ET1_peri0 from equilibrium constraints:
  b = Qint_cent*ET1_cent0/(Kd_ET1 + ET1_cent0)  #Used for substitution
```

```
  a = ECE_activity*V_peri - b                #Used for substitution

  Qint_peri = a*Kd_ET1*Q_ET1_pc/(b+Q_ET1_cp*ET1_cent0) + a   #pmol/min

  ET1_peri0 = a*Kd_ET1/(Qint_peri - a)  #pmol/L

  #ETB receptor concentrations, calculated from estimated internalization rates and volumes

  ETB_total_peri0 = Qint_peri*ETB_internalization_fraction/(V_peri*KintB)   #pmol/L total receptor concenntration

  ETB_total_cent0 = Qint_cent*ETB_internalization_fraction/(V_cent*KintB)

  ETA_total_peri0 = Qint_peri*(1-ETB_internalization_fraction)/(V_peri*KintB)   #pmol/L total receptor concenntration

  ETA_total_cent0 = Qint_cent*(1-ETB_internalization_fraction)/(V_cent*KintB)

  BigET_prod_rate = ECE_activity*V_peri  #pmol/L/min

  #Calculate Ligend-receptor complex

  ET1_ETA_cent0 = ETA_total_cent0*ET1_cent0/(Kd_ET1 + ET1_cent0) #pmol/L

  ET1_ETB_cent0 = ETB_total_cent0*ET1_cent0/(Kd_ET1 + ET1_cent0) #pmol/L

  ET1_total_cent0 = ET1_cent0 + ET1_ETA_cent0 +  ET1_ETB_cent0 #pmol/L(total = free + bound)

  #pmol/L

  ET1_ETA_peri0 = ETA_total_peri0*ET1_peri0/(Kd_ET1 + ET1_peri0) #

  ET1_ETB_peri0 = ETB_total_peri0*ET1_peri0/(Kd_ET1 + ET1_peri0) #pmol/L

  ET1_total_peri0 = ET1_peri0 + ET1_ETA_peri0 +  ET1_ETB_peri0 #(total = free + bound)

  #Drug effects - 0 = turned off

  ETA_inhibition = 0

  ETB_inhibition = 0

  ETA_inhibition_peri = 0

  ETB_inhibition_peri = 0

  ETA_inhib_slope = 30 #min

  ETB_inhib_slope = 30 #min

  ETA_ramp = 0

  ETB_ramp = 0
```

ETA_halflife = 12 #12 min

ETB_halflife = 60 #60 min

ET1_infusion_rate_cent = 0

#ETA inhibition for VML 588 0.05, 0.2, and 0.4 doses from Vuurmans 2004

#Study noted lack of dose-response

ETA_inhibition_pct_Vlow = 0.4215

ETA_inhibition_pct_Vmed = 0.2054

ETA_inhibition_pct_Vhigh = 0.5575

################## ET-1 effects ###################################

ET1_ETA_eff_slope = 1

ET1_ETB_eff_slope = 1

ET1_ETA_pt_slope = 1

ET1_ETA_venous_compliance_scale = 0

ET1_ETA_venous_resistance_scale = 0

ET1_ETA_venous_capacity_scale = -.07#.904

ET1_ETA_venous_slope = 0.128

ET1_ETA_svr_scale1   = 0.36053 #0.45148

ET1_ETA_preaff_scale1   = 2.8672 #0.242856

ET1_ETA_aff_scale1   = 5.0927 #3.76985

ET1_ETA_eff_scale1   = 1.0395#0.7687286

ET1_ETA_PT_scale1   = 0.2503 #0.11241

ET1_ETB_svr_scale1 = 0#-0.01904

ET1_ETB_aff_scale   = 0#0

ET1_ETB_eff_scale   = -0.02362 #-0.1218

ET1_ETB_CD_scale   = -0.15825*1e-3#-0.3167996e-6

ET1_ETB_CD_water_scale = -2.1864*1e-3 # -0.049444*1e-6

#Turned off:

ET1_ETA_peritubular_scale1 =0#8# 6# increasing scale can decrease both GFR and RBF

ET1_ETA_peritubular_scale2 =0#8 # 1.5 increasing scale can decrease both GFR and RBF

ET1_ETA_peritubular_slope = 2

ET1_effect_ECE_scale=0#0.0005 #0.0005

ET1_effect_ECE_slope=1

ET1_effect_cent_ETB_scale =0#1.5#1.5

ET1_effect_ETB_cent_slope = 1

ET1_effect_peri_ETB_scale =0

ET1_effect_ETB_peri_slope =1

##

####RAAS Pathway parameters

#Values for half lives and equilibrium concentrations of RAAS peptides available in the literature and

defined above to calculate nominal values for other RAAS parameters not available in the literature:

#ACE activity

#Chymase activity

#AT1 receptor binding rate

#AT2 receptor binding rate

#equilibrium AT1_bound_AngII

#These values are then assumed to be fixed unless specified otherwise.

#Calculating these nominal parameter values initially in a separate file is required so that these parameters can then be varied independently in the main model

nominal_equilibrium_PRC = nominal_equilibrium_PRA/concentration_to_renin_activity_conversion_plasma

nominal_AngI_degradation_rate = log(2)/nominal_AngI_half_life #/hr

nominal_AngII_degradation_rate = log(2)/nominal_AngII_half_life #/hr

nominal_AT1_bound_AngII_degradation_rate = log(2)/nominal_AT1_bound_AngII_half_life

nominal_AT2_bound_AngII_degradation_rate = log(2)/nominal_AT2_bound_AngII_half_life

#ACE converts 95% of AngI, chymase converts the rest

nominal_ACE_activity = (ACE_chymase_fraction*(nominal_equilibrium_PRA - nominal_AngI_degradation_rate*nominal_equilibrium_AngI)/nominal_equilibrium_AngI)#Therapy_effect_on_ACE

nominal_chymase_activity = (1-ACE_chymase_fraction)*(nominal_equilibrium_PRA - nominal_AngI_degradation_rate*nominal_equilibrium_AngI)/nominal_equilibrium_AngI

#75% of bound AngII is AT1, the rest is AT2

nominal_AT1_receptor_binding_rate = fraction_AT1_bound_AngII*(nominal_equilibrium_AngI*(nominal_ACE_activity+nominal_chymase_activity)-nominal_AngII_degradation_rate*nominal_equilibrium_AngII)/nominal_equilibrium_AngII

nominal_AT2_receptor_binding_rate = (1-fraction_AT1_bound_AngII)*(nominal_equilibrium_AngI*(nominal_ACE_activity+nominal_chymase_activity)-nominal_AngII_degradation_rate*nominal_equilibrium_AngII)/nominal_equilibrium_AngII

nominal_equilibrium_AT1_bound_AngII = nominal_equilibrium_AngII*nominal_AT1_receptor_binding_rate/nominal_AT1_bound_AngII_degradation_rate

nominal_equilibrium_AT2_bound_AngII = nominal_equilibrium_AngII*nominal_AT2_receptor_binding_rate/nominal_AT2_bound_AngII_degradation_rate

##########

#The following parameters were determined indirectly from many different literature studies on the response

#various changes in the system (e.g. drug treatments, infusions of peptide, fluid, sodium, etc.....)

##########

#Effects of AT1-bound AngII on preafferent, afferent, and efferent resistance, and aldosterone secretion

AT1_svr_slope = 0

AT1_effect_slope = 7

AT1_preaff_scale = 0.778 #Fit to Schmidlin and Molstrom RSE 39.9%

AT1_aff_scale= 0.481 #Fit to Schmidlin and Molstrom RSE 87.6%

AT1_eff_scale=0.771 #Fit to Schmidlin and Molstrom RSE 147%

AT1_PT_scale = 0.075

AT1_aldo_slope = 0.04340511#0.0505 #Fit to Dluhy and Williams 1972

AT1_NKCC_scale = 0.001#0

AT1_NCC_scale =0.33904493#0

AT1_ENAC_scale = 0

#Effects of Aldosterone on distal and collecting duct sodium reabsorption

nominal_aldosterone_concentration=85

K_Na_ratio_effect_on_aldo = 1

aldo_DCT_slope = 0.5

aldo_CD_slope = 0.5

aldo_ROMK_scale=0.84620333#0#0.9169 #Fit to Dluhy and Williams 1972

aldo_ENAC_scale = 0.15190523##Fit to Dluhy and Williams 1972

C_aldo_on_ENAC=0.34352298 #1 #Fit to Dluhy and Williams 1972

#Effects of Atrial Natriuretic Peptide (ANP)preafferent, afferent, and efferent resistance and collectin duct sodium reabsorption

#Raine et al NEJM 1986 315(9):533-7

#measured ANP and right atrial pressure in CHF patients with normal and elevated rigth atrial pressure ANP = 13.6 * RAP - 16.7

nom_ANP=24.1 #pmol/L Raine et al NEJM 1986 315(9):533-7

rap_anp_slope=13.6 #pmol/L/mmHg

rap_anp_intercept = 16.7

#Effects of Atrial Natriuretic Peptide (ANP)preafferent, afferent, and efferent resistance and collectin duct sodium reabsorption

ANP_effect_slope = 11.64 #Fit to Schmidlin and Molstrom RSE 14.3%

ANP_preaff_scale = 0.3974 #Fit to Schmidlin and Molstrom RSE 117%

ANP_aff_scale = 0.4217 #Fit to Schmidlin and Molstrom RSE 152%

ANP_eff_scale = 0 #Fit to Schmidlin and Molstrom RSE - estimated to be very close to zero with huge RSE

anp_CD_scale =0.0313 #Fit to Schmidlin and Molstrom RSE 119%

ANP_svr_scale = 0.9032 #Fit to Schmidlin and Molstrom RSE 41.3%

#Effects of Renal Sympathetic Nerve Activity on preafferent resistance, renin secretion, and PT sodium reabsorption

nom_rsna = 1

map_rsna_slope=5

RAP_rsna_slope=1

RAP_rsna_scale=4.238 #Fit to Schmidlin and Molstrom RSE 18.7%%

#Different values fit Schmidlin vs Molstrom better. Schmidlin: 4.238, Molstrom 2.416 (RSE = 40%)

rsna_preaff_scale = 0.619 ##Fit to Schmidlin and Molstrom RSE 192% poorly identified

rsna_preaff_slope = 0.25

rsna_PT_scale=0.2223 #Fit to Schmidlin and Molstrom RSE 117.5%

rsna_PT_slope=0.25

rsna_CD_scale = 0

rsna_CD_slope = 0.25

rsna_renin_slope=0.2   #Fit to Schmidlin and Molstrom, RSE = 162% (poorly idenfied)

rsna_svr_slope = 0.25

rsna_svr_scale = 1.112 #Fit to Schmidlin and Molstrom RSE 51.35%

rsna_HR_scale = 0.1725 #Fit to Schmidlin and Molstrom RSE 39.8%

rsna_HR_slope = 0.25

map_reset_rate = 1#10^(-4.9)   #Exponent fit to Schmidlin, RSE 28.4%)

filling_pressure_reset_rate = 0.0001

RAP_reset_rate = 1#10^(-4.14)   #Exponent fit to Schmidlin RSE 0.18%

Kp_baroreceptor = 0

sna_stiffness_slope = 0.1

sna_venous_stiffness_scale = -0.532   #Fit to Schmidlin RSE 538% (not identified)

MAP_rsna_scale = 1.635 #Fit to Schmidlin and Molstrom RSE 16.2%

#Na and water transfer between blood, ECF

Q_water = 1

Q_Na = 17.49701249#1

Q_Na_store = 0.001 #fit to Schmidlin, Molstrom, and Dapa

#old value: 0.0691 #Fit to Schmidlin and Molstrom RSE 28.35%

max_stored_sodium = 500 #mmol

#Osmolarity control of vasopressin secretion

Na_controller_gain=0.05

Kp_VP = 2.753 #Fit to Schmidlin and Molstrom RSE 11.9%

Ki_VP = 0.002 #Fit to Schmidlin and Molstrom RSE 85.1%

#Effects of Vasopressin on water intake and reabsorption

nominal_vasopressin_conc=4

water_intake_vasopressin_scale = 0.25#1.5

water_intake_vasopressin_slope = -0.5

vasopressin_perm_slope = 0.1066 #Fit to Schmidlin, Molstrom, and DAPA, RSE = 4.1%

vasopressin_perm_scale = 1.145 #0.936 #Fit to Schmidlin, Molstrom, and DAPA, RSE = 2.67% (if estimated greater than 1, need to check equation at extremes)

right_atrial_pressure_vasopressin_slope = 0.7803 #Fit to Schmidlin and Molstrom RSE 41.8%

#Magnitude and Steepness of tubuloglomerular feedback

S_tubulo_glomerular_feedback=0.7

F_md_scale_tubulo_glomerular_feedback=6

MD_Na_concentration_setpoint = 63.094 # should be set after running to equilibrium

#Effect of macula densa sodium flow on renin secretion

md_renin_A = 1

md_renin_tau = 6.48999762#2.79 #Fit to Dluhy and Williams 1972

renin_hyperactivity = 1

md_renin_tau_K = 0 #potential effect of potassium on renin secret - turned off if zero

nom_LoH_K_outflow = (1.284979e-08)*baseline_nephrons

#Responsiveness of renal vasculature to regulatory signals

preaff_diameter_range=0.25

afferent_diameter_range=1.2e-05

efferent_diameter_range=3e-06

preaff_signal_nonlin_slope = 1.0792#1/3

preaff_signal_nonlin_scale = 3.3588 #1

eff_signal_nonlin_slope = 1/3

eff_signal_nonlin_scale = 1.1147

aff_signal_nonlin_slope =1.5# 1/3 #1.5----------

aff_signal_nonlin_scale = 2.966 #4----------

#Limit on PT sodium reabsorption

renal_threshold_Na_reabs = 16e-6

#Empirical relationship between blood volume and cardiac filling pressure - from Guyton

BV_filling_pressure_slope=7.436

#RAAS pathway (these parameters can be set to different values than used to calculate the equilibrium state above)

AngI_half_life=0.008333

AngII_half_life=0.011

AT1_bound_AngII_half_life=0.2

AT1_PRC_slope=-0.9 #1.2

AT1_PRC_yint=0

AT2_bound_AngII_half_life=0.2

concentration_to_renin_activity_conversion_plasma = concentration_to_renin_activity_conversion_plasma

fraction_AT1_bound_AngII = fraction_AT1_bound_AngII

nominal_ACE_activity = nominal_ACE_activity #48.9

nominal_AT1_receptor_binding_rate= nominal_AT1_receptor_binding_rate #12.1

nominal_AT2_receptor_binding_rate= nominal_AT2_receptor_binding_rate#4.0

nominal_chymase_activity= nominal_chymase_activity #1.25

nominal_equilibrium_AT1_bound_AngII= nominal_equilibrium_AT1_bound_AngII #16.6315288606173

nominal_equilibrium_PRC= nominal_equilibrium_PRC #16.4

renin_half_life=0.1733

#Transfer constants for ODEs - determine speed of processes

C_aldo_secretion=1000

C_prerenal_blood_pressure=1000

C_P_bowmans = 1000

C_P_oncotic = 1000

C_rbf=1000

C_pt_water=1000

C_rsna = 10^(-3.02) # Exponent Fit to Schmidlin and Molstrom, RSE = 9.38%

C_rsna2 = 10^(-2.84) #Exponent #Fit to Schmidlin and Molstrom, RSE = 15.4%

C_tgf_reset=0

C_cardiac_output_delayed=.001

C_co_error=0.00001

C_vasopressin_delay = 0.01

C_md_flow = 1 #0.001#Time delay between MD sodium flow and renin secretion

C_rihp = 10^(-3.5) #Exponent fit to Schmidlin and Molstrom, RSE = 3.23% #time delay between peritubular pressure and RIHP

C_tgf=1#/30 #1000

C_Na_error=0.1#0

C_serum_creatinine = 1#/2#/60

C_hydrostatic = 0.05

C_map_delay = 100

C_rap = 0.1 #right atrial pressure delay

C_anp = 100

#Therapy effects

HCTZ_effect_on_DT_Na_reabs = 1

HCTZ_effect_on_renin_secretion = 1

DRI_effect_on_PRA = 1

CCB_effect_on_preafferent_resistance = 1

CCB_effect_on_afferent_resistance = 1

CCB_effect_on_efferent_resistance = 1

MR_antagonist_effect_on_aldo_MR = 1

##

#These parameters are by default set to ensure strong autoregulation of cardiac output, RBF, glomerular pressure, and MAP

#However, reducing these parameters reduces the ability of the system to autoregulate, and is necessary for modeling the development of hypertension, etc.

##

#Metabololic tissue autoregulation of cardiac output

tissue_autoreg_scale=0.2917 #Fit to Schmidlin RSE 4.9%

Kp_CO=0.1 #1.5

Ki_CO= 27.2 #Fit to Schmidlin RSE 24.6%

#Renal autoregulation of glomerular pressure

gp_autoreg_scale=0

preaff_autoreg_scale = 0

myogenic_steepness=2

#Renal autoregulation of renal blood flow

RBF_autoreg_scale = 0#3

RBF_autoreg_steepness=0.001

RBF_efferent_autoreg_start = 0.5 #L/min

RBF_autoreg_scale_eff = 0

RBF_efferent_autoreg_steepness = 0.001

#Pressure natiuresis effect through collecting duct sodium reabsorption

#Parameters selected based on Isaksson 2014:

#For a 10X increase in salt intake:MAP increases by 5mmHg, Renin decreases by 45%

#GFR increases by 1.4ml/min

#Strong CD effect required to minimize BP rise

#PT effect + LoH effect required to produce renin response

#If PT effect is too big, GFR will decrease instead of increase.

#So LoH must make up for the rest

max_pt_reabs_rate = 0.995

pressure_natriuresis_PT_scale = 0.115 #Fit to Schmidlin, Molstrom, and DAPA RSE 27.2%

pressure_natriuresis_PT_slope = 1

pressure_natriuresis_LoH_scale = 0

pressure_natriuresis_LoH_slope = 1

pressure_natriuresis_DCT_scale = 0

pressure_natriuresis_DCT_slope = 1

max_cd_reabs_rate = 0.995

pressure_natriuresis_CD_scale = 0.69 #Fit to Schmidlin, Molstrom, and DAPA RSE 4.88%

pressure_natriuresis_CD_slope=1

rbf_natriuresis_setpoint = 0.5 #L/min

RBF_PT_scale = 0

RBF_PT_slope = 1

RBF_CD_scale = 0

RBF_CD_slope = 1

#Glomerular pressure effect on glomerular hypertrophy

maximal_glom_surface_area_increase = 0.25

T_glomerular_pressure_increases_Kf = 2000

glomerular_pressure_increment = 2

#PT sodium reabsorption effects on tubular hypertrophy

maximal_tubule_length_increase = 0#.5

maximal_tubule_diameter_increase = 0#.25

T_PT_Na_reabs_PT_length = 1e10

T_PT_Na_reabs_PT_diameter = 1e10

####Proteinuria

#reabsorptive capacity based on observation of no proteinuria after UNX

nom_glomerular_albumin_sieving_coefficient = 1e-4

Emax_seiving = 4

Gamma_seiving = 3

Km_seiving = 25

max_PT_albumin_reabsorption_rate = 0.1

nom_albumin_excretion_rate = 3.5e-9

SN_albumin_reabsorptive_capacity = 1.4e-6 #mg/min/tubule

c_albumin = 0.0231 #min/nl, from Dean and Lazzara

seiving_inf = 4.25e-4 #from Dean and Lazarra, calculated for seiving coeff =0.00062 when SNGFR = 50 nl/min

nom_GP_seiving_damage = 62.5

#Reduce Kf due to glomerulosclerosis

disease_effects_decreasing_Kf = 0

#Disease effects

disease_effect_on_nephrons = 0

IgA_on = 0

FSGS_on = 0

T_age_fibrosis = 1e16#7e7 #3.5e7

T_age_sclerosis = 1e16#7e7#3.5e7

T_albumin_fibrosis = 1e15

T_pressure_glomerulosclerosis = 1e15

T_IgA_sclerosis = 1e15

T_IgA_seiving = 1e20

T_FSGS_sclerosis = 1e15

T_FSGS_nephronloss = 1e15

T_FSGS_seiving = 1e20

T_PT_overload_fibrosis = 1e15

#PT overload effect

PT_overload_threshold = 1.2

excess_flow_pt_scale = 0 # no effect if set to zero

max_s1_Na_reabs = 7.5e-6

CD_Na_reabs_threshold = 2.5e-7

nom_water_in_CD = 6.38e-9

#Rate at which the tubular pressure natriuresis mechanism is lost in diabetes (should be zero or negative number)

CD_PN_loss_rate = 0

#parameter that can be used to fix renal venous pressure

fix_ren_venous_pressure = 0

beta_gs_nl = 0 #0.3 # 0.75

beta_tf_nl = 0

beta_gs_gl = 1

#Treatment Parameters

BB_effect_on_RSNA = 1

CA_inhibitor = 1

ACEi_effect_on_ACE = 1

loop_diuretic_effect = 1

SGLT2_inhibition = 1

SGLT1_inhibition = 1

C_sglt2_delay = 0.01 #0.0025

C_sglt2i_glucose_delay = 10^(-3.63)

C_ruge = 0.003#0.1#.5

Anhe3 = 0.12 #0.148 #Fit to Dapa RSE 5.8%

SGLT2i_pct = 0.21 #0.05 #0.146   #Fit to Dapa RSE 3.17%

Emax_SGLT2i_glucose = 0.15

#Oral Na nad water reabsorption rate - used to model an oral load of sodium and/or water

Ka_Na = 0

Ka_water = 0

#######Bohm study - ETA and ETB antagonists

ETA_inhibition_pct = 0.4744#Bohm

ETA_inhibition_pct_peri = 0.4744

ETB_inhibition_pct = 0.6643 #Bohm

ETB_inhibition_pct_peri = 0.6643 #Bohm

Kout_ET1_ETA = 1# 0.0393

Kin_ET1_ETA = Kout_ET1_ETA

Kout_ET1_ETB = 1#0.022

Kin_ET1_ETB = Kout_ET1_ETB

Kout_ET1_ETB_peri = 1#0.0238

Kin_ET1_ETB_peri = Kout_ET1_ETB_peri

##
##

#Constants

F= 97 #Faraday constant C/mmol

R= 8.3145 #universal constant for all gases #J/mol.K

T= 310.6 #normal body temperature #K

#Intake Rates

Kin= 0.08 #(mEq/min), normal rate of potassium intake for a healthy adult is between 0.073-0.084 mEq/min.#0.08

Nain=100/24/60 #mEq/min, sodium intake rate

Kinfusion = 0 #(mEq/min), potassium infusion rate into extracellular compartment

#Normal Concentrations

norm_Aldo= 0.49 #nmol/L ##0.13-0.83

nom_intracellular_K_conc = 0.150 #meq/mL

norm_plasma_Na = 140 #mEq/L

principal_cell_intracellular_Na = (12/1000)#mEq/mL

#Compartment volumes

V_ecf = 15000 #(ml)

V_ic = 25000 #mL

#Renal geometry and function

GFR=105 #(ml/min)

number_of_nephrons=2000000

CNT_diameter=2*12*10^-4 #cm

CNT_length= 0.4 #cm #Layton, Anita T., and Harold E. Layton. "A computational model of epithelial solute and water transport along a human nephron." PLoS computational biology 15.2 (2019): e1006108.

DCT_diameter=0.0015 #cm

DCT_length=0.5 #cm

CCD_diameter=0.0025 #cm

CCD_length=0.2 #cm

MCD_diameter = 0.0025

MCD_length = 0.8

SV_CNT=6*10e-4# CNT ratio per volume m^3/m^2 #https://journals.physiology.org/doi/pdf/10.1152/ajprenal.00044.2005

SV_DCT=0.75*10e-3# DCT ratio per volume m^3/m^2

SV_CCD=4*10e-4# CCD ratio per volume m^3/m^2

SV_MCD=4*10e-4# MCD ratio per volume m^3/m^2

principal_fraction_CNT=0.6 #fraction of principal cells in CNT

principal_fraction_CCD=0.75 #fraction of principal cells in CCD

principal_fraction_MCD=0.75 #fraction of principal cells in MCD

DCT1_volume = pi*((DCT_diameter/2)^2)*DCT_length/2 #ml = cm3

DCT2_volume = pi*((DCT_diameter/2)^2)*DCT_length/2 #ml = cm3

CNT_volume = pi*((CNT_diameter/2)^2)*CNT_length #ml

CCD_volume = pi*((CCD_diameter/2)^2)*CCD_length #ml

DCT1_luminal_Na_conc0=0.05608 #0.4082670 #mEq/ml

DCT2_luminal_Na_conc0=0.04626 #0.4082670 #mEq/ml

```
  CNT_luminal_Na_conc0=0.071 #0.297

  CCD_luminal_Na_conc0=0.076# 0.1837201 #mEq/ml

  MCD_luminal_Na_conc0 = 0.0512

  AscLoH_luminal_K_conc0 = 0.002

  DCT1_luminal_K_conc0 = 0.00227 #0.0055  #mEq/ml

  DCT2_luminal_K_conc0 = 0.00319 #0.0055  #mEq/ml

  CNT_luminal_K_conc0 = 0.00888 #0.0072 #mEq/ml

  CCD_luminal_K_conc0 = 0.0207 #0.032 #mEq/ml

  MCD_luminal_K_conc0 = 0.0423

  CNT_water_reabs_fraction = 0.7

  CCD_water_reabs_fraction = 0.337

  AscLoH_paracellular_K_SA =0# 5e-6

  water_in_DCT0 = 1000*6.98e-9

  water_in_DCT20 = 1000*6.98e-9

  water_in_CNT0 = water_in_DCT0

  water_in_CCD0 = water_in_CNT0*(1-CNT_water_reabs_fraction)

  water_out_CCD0 = water_in_CCD0*(1-CCD_water_reabs_fraction)

  norm_Na_reabsorption_MCD = nom_CCD_Na_outflow*nominal_mcd_na_reabsorption

  norm_K_secretion_MCD = norm_Na_reabsorption_MCD*2/3

  potassium_in_MCD0 = filtered_K_load*((1-nominal_pt_na_reabsorption_total)*(1-
nominal_loh_na_reabsorption) + (.12)) #net of reabsorbed and secreted (assumed 8%secretion)

  #Single-nephron MCD potassium reabsorption rate required for potassium balance (mEq/min)

  K_reabsorption_MCD_rate0 = (potassium_in_MCD0 - Kin)/number_of_nephrons #excretion
must equal intake for K+ balance

  #fractional MCD potassium reasorption

  eta_MCD0 = K_reabsorption_MCD_rate0 / (potassium_in_MCD0/number_of_nephrons)

  slope_plasmaK_MCD = K_reabsorption_MCD_rate0 / (CCD_luminal_K_conc0 -
norm_plasma_K) #reabsorption per unit potassium gradient

  #Tubular surface areas
```

DCT_SA = pi*DCT_diameter*DCT_length

CNT_SA = pi*CNT_diameter*CNT_length

CCD_SA = pi*CCD_diameter*CCD_length

MCD_SA = pi*MCD_diameter*MCD_length

DCT_Na_reabs_coefficient=1

CNT_Na_reabs_coefficient=1

CCD_Na_reabs_coefficient=1

DCT_cell_K_conc0 = 0.15

CNT_cell_K_conc0 = 0.15

CCD_cell_K_conc0 = 0.15

MCD_cell_K_conc0 = 0.15

DCT_cell_Na_conc0 = 0.012

CNT_cell_Na_conc0 = 0.012

CCD_cell_Na_conc0 = 0.012

MCD_cell_Na_conc0 = 0.012

########### DCT Na+ Reabsorption

K_Na_DCT0= (0.2*(1+DCT_cell_K_conc0*1000/8.33))/1000

K_K0 = (0.1*(1+ref_Na_concentration /18.5))/1000 #assume plasma Na+ of 140 mEq/L

#DCT net Na+ reabsorption

DCT_net_Na_reaborption0= nom_LoH_Na_outflow*nominal_dt_na_reabsorption/baseline_nephrons/DCT_SA

Na_luminal_fraction_DCT = 0.71 #fraction of sodium entering DCT from luminal side (the rest enters basolaterally)

J_Na_active_DCT10 = DCT_net_Na_reaborption0/Na_luminal_fraction_DCT #Active transport out equals the total passive transport in

J_Na_active_DCT20 = DCT_net_Na_reaborption0/Na_luminal_fraction_DCT

J_Na_active_max_DCT1 = J_Na_active_DCT10/(((DCT_cell_Na_conc0/(DCT_cell_Na_conc0 + K_Na_DCT0))^3)*((norm_plasma_K/(norm_plasma_K + K_K0))^2))

J_Na_active_max_DCT2 = J_Na_active_DCT20/(((DCT_cell_Na_conc0/(DCT_cell_Na_conc0 + K_Na_DCT0))^3)*((norm_plasma_K/(norm_plasma_K + K_K0))^2))

Na_luminal_permeability_dct1 = Na_luminal_fraction_DCT*J_Na_active_DCT10/ (DCT1_luminal_Na_conc0 - DCT_cell_Na_conc0)

Na_luminal_permeability_dct2 = Na_luminal_fraction_DCT*J_Na_active_DCT20/ (DCT2_luminal_Na_conc0 - DCT_cell_Na_conc0)

Na_basolateral_permeability_dct1 = (1-Na_luminal_fraction_DCT)*J_Na_active_DCT10/ (ref_Na_concentration/1000 - DCT_cell_Na_conc0)

Na_basolateral_permeability_dct2 = (1-Na_luminal_fraction_DCT)*J_Na_active_DCT20/ (ref_Na_concentration/1000 - DCT_cell_Na_conc0)

########## CNT Na+ Reabsorption

K_Na_CNT0 = (0.2*(1+CNT_cell_K_conc0*1000/8.33))/1000;

CNT_net_Na_reaborption0= nom_DT_Na_outflow*nominal_cnt_na_reabsorption/baseline_nephrons/CNT_SA

Na_luminal_fraction_CNT = 0.9 #fraction of sodium entering DCT from luminal side (the rest enters basolaterally)

J_Na_active_CNT0 = CNT_net_Na_reaborption0/Na_luminal_fraction_CNT #Active transport out equals the total passive transport in

J_Na_active_max_CNT = J_Na_active_CNT0/(((CNT_cell_Na_conc0/(CNT_cell_Na_conc0 + K_Na_CNT0))^3)*((norm_plasma_K/(norm_plasma_K + K_K0))^2))

Na_luminal_permeability_cnt = Na_luminal_fraction_CNT*J_Na_active_CNT0/ (CNT_luminal_Na_conc0 - CNT_cell_Na_conc0)

Na_basolateral_permeability_cnt = (1-Na_luminal_fraction_CNT)*J_Na_active_CNT0/ (ref_Na_concentration/1000 - CNT_cell_Na_conc0);

########## CCD Na+ Reabsorption

K_Na_CCD0 = (0.2*(1+CCD_cell_K_conc0*1000/8.33))/1000

CCD_net_Na_reaborption0= nom_CNT_Na_outflow*nominal_ccd_na_reabsorption/baseline_nephrons/CCD_SA

Na_luminal_fraction_CCD = 0.82 #fraction of sodium entering DCT from luminal side (the rest enters basolaterally)

J_Na_active_CCD0 = CCD_net_Na_reaborption0/Na_luminal_fraction_CCD #Active transport out equals the total passive transport in

J_Na_active_max_CCD = J_Na_active_CCD0/(((CCD_cell_Na_conc0/(CCD_cell_Na_conc0 + K_Na_CCD0))^3)*((norm_plasma_K/(norm_plasma_K + K_K0))^2))

Na_luminal_permeability_ccd = Na_luminal_fraction_CCD*J_Na_active_CCD0/ (CCD_luminal_Na_conc0 - CCD_cell_Na_conc0);

Na_basolateral_permeability_ccd = (1-Na_luminal_fraction_CCD)*J_Na_active_CCD0/ (ref_Na_concentration/1000 - CCD_cell_Na_conc0);

########### MCD Na+ Reabsorption

K_Na_MCD0 = (0.2*(1+MCD_cell_K_conc0*1000/8.33))/1000

MCD_net_Na_reaborption0= nom_CCD_Na_outflow*nominal_mcd_na_reabsorption/baseline_nephrons/MCD_SA

Na_luminal_fraction_MCD = 0.82 #fraction of sodium entering DCT from luminal side (the rest enters basolaterally)

J_Na_active_MCD0 = MCD_net_Na_reaborption0/Na_luminal_fraction_MCD #Active transport out equals the total passive transport in

J_Na_active_max_MCD = J_Na_active_MCD0/(((MCD_cell_Na_conc0/(MCD_cell_Na_conc0 + K_Na_MCD0))^3)*((norm_plasma_K/(norm_plasma_K + K_K0))^2))

Na_luminal_permeability_mcd = Na_luminal_fraction_MCD*J_Na_active_MCD0/ (MCD_luminal_Na_conc0 - MCD_cell_Na_conc0);

Na_basolateral_permeability_mcd = (1-Na_luminal_fraction_MCD)*J_Na_active_MCD0/ (ref_Na_concentration/1000 - MCD_cell_Na_conc0);

luminal_potential_difference= -18.4 #mV #Weinstien 2001 p.F1078

basolateral_potential_difference= -78.2 #mV #Weinstien 2001 p.F1078

#Potassium Secretion Parameters

#Adjust

baseline_K_luminal_permeability_DCT =1.575e-5 #2.6e-5 #cm/s

K_basolateral_permeability_DCT=10.43e-5#3.842e-5#5e-7##8.396e-4 #cm/s

baseline_K_luminal_permeability_CNT =0.90e-5 #2.6e-5 #cm/s

K_basolateral_permeability_CNT=4.46e-5#3.842e-5#5e-7##8.396e-4 #cm/s

baseline_K_luminal_permeability_CCD =6.4e-5 #2.6e-5 #cm/s

K_basolateral_permeability_CCD=12.65e-5#3.842e-5#5e-7##8.396e-4 #cm/s

#Na_permeability_dct=10e-5#0.34e-5

#Na_permeability_CCD=33.5e-5#0.43e-5

luminal_permeability = 2.55e-5

basolateral_permeability = 10e-5

baseline_K_luminal_permeability_DCT =luminal_permeability

K_basolateral_permeability_DCT=basolateral_permeability

baseline_K_luminal_permeability_CNT =luminal_permeability*J_Na_active_CNT0/J_Na_active_DCT10

K_basolateral_permeability_CNT=basolateral_permeability*J_Na_active_CNT0/J_Na_active_DCT10

baseline_K_luminal_permeability_CCD = luminal_permeability*J_Na_active_CCD0/J_Na_active_DCT10

K_basolateral_permeability_CCD=basolateral_permeability*J_Na_active_CCD0/J_Na_active_DCT10

baseline_K_luminal_permeability_MCD = luminal_permeability*J_Na_active_MCD0/J_Na_active_DCT10

K_basolateral_permeability_MCD=basolateral_permeability*J_Na_active_MCD0/J_Na_active_DCT10

#Fitting Constants

m_osm_ALDO = 0.30819925 #effect of plasma osmolaltiy on plasma aldosterone, mL/mEq#Fit to Dluhy 1972 & williams 1972

m_K_ALDO= 14.03830959*100#951.2 #Slope of plasma K+ effect on plasma aldosterone, mL/mEq

m_Na_ALDO=0.00010000*10#1.02 #15.569 #Slope of Na intake effect on plasma aldosterone, min/mEq

Aldo_KSec_scale=0.01042546*100#839.35 #103.5 #L/nmol

Aldo_Nareab_scale= 0

m_plasmaK_MCD = 3.33188213*1e-7#4.07e-7 #unitless

C_MCD_K = 1

m_flow_K = 0 #effect of flow rate on potassium secretion

m_Na_MCD = 3.438#0.69775 #min/mEq

K_NKCC_scale = 0

Ki_K_aldo = 0

Q_K_intracellular = 0.19164617 #1284 #L/min

#Disease effectsplasma_K

hyperaldo_effect = 0

#Drug effects

E_MAX_spiro= 1# #MRA Imax

EC50_spiro = 0.57593467#fit into McInnes 1982

E_MAX_epl= 0.9978#0.9988 #MRA Imax

EC50_epl = 2.38#ug/L or ng/ml

Koff_MRA = 3.4035 #0.0128

#Spironolactone pharmacokinetics

Ka_spiro = 0.01524458

V1_spiro = 7.15696

CL_spiro = 8.07626

CL_canrenone = 0.222487

V_canrenone = 70.47

V2_canrenone = 8.021

Q_canrenone = 0.110275

Spiro_Fmetabolized = 0.19311

Spiro_bioavailability = 0.91097

#Eplerenone Pharmacokinetics

Ka_epl = 0.00427#0.002673

V1_epl = 13.8#5.6

V2_epl = 0.59#1.6

```
  CL_epl = 0.1786# 0.1527
  Q_epl = 0.9769 #1.06
  t=sort(ls())
  param=sapply(t,names)
  for (i in 1:length(t)){
    param[i]=get(t[i])
  }
  param$param=NULL
  param = data.frame(param)
  return(param)
}
```

C-4. Run file code

```
# load packages
library(tidyverse)
library(gridExtra)
library(RxODE)
library(tidyr)
library(cowplot)
library(MASS)
library(lattice)
library(grid)
library(mvtnorm)
library(MESS)
library(plyr)
library(dplyr)
library(ggpubr)
source("modelfile-clean.R")
cvrsim <- RxODE(model = ode)
```

```
#load basecase
source("Parameters-clean.R")
theta=calcNomParams_human()
source("Inits-clean.R")

times = seq(0,100000,by=100)
ev=eventTable(time.units = 'minutes')
ev$add.sampling(times)
#turn off all feedbacks
theta$RAP_rsna_scale = 0
theta$MAP_rsna_scale = 0
theta$md_renin_tau = 0
theta$AT1_aldo_slope = 0
theta$pressure_natriuresis_CD_scale = 0
theta$pressure_natriuresis_DCT_scale = 0
theta$pressure_natriuresis_PT_scale = 0
theta$pressure_natriuresis_LoH_scale = 0
theta$tissue_autoreg_scale = 0
theta$ANP_preaff_scale = 0
theta$ANP_aff_scale = 0
theta$ANP_eff_scale = 0
theta$ANP_svr_scale = 0
theta$anp_CD_scale = 0
theta$S_tubulo_glomerular_feedback = 0
theta$ET1_ETA_svr_scale1 = 0
theta$ET1_ETA_svr_scale2 = 0
theta$ET1_ETA_preaff_scale1 = 0
```

```
theta$ET1_ETA_preaff_scale2 = 0
theta$ET1_ETA_aff_scale1 = 0
theta$ET1_ETA_aff_scale2 = 0
theta$ET1_ETA_eff_scale1 = 0
theta$ET1_ETA_eff_scale2 = 0
theta$ET1_ETB_aff_scale = 0
theta$ET1_ETB_eff_scale = 0
theta$ET1_ETA_peritubular_scale1 = 0
theta$ET1_ETA_peritubular_scale2 = 0
theta$ET1_ETA_PT_scale= 0
theta$ET1_ETB_CD_scale = 0
theta$ET1_ETB_CD_water_scale = 0
theta$ET1_effect_ECE_scale = 0
theta$Anhe3 = 0
theta$m_K_ALDO = 0
theta$m_Na_ALDO = 0
theta$m_Na_MCD = 0
theta$Aldo_KSec_scale = 0
theta$m_plasmaK_MCD = 0
theta$aldo_DCT_scale  = 0
theta$aldo_CD_scale = 0
theta$m_osm_ALDO=0
theta$AT1_aldo_slope = 0
theta$aldo_ROMK_scale=0
theta$aldo_ENAC_scale=0
theta$C_aldo_on_ENAC=0
theta$Q_K_intracellular=0
theta$Q_Na=0
```

```
theta$aldo_CD_slope=0

theta$aldo_DCT_slope=0

theta$AT1_NKCC_scale=0

theta$AT1_NCC_scale=0

theta$DCT_Na_reabs_coefficient=1

theta$CNT_Na_reabs_coefficient=1

theta$CCD_Na_reabs_coefficient=1

theta$E_esax=0

h <-data.frame(cvrsim$run(theta, ev, inits))

p1 = ggplot(h)+geom_path(mapping = aes(x=time,y=plasma_K))+xlab("Time(minutes)") +

  geom_hline(yintercept = 0.0042, color = "gray" , type = "dashed")+theme_bw()

p2 = ggplot(h)+geom_path(mapping = aes(x=time, y= Aldo))+xlab("Time(minutes)") +

  ylim(c(0.47, .52))+theme_bw()

p3 = ggplot(h)+geom_path(mapping = aes(x=time, y=
DCT1_cell_Na_conc))+xlab("Time(minutes)") +

  ylim(c(.01, .013))+theme_bw()

p4 = ggplot(h)+geom_path(mapping = aes(x=time, y=
CNT_cell_Na_conc))+xlab("Time(minutes)") +

  ylim(c(.01, .013))+theme_bw()

p5 = ggplot(h)+geom_path(mapping = aes(x=time, y=
CCD_cell_Na_conc))+xlab("Time(minutes)") +

  ylim(c(.01, .013))+theme_bw()

p6= ggplot(h)+geom_path(mapping = aes(x=time, y=
DCT1_cell_K_conc))+xlab("Time(minutes)") +

  ylim(c(.145, .155))+theme_bw()

p7= ggplot(h)+geom_path(mapping = aes(x=time, y=
CNT_cell_K_conc))+xlab("Time(minutes)") +

  ylim(c(.145, .155))+theme_bw()

p8= ggplot(h)+geom_path(mapping = aes(x=time, y=
CCD_cell_K_conc))+xlab("Time(minutes)") +
```

```
  ylim(c(.145, .155))+theme_bw()

p9 =  ggplot(h)+geom_path(mapping = aes(x=time, y=
Na_balance))+xlab("Time(minutes)")+theme_bw()

grid.arrange(p1,p2,p3,p4,p5,p6,p7,p8,p9,nrow=3)

inits = h[dim(h)[1], names(h) %in% names(inits)]

inits["CO_error"] = 0

theta  = calcNomParams_human()

########Run to equilibrium

times=seq(0,200000,200)

ev=eventTable(time.units = 'minutes')

ev$add.sampling(times)

x <- cvrsim$run(theta, ev, inits=inits)

x=data.frame(x)

p1 = ggplot(x)+

  geom_hline(yintercept = theta$norm_plasma_K, color = "red" , type = "dashed") +

  geom_path(mapping = aes(x=time,y=plasma_K))+xlab("Time(minutes)") +

  ylab("plasma potassium concentration (mEq/ml)")+theme_bw()# +

#ylim(c(theta$norm_plasma_K-0.0005, theta$norm_plasma_K+0.0005))

p2 = ggplot(x)+geom_path(mapping = aes(x=time, y= Aldo))+xlab("Time(minutes)")+

  ylim(c(0.45, .62))+geom_hline(yintercept = theta$norm_Aldo, color = "red" , type = "dashed")
+

  xlab("Time(minutes)") +

  ylab("Aldosterone concentration (nmol/l)")+theme_bw()

p3 = ggplot(x)+geom_path(mapping = aes(x=time, y=
DCT1_cell_Na_conc))+xlab("Time(minutes)")+

  ylim(c(.01, .015))+theme_bw()

p4 = ggplot(x)+geom_path(mapping = aes(x=time, y=
CNT_cell_Na_conc))+xlab("Time(minutes)")+

  ylim(c(.01, .015))+theme_bw()
```

```
p5 = ggplot(x)+geom_path(mapping = aes(x=time, y=
CCD_cell_Na_conc))+xlab("Time(minutes)")+

  ylim(c(.01, .015))+theme_bw()

p6= ggplot(x)+geom_path(mapping = aes(x=time, y=
DCT1_cell_K_conc))+xlab("Time(minutes)")+

  ylim(c(.13, .16))+theme_bw()

p7= ggplot(x)+geom_path(mapping = aes(x=time, y=
CNT_cell_K_conc))+xlab("Time(minutes)")+

  ylim(c(.13, .16))+theme_bw()

p8= ggplot(x)+geom_path(mapping = aes(x=time, y=
CCD_cell_K_conc))+xlab("Time(minutes)")+

  ylim(c(.13, .16))+theme_bw()

p9= ggplot(x)+geom_path(mapping = aes(x=time,
y=mean_arterial_pressure_MAP))+xlab("Time(minutes)")+theme_bw()


grid.arrange(p1,p2,p3,p4,p5,p6,p7,p8,p9, nrow=4)


inits = x[dim(x)[1], names(x) %in% names(inits)]

theta_orig=theta

inits_orig=as.list(inits)

x_orig = data.frame(x)
```

C-5. Calibration code

```
############################## Sodium-Potassium Homeostasis Model
########################################### Fig 4.3 , 4.4 and 4.5


#Authors: Erfan Maddah, KM Hallow, University of Georgia

#September 2022

#This file optimizes model feedback parameters to fit the following studies and generates Fig
4.3, 4.4, and 4.5:
```

#McInnes, G., et al. (1982). "Spironolactone doseâ€• response relationships in healthy subjects."

#British journal of clinical pharmacology 13(4): 513-518.

#Dluhy, R. G., et al. (1972). "Studies of the control of plasma aldosterone concentration in

#normal man: II. Effect of dietary potassium and acute potassium infusion."

#The Journal of Clinical Investigation 51(8): 1950-1957.

##Williams, Gordon H., et al. "Studies of the control of plasma aldosterone concentration in normal man: I. Response to posture, acute

#and chronic volume depletion, and sodium loading." The Journal of Clinical Investigation 51.7 (1972): 1731-1742. ###############

#Before running this file, run the file "runToEquilibrium.R"

####################

#Load base parameters and initial conditions

theta = theta_orig

inits = inits_orig

#Load helper files for simulating each study

source("helperfiles/simDluly.R")

source("helperfiles/simwilliams.R")

source("helperfiles/simMcInnes_PK.R")

#Load datasets digitized from each study

potassiumexcretionSPR = read.csv("data/McInnes1982.csv") #McInnes 1982

Normalpotassiumaldo = read.csv("Normalpotassiumaldo.csv") #Dluly 1972

plasmaKreninmeandata1=read.csv("plasmaKreninmeandata2.csv") #Williams 1972

################## No Feedback ############################

################################## Fit Aldo-potassium effects only ##################33

```
#Define Objective Function
obj <- function(beta){
  tryCatch({
    print(beta)

    #reset parameters and intial conditions
    theta = theta_orig
    inits = inits_orig

    #Update parameters with current beta values
    theta[intersect(names(theta), names(beta))] = beta[intersect(names(theta), names(beta))]



    #Scale parameters

    theta["m_K_ALDO"] = theta["m_K_ALDO"]*100
    theta["m_plasmaK_MCD"] = theta["m_plasmaK_MCD"]*1e-7#00
    theta["EC50_spiro"] =theta["EC50_spiro"]*10#30
    theta["Aldo_KSec_scale"] =theta["Aldo_KSec_scale"]*100#30
    theta["m_Na_ALDO"] = theta["m_Na_ALDO"]*10



    #Simulate Williams
    out = simwilliams(theta)
    obj1 = out$obj



    #Simulate Dluly
```

```
  out2 = simDluly(theta)
  obj2 = (out2$obj)#/10


  #Simulate McInnes
  out3 = simMcInnes(theta)
  obj3 = out3$obj

  #Weight and combine objective function values
  obj = obj1 + obj2 + obj3/4


  if (is.na(obj)) {
    obj = 1e10
  }

  print(paste("williams:", obj1))
  print(paste("Dluhy:", obj2))
  print(paste("McInnes", obj3/4))
  print(paste("Total:", obj))
  return(obj)
},


error =  function(err) {
  ssq = 10e10
  return(ssq)
})
```

```
}

beta =c(m_K_ALDO= 13.93,#12.2205732, #fixed
        m_osm_ALDO = 0.31,#0.3782274, #fixed
        Aldo_KSec_scale = 0.01049313,#0.0257460,
        m_plasmaK_MCD = 2.13,#3.7229754,
        Q_K_intracellular = 0.177,#0.1071755,
        Q_Na = 17.45,#13.8533909, #fixed
        aldo_ROMK_scale = 0.78633683,#0.2439132,
        aldo_ENAC_scale =0.15007451,#0.0825,
        AT1_NKCC_scale = 0.00010000,#0.2003847,
        AT1_NCC_scale = 0.34663599,#1.1820732,
        AT1_aldo_slope =0.01000000,#0.055,
        md_renin_tau= 6.5,#4.1425466,
        E_MAX_spiro = 1,#0.9,
        EC50_spiro=0.56,#1,
        C_aldo_on_ENAC=0.35,#.99
        m_Na_ALDO=0.001
)


lower =c(   m_K_ALDO=0,
            m_osm_ALDO = 0,
            Aldo_KSec_scale = 0,
            m_plasmaK_MCD = 0,
            Q_K_intracellular = 0.01,
            Q_Na = 5,
```

```
        aldo_ROMK_scale = 0.0001,
        aldo_ENAC_scale =0.0001,
        AT1_NKCC_scale = 0.001,
        AT1_NCC_scale = 0.001,
        AT1_aldo_slope =0.001,
        md_renin_tau= 6,
        E_MAX_spiro = 0.5,
        EC50_spiro=0.4,
        C_aldo_on_ENAC=0.0001,
        m_Na_ALDO=0.0001
)

upper =c( m_K_ALDO=15,#15,
        m_osm_ALDO = 0.9,
        Aldo_KSec_scale = .9,
        m_plasmaK_MCD = 5,
        Q_K_intracellular = 1,
        Q_Na = 20,
        aldo_ROMK_scale = 4,
        aldo_ENAC_scale =0.2,
        AT1_NKCC_scale = 0.5,
        AT1_NCC_scale = 1.25,
        AT1_aldo_slope =0.08,
        md_renin_tau= 7.5,
        E_MAX_spiro = 1,
        EC50_spiro=1.4,
        C_aldo_on_ENAC=1,
        m_Na_ALDO=0.2
```

```
)


#Optimize
fit = optim(beta, obj, method = "L-BFGS-B", lower = lower, upper = upper,hessian = T)

##############

#save(file = "fitlast54", fit)
load("fitlast54")

beta = fit$par

hessian<-fit$hessian
F<-solve(hessian,tol=1e-100)
st_errors<- diag(sqrt(F))
st_errors


theta = theta_orig
inits = inits_orig

theta[intersect(names(theta), names(beta))] = beta[intersect(names(theta), names(beta))]

#Scale estimated parameters
theta["m_K_ALDO"] = theta["m_K_ALDO"]*100
theta["m_plasmaK_MCD"] = theta["m_plasmaK_MCD"]*1e-7#00
#theta["EC50_spiro"] = 30
```

```
theta["EC50_spiro"] =theta["EC50_spiro"]*10#30
theta["Aldo_KSec_scale"] =theta["Aldo_KSec_scale"]*100#30
theta["aldo_ENAC_scale"]=theta["aldo_ENAC_scale"]

theta["C_aldo_on_ENAC"] = theta["C_aldo_on_ENAC"]
theta["m_Na_ALDO"] =theta["m_Na_ALDO"]

theta["md_renin_tau"] =theta["md_renin_tau"]

out = simDluly(theta)
allCaseResults = out$allCaseResults

out1=simwilliams(theta)
allCaseResults1=out1$allCaseResults1

h1 =  ggplot() +
  geom_path(data = allCaseResults, mapping = aes(x=time, y = Aldo,colour="Model" )) +
  geom_point(data = Normalpotassiumaldo, mapping = aes(x=time, y =
Aldo/36.044,colour="Dluly 1972")) +
  geom_errorbar(data = Normalpotassiumaldo, mapping = aes(x=time, ymin =(Aldo -
AldoSD)/36.044, ymax = (Aldo + AldoSD)/36.044))+
  facet_grid(rows = ~case)+xlab("Time (minutes)") +
  ylab("Plasma aldo (nmol/l)")+
  #expand_limits(y=0) +                    # Expand y range
  ylim(c(0,8)) +
  scale_y_continuous() + scale_x_continuous()+        # Set tick every 4
  theme_bw() +scale_colour_manual("",
                             breaks = c("Dluly 1972", "Model"),
                             values = c("Black", "Red"))
```

```
h2 =  ggplot()+
  geom_path(data = allCaseResults, mapping = aes(x=time, y = plasma_K*1000,colour="Model")) +
  geom_point(data = Normalpotassiumaldo, mapping = aes(x=time, y = Potassium,colour="Dluly 1972")) +
  geom_errorbar(data = Normalpotassiumaldo, mapping = aes(x=time, ymin =Potassium -KSD, ymax = Potassium + KSD))+
  facet_grid(rows = ~case)+xlab("Time (minutes)") +
  ylab("Plasma K+ (mEq/l)")+expand_limits(y=2) +
  scale_y_continuous() + scale_x_continuous()+
  theme_bw()+scale_colour_manual("",
                         breaks = c("Dluly 1972", "Model"),
                         values = c("Black", "Red"))+ylim(0,8)


h3 =  ggplot() +
  geom_path(data = allCaseResults1, mapping = aes(x=time/(24*60), y = Aldo,colour="Model" )) +
  geom_point(data = plasmaKreninmeandata1, mapping = aes(x=time/(24*60), y = Aldo/36.044,colour="Williams 1972")) +
  geom_errorbar(data = plasmaKreninmeandata1, mapping = aes(x=time/(24*60), ymin =(Aldo.MinusSE)/36.044, ymax = (Aldo.PlusSE)/36.044))+
  facet_grid(rows = ~case)+xlab("Time (Days)") +
  ylab("")+
  expand_limits(y=0) +ggtitle("Plasma aldo (nmol/l)")+ theme(plot.title = element_text(hjust = 0.5))+                    # Expand y range
  scale_y_continuous() + scale_x_continuous()+        # Set tick every 4
  theme_bw()+scale_colour_manual("",
                         breaks = c("Williams 1972", "Model"),
                         values = c("Black", "Red"))
```

```
h4 =  ggplot() +
  geom_path(data = allCaseResults1, mapping = aes(x=time/(24*60), y = plasma_renin_activity,colour="Model" )) +
  geom_point(data = plasmaKreninmeandata1, mapping = aes(x=time/(24*60), y = Renin*2.57,colour="Williams 1972")) +
  geom_errorbar(data = plasmaKreninmeandata1, mapping = aes(x=time/(24*60), ymin =( Renin.MinusSE*2.57), ymax = (Renin.PlusSE*2.57)))+
  facet_grid(rows = ~case)+xlab("Time (Days)") +
  ylab("")+
  expand_limits(y=0) + ggtitle("Renin (pmol/l-hr)") + theme(plot.title = element_text(hjust = 0.5))+                    # Expand y range
  scale_y_continuous() + scale_x_continuous()+        # Set tick every 4
  theme_bw()+scale_colour_manual("",
                          breaks = c("Williams 1972", "Model"),
                          values = c("Black", "Red"))


h5= ggplot() +
  geom_path(data = allCaseResults1, mapping = aes(x=time/(24*60), y = plasma_K*1000 - 0.000225*1000,colour="Model" )) +
  geom_point(data = plasmaKreninmeandata1, mapping = aes(x=time/(24*60), y = PlasmaK,colour="Williams 1972")) +
  geom_errorbar(data = plasmaKreninmeandata1, mapping = aes(x=time/(24*60), ymin =( PlasmaK.MinusSE), ymax = (PlasmaK.PlusSE)))+
  facet_grid(rows = ~case)+xlab("Time (Days)") +
  ylab("")+ggtitle("Plasma K+ (mmol/l)")+theme(plot.title = element_text(hjust = 0.5))+
  #expand_limits(y=0) +                    # Expand y range
  ylim(c(0.003*1000, 0.007*1000)) +
  scale_x_continuous()+        # Set tick every 4
  theme_bw()+scale_colour_manual("",
                          breaks = c("Williams 1972", "Model"),
                          values = c("Black", "Red"))
```

```
grid.arrange(h1, h2,h3,h4,h5, nrow = 3)


############################## Simulate McInnes


out = simMcInnes(theta)
allCaseResults = out$allCaseResults



G1=ggplot()+geom_path(data = allCaseResults, mapping = aes(x=Dose ,y =
K2_10_pctdeltaPlac,colour="Model" ))+
  geom_point(data = potassiumexcretionSPR, mapping = aes(x=Dose, y =
pctChange210,colour="McInnes 1982"))+
  geom_errorbar(data = potassiumexcretionSPR, mapping = aes(x=Dose, ymin =(pctChange210
-SE), ymax = (pctChange210  + SE),colour="McInnes 1982")) +
  ylab("") +
  scale_colour_manual("",
                      breaks = c("McInnes 1982", "Model"),
                      values = c("Black", "Red"))+
  xlab("Dose (mg)") +


  ggtitle("K+ Excretion (% Change) in 2-10 hrs") +
  #ylim(c(-12,6)) +                    # Expand y range
  scale_y_continuous() + scale_x_continuous()+        # Set tick every 4
  theme_bw() +
  theme(plot.title = element_text(hjust=0.5))+theme(axis.text=element_text(size=9,face =
"bold"),
```

```
                                    axis.title=element_text(size=9,face = "bold")) #, legend.position
= c(0.8,0.8))

G2=ggplot()+geom_path(data = allCaseResults, mapping = aes(x=Dose ,y =
K12_16_pctdeltaPlac,colour="Model" ))+

  geom_point(data = potassiumexcretionSPR, mapping = aes(x=Dose, y =
pctChange1216,colour="McInnes 1982"))+

  geom_errorbar(data = potassiumexcretionSPR, mapping = aes(x=Dose, ymin =(pctChange1216
-SE1), ymax = (pctChange1216 + SE1),colour="McInnes 1982")) +

  ylab("") + scale_colour_manual("", breaks = c("McInnes 1982", "Model"),values = c("Black",
"Red"))+

  xlab("Dose (mg)") +

    ggtitle("K+ Excretion (% Change) in 12-16 hrs") +

  ylim(c(-0.4,0.4)) +        # Set tick every 4

  theme_bw() +

  theme(plot.title = element_text(hjust=0.5))+theme(axis.text=element_text(size=9,face =
"bold"),

                                    axis.title=element_text(size=9,face = "bold"))#, legend.position
= c(0.8,0.8))

G3=ggplot()+geom_path(data = allCaseResults, mapping = aes(x=Dose ,y =
Na2_10_pctdeltaPlac,colour="Model" ))+

  geom_point(data = potassiumexcretionSPR, mapping = aes(x=Dose, y =
pctChange210Na,colour="McInnes 1982"))+

  geom_errorbar(data = potassiumexcretionSPR, mapping = aes(x=Dose, ymin
=(pctChange210Na  -SE_Na), ymax = (pctChange210Na  + SE_Na),colour="McInnes 1982")) +

  ylab("") +

  scale_colour_manual("",

                      breaks = c("McInnes 1982", "Model"),

                      values = c("Black", "Red"))+

  xlab("Dose (mg)") +


  ggtitle("Na+ Excretion (% Change) in 2-10 hrs") +

  #ylim(c(-12,6)) +                    # Expand y range
```

```
  scale_y_continuous() + scale_x_continuous()+        # Set tick every 4

  theme_bw() +

  theme(plot.title = element_text(hjust=0.5))+theme(axis.text=element_text(size=9,face =
"bold"),

                                    axis.title=element_text(size=9,face = "bold")) #, legend.position
= c(0.8,0.8))


G4=ggplot()+geom_path(data = allCaseResults, mapping = aes(x=Dose ,y =
Na12_16_pctdeltaPlac,colour="Model" ))+

  geom_point(data = potassiumexcretionSPR, mapping = aes(x=Dose, y =
pctChange1216Na,colour="McInnes 1982"))+

  geom_errorbar(data = potassiumexcretionSPR, mapping = aes(x=Dose, ymin
=(pctChange1216Na -SE1_Na), ymax = (pctChange1216Na + SE1_Na),colour="McInnes
1982")) +

  ylab("") + scale_colour_manual("", breaks = c("McInnes 1982", "Model"),values = c("Black",
"Red"))+

  xlab("Dose (mg)") +

  ggtitle("Na+ Excretion (% Change) in 12-16 hrs") +

  ylim(c(-1,4)) +        # Set tick every 4

  theme_bw() +

  theme(plot.title = element_text(hjust=0.5))+theme(axis.text=element_text(size=9,face =
"bold"),

                                    axis.title=element_text(size=9,face = "bold"))#, legend.position
= c(0.8,0.8))


ggarrange(G1,G2,G3,G4, nrow = 4, common.legend = TRUE, legend = "top")

out$obj
```

C-6. Helper files

```
############################## Potassium Homeostasis Model
########################################
```

```
#Authors: Erfan Maddah, KM Hallow, University of Georgia

#November 22, 2021



#This file simulates and calculates and objective function for:


#McInnes, G., et al. (1982). "Spironolactone doseâ€• response relationships in healthy
subjects."

#British journal of clinical pharmacology 13(4): 513-518.


#Before running this file, run the file "runToEquilibrium.R"


#This file is called by "optimization_McInnesDluly_simultaneous.R"


################################################################################
####################



simDluly = function(theta) {


  obj = 0


  allCaseResults = NULL


  cases = unique(Normalpotassiumaldo$case)


  ############################## Simulate Study Design ######################
```

```
 #simulate each Na/K intake case


for (i in 1:4) {
    thiscase = cases[i]
 thisdata = Normalpotassiumaldo[Normalpotassiumaldo$case == thiscase,]
    inits = inits_orig
    #Run for 3 days on specified diet
 times=seq(0,24*60*3,24*60)#24*60)
 ev=eventTable(time.units = 'minutes')
 ev$add.sampling(times)
 baseline <-data.frame(cvrsim$run(theta, ev, inits))
    #Get new starting point
 inits = as.list(baseline[dim(baseline)[1], names(baseline) %in% names(inits_orig)])
    #Set sodium and potassium intake
 theta$Kin = thisdata$KIntake[1]/24/60
 theta$Na_intake_rate = thisdata$NaIntake[1]/24/60
 theta$Kinfusion=0 #mEq/min
 baseline <-data.frame(cvrsim$run(theta, ev, inits))
    #Get new starting point
 inits = as.list(baseline[dim(baseline)[1], names(baseline) %in% names(inits_orig)])


    #Turn on infusion and simulate for two hours
 theta$Kinfusion = 0.62 #mEq/min
  times=seq(0, 2*60,10)
 ev=eventTable(time.units = 'minutes')
 ev$add.sampling(times)
 infusion <-data.frame(cvrsim$run(theta, ev, inits))
    #Get new starting point
```

```
 inits2 = as.list(infusion[dim(infusion)[1], names(infusion) %in% names(inits_orig)])
    #Turn off infusion and simulation for 3 hours
 theta$Kinfusion = 0
    times=seq(1, 3*60,1)
 ev=eventTable(time.units = 'minutes')
 ev$add.sampling(times)
 infusion_off <-data.frame(cvrsim$run(theta, ev, inits2))
    infusion_off$time = infusion_off$time + 120
    infusion = rbind(infusion, infusion_off)
    #save this case name
 infusion$case = cases[i]
 allCaseResults = rbind(allCaseResults,infusion )
    ########## Calculate Contribution to Objective function ##########
# K_scale = 1e6  #Scaling factor for potassium, to account for differences in units
 K_scale = 1e4
 #Get simulation data matching experimental observation times
 sim_at_data_times = infusion[infusion$time %in% thisdata$time, ]
    #Calculate resisuals and  weighted sum of the squares
 residuals_K = sim_at_data_times$plasma_K - thisdata$Potassium/1000
 obj_K = K_scale*(sum((thisdata$weights*residuals_K)^2))
  residuals_Aldo = sim_at_data_times$Aldo - thisdata$Aldo/36.044
  obj_aldo = (sum((thisdata$weights_aldo*residuals_Aldo)^2))
 #sum up objective function values
 obj = obj + obj_K + obj_aldo/10
 #print(paste("obj_K:",obj_K))
 #print(paste("obj_aldo:",obj_aldo/10))
 }
print(paste("OBJ:",obj))
```

```
  return(list(obj = obj, allCaseResults = allCaseResults))
}


############################## Potassium Homeostasis Model
########################################

#Authors: Erfan Maddah, KM Hallow, University of Georgia
#November 22, 2021


#This file simulates and calculates and objective function for:

#Dluhy, R. G., et al. (1972). "Studies of the control of plasma aldosterone concentration in
#normal man: II. Effect of dietary potassium and acute potassium infusion."
#The Journal of Clinical Investigation 51(8): 1950-1957.

#Before running this file, run the file "runToEquilibrium.R"

#This file is called by "optimization_McInnesDluly_simultaneous.R"

##############################################################################
####################
simMcInnes = function(theta) {

  Spiro_bioavailability = theta$Spiro_bioavailability

  obj = 0
  allCaseResults1=NULL
```

```
allCaseResults = NULL

 ############################## Simulate Study Design ####################
 #Doses to simulate
Dose<-c(0,25,50,100,200,400)

#Event Tables
times=seq(0,60*2,1)
evF=eventTable(time.units = 'minutes')
evF$add.sampling(times)

times=seq(0,60*16,1)
ev16=eventTable(time.units = 'minutes')
ev16$add.sampling(times)

cases = unique(potassiumexcretionSPR$Dose)

#### Simulate fludricortisone (same for all doses)

#set fludrocortisone
theta$D_FLU=1      #mg

#Run for 2 hours
Flud_ON <-data.frame(cvrsim$run(theta, evF, inits))

#Get new starting point
inits = as.list(Flud_ON[dim(Flud_ON)[1], names(Flud_ON) %in% names(inits)])
#Simulate placebo
```

```
  plac <-data.frame(cvrsim$run(theta, ev16, inits))
  #simulate each Dose


  for (i in 1:6) {#6) {#length(cases))
    thiscase = cases[i]
    thisdata = potassiumexcretionSPR[potassiumexcretionSPR$Dose == thiscase,]
      #Set sprinolactone dose
    #theta$D_MRA= thisdata$Dose #mg
   # inits["spiro_depot"] = thisdata$Dose*1000*Spiro_bioavailability #mg to ug
    ev16=eventTable(time.units = 'minutes')
    ev16$add.sampling(times)
       ev16$add.dosing(dose = thisdata$Dose*1000*Spiro_bioavailability, nbr.doses = 1,
dosing.interval = 24, dosing.to = "spiro_t1")


    #Run for 16 hours
    SPR_ON <-data.frame(cvrsim$run(theta, ev16, inits))
       #Calculate cumulative K+ & Na+ excretion during each measurement period
    SPR_ON$K2_10 = SPR_ON$potassium_excretion_rate[SPR_ON$time == 10*60]-
SPR_ON$potassium_excretion_rate[SPR_ON$time == 2*60]
    SPR_ON$K12_16 = SPR_ON$potassium_excretion_rate[SPR_ON$time == 16*60]-
SPR_ON$potassium_excretion_rate[SPR_ON$time == 12*60]


    SPR_ON$Na2_10 = SPR_ON$cumNaExcretion[SPR_ON$time == 10*60]-
SPR_ON$cumNaExcretion[SPR_ON$time == 2*60]
    SPR_ON$Na12_16 = SPR_ON$cumNaExcretion[SPR_ON$time == 16*60]-
SPR_ON$cumNaExcretion[SPR_ON$time == 12*60]


    #Calculate cumulative K+ & Na+ excretion on placebo during each measurement period
    plac$K2_10 = plac$potassium_excretion_rate[plac$time == 10*60]-
plac$potassium_excretion_rate[plac$time == 2*60]
```

```
   plac$K12_16 = plac$potassium_excretion_rate[plac$time == 16*60]-
plac$potassium_excretion_rate[plac$time == 12*60]


   plac$Na2_10 = plac$cumNaExcretion[plac$time == 10*60]-plac$cumNaExcretion[plac$time
== 2*60]
   plac$Na12_16 = plac$cumNaExcretion[plac$time == 16*60]-
plac$cumNaExcretion[plac$time == 12*60]


      #Calculate change from placebo
   SPR_ON$K2_10_deltaPlac = SPR_ON$K2_10 - plac$K2_10
   SPR_ON$K12_16_deltaPlac = SPR_ON$K12_16 - plac$K12_16


   SPR_ON$Na2_10_deltaPlac = SPR_ON$Na2_10 - plac$Na2_10
   SPR_ON$Na12_16_deltaPlac = SPR_ON$Na12_16 - plac$Na12_16
                  #Calculate percent change from placebo
   SPR_ON$K2_10_pctdeltaPlac = SPR_ON$K2_10_deltaPlac/plac$K2_10
   SPR_ON$K12_16_pctdeltaPlac = SPR_ON$K12_16_deltaPlac/plac$K12_16
      SPR_ON$Na2_10_pctdeltaPlac = SPR_ON$Na2_10_deltaPlac/plac$Na2_10
   SPR_ON$Na12_16_pctdeltaPlac = SPR_ON$Na12_16_deltaPlac/plac$Na12_16
                    #save this case name
   SPR_ON$Dose = Dose[i]
   allCaseResults = rbind(allCaseResults,SPR_ON[SPR_ON$time == max(SPR_ON$time),] )
    # #Calculate residual
 #residuals_K210_diff = SPR_ON$K2_10_deltaPlac[1] - thisdata$Change210
 #residuals_K1216_diff = SPR_ON$K12_16_deltaPlac[1] - thisdata$Change1216


 residuals_K210_diff = 100*(SPR_ON$K2_10_pctdeltaPlac[1] - thisdata$pctChange210)
 residuals_K1216_diff = 100*(SPR_ON$K12_16_pctdeltaPlac[1] - thisdata$pctChange1216)
  residuals_Na210_diff = 100*(SPR_ON$Na2_10_pctdeltaPlac[1] - thisdata$pctChange210Na)
```

```
  residuals_Na1216_diff = 100*(SPR_ON$Na12_16_pctdeltaPlac[1] -
thisdata$pctChange1216Na)

     obj1_diff= (sum((residuals_K210_diff*thisdata$weights210)^2))

     #multiplying by thisdata$weights allows different timepoints to be weighted differently

  obj2_diff = (sum((residuals_K1216_diff*thisdata$weights1216)^2))

     obj3_diff= (sum((residuals_Na210_diff*thisdata$weights210Na)^2))

     obj4_diff = (sum((residuals_Na1216_diff*thisdata$weights1216Na)^2))

   #sum up objective function values

  obj = obj + obj1_diff + obj2_diff+obj3_diff+obj4_diff

  }

return(list(obj = obj, allCaseResults = allCaseResults))


}

############################### Potassium & Sodium Homeostasis Model
########################################

#Authors: Erfan Maddah, KM Hallow, University of Georgia

#September 22, 2022

#This file simulates and calculates and objective function for:

#Williams, Gordon H., et al. "Studies of the control of plasma aldosterone concentration in
normal man: I. Response to posture, acute and chronic volume depletion, and sodium loading."
The Journal of Clinical Investigation 51.7 (1972): 1731-1742.

#Before running this file, run the file "runToEquilibrium.R"

#This file is called by "optimization_McInnesDluly_simultaneous.R"

##########################################################################################
####################

simwilliams = function(theta) {

    theta_start = theta

    obj = 0

    allCaseResults1 = NULL

    cases = unique(plasmaKreninmeandata1$case)
```

```
############################## Simulate Study Design ######################
#simulate each Na/K intake case
for (i in 1:2) {

thiscase = cases[i]
thisdata = plasmaKreninmeandata1[plasmaKreninmeandata1$case == thiscase,]
   theta = theta_start
inits = inits_orig
   #Run for 5 days on specified diet
times=seq(0,24*60*5,24*60)
ev=eventTable(time.units = 'minutes')
ev$add.sampling(times)
baseline <-data.frame(cvrsim$run(theta, ev, inits))
   #Get new starting point
inits = as.list(baseline[dim(baseline)[1], names(baseline) %in% names(inits_orig)])
   #Set sodium and potassium intake
theta$Kin =100/24/60 #thisdata$KIntake[1]/24/60
theta$Na_intake_rate = thisdata$NaIntake[1]/24/60
# baseline <-data.frame(cvrsim$run(theta, ev, inits))
#
# #Get new starting point
# inits = as.list(baseline[dim(baseline)[1], names(baseline) %in% names(inits_orig)])
#

# simulate for one day
# simulate for three days
      times=seq(0, 8*24*60,720)
ev=eventTable(time.units = 'minutes')
```

```
ev$add.sampling(times)
#Day1 <-data.frame(cvrsim$run(theta, ev, inits))
AllDay <-data.frame(cvrsim$run(theta, ev, inits))

#Get new starting point
#inits2 = as.list(Day1[dim(Day1)[1], names(Day1) %in% names(inits_orig)])
   #times=seq(0, 24*60,720)
#ev=eventTable(time.units = 'minutes')
#ev$add.sampling(times)
#Day2 <-data.frame(cvrsim$run(theta, ev, inits2))
   #Get new starting point
#inits3 = as.list(Day2[dim(Day2)[1], names(Day2) %in% names(inits_orig)])
      #times=seq(0, 24*60,720)
#ev=eventTable(time.units = 'minutes')
#ev$add.sampling(times)
#Day3 <-data.frame(cvrsim$run(theta, ev, inits3))
      #AllDay = rbind(Day1,Day2,Day3)
#save this case name
AllDay$case = cases[i]
AllDay$time = AllDay$time - 5*24*60
allCaseResults1 = rbind(allCaseResults1,AllDay )

########## Calculate Contribution to Objective function ##########
K_scale =2.5e4# 1e6  #Scaling factor for potassium, to account for differences in units
renin_scale=1e-2#500
aldo_scale=10

   #Get simulation data matching experimental observation times
```

```
   sim_at_data_times = AllDay[AllDay$time %in% thisdata$time,]
      #Calculate resisuals and  weighted sum of the squares
   residuals_K = K_scale*(sim_at_data_times$plasma_K -0.000225 - thisdata$PlasmaK/1000)
   obj_K1 = (sum((residuals_K)^2))
      residuals_Aldo =aldo_scale*( sim_at_data_times$Aldo - thisdata$Aldo/36.044)
   obj_aldo1 = (sum((residuals_Aldo)^2))
      residuals_renin = renin_scale*(sim_at_data_times$plasma_renin_activity -
(thisdata$Renin)*2.57)
   obj_renin = (sum((residuals_renin)^2))
   print(paste("obj_K1:",obj_K1))
   print(paste("obj_aldo1:",obj_aldo1))
   print(paste("obj_renin:",obj_renin))
   #sum up objective function values
   obj = obj + obj_K1 + obj_aldo1 + obj_renin
  }
   print(paste("OBJ:",obj))
  return(list(obj = obj, allCaseResults1 = allCaseResults1))

}
```

C-7. Virtual patient simulation code

```
set.seed(10005)
nsub=100
theta=theta_orig
df=data.frame(theta)
theta.all=do.call("rbind", replicate(nsub, df, simplify = FALSE))
#RSNA reset rates
theta.all$RAP_reset_rate = 1
theta.all$map_reset_rate = 1
```

```
######################### Make Normotensive-hypertensive spectrum
#########################
# #Sympathetic activation level
theta.all$nom_rsna = runif(nsub, 1, 2)
#
#Sodium intake
theta.all$Na_intake_rate=runif(nsub,0.06,0.14)
# #Basal renal vascular resistances
theta.all$nom_preafferent_arteriole_resistance = runif(nsub, 11,23)
theta.all$nom_afferent_diameter = runif(nsub, 1.57e-5, 1.7e-5)
#Glomerulosclerosis
theta.all$disease_effects_decreasing_Kf = runif(nsub, 0.1,.3)+ rnorm(nsub, mean = 0, sd = 0.15)
theta.all$disease_effects_decreasing_Kf[theta.all$disease_effects_decreasing_Kf <0] = 0
theta.all$disease_effects_decreasing_Kf[theta.all$disease_effects_decreasing_Kf >0.9] = 0.9
# #Basal tubular reabsorption rates
R = runif(nsub,.9,3)
theta.all$Na_luminal_permeability_mcd= theta_orig$Na_luminal_permeability_mcd * R
theta.all$Na_luminal_permeability_ccd= theta_orig$Na_luminal_permeability_ccd * R
theta.all$Na_luminal_permeability_cnt= theta_orig$Na_luminal_permeability_cnt * R
theta.all$Na_luminal_permeability_dct2= theta_orig$Na_luminal_permeability_dct2 * R
theta.all$Na_luminal_permeability_dct1= theta_orig$Na_luminal_permeability_dct1 * R
theta.all$nominal_pt_na_reabsorption_nonSGLT = runif(nsub,0.64,0.8)
#Tubular pressure-natriuresis signal sensitivity
theta.all$pressure_natriuresis_CD_scale = runif(nsub,0,0.5)
theta.all$pressure_natriuresis_PT_scale = theta.all$pressure_natriuresis_CD_scale/5
# #Setpoint for renal interstitial hydrostatic pressure
theta.all$RIHP0 = runif(nsub, 5,5.5)
# ######################Make salt-sensitive
SS = runif(nsub,0.5,1)
```

```
#Venous stiffness

theta.all$venous_compliance = (0.45 - theta_orig$venous_compliance)*SS +
theta_orig$venous_compliance

######################### Give existing Kidney Injury ########################

#Nephron loss

theta.all$disease_effect_on_nephrons = runif(nsub,0,.3)

#Glomerular hypertrophy  - assume already maxed out

theta.all$disease_effects_increasing_Kf = runif(nsub, 0.2, 0.3)

theta.all$maximal_glom_surface_area_increase = theta.all$disease_effects_increasing_Kf

#Glomerulosclerosis

theta.all$disease_effects_decreasing_Kf = runif(nsub, 0.1,.4)+ rnorm(nsub, mean = 0, sd = 0.15)

theta.all$disease_effects_decreasing_Kf[theta.all$disease_effects_decreasing_Kf <0] = 0

theta.all$disease_effects_decreasing_Kf[theta.all$disease_effects_decreasing_Kf >0.9] = 0.9

#Glomeruli loss, a function of glomerulosclerosis

theta.all$disease_effect_losing_glomeruli =  theta.all$disease_effects_decreasing_Kf +
rnorm(nsub, mean = 0, sd = 0.15)

theta.all$disease_effect_losing_glomeruli[theta.all$disease_effect_losing_glomeruli <0] = 0

theta.all$disease_effect_losing_glomeruli[theta.all$disease_effect_losing_glomeruli >0.9] = 0.9

#Progressoin variability

theta.all$renal_progression_variability = 1+(rnorm(nsub, mean = 0, sd = 0.04))

theta.all$relative_fibrosis_sclerosis = theta.all$disease_effect_on_nephrons-
theta.all$disease_effects_decreasing_Kf

theta.all$disease_severity
=(theta.all$disease_effect_on_nephrons+theta.all$disease_effects_decreasing_Kf+theta.all$disea
se_effect_losing_glomeruli)/3

#reabsorptive capacity decreases with increasing injury

#scale between 1.2 and 1.8 e-6

theta.all$SN_albumin_reabsorptive_capacity=0.9e-6+((1-theta.all$disease_severity)/0.6)*0.6e-6

######################### Simulate All Virtual patients ######################

VPdf_acei=NULL
```

```
ev <- eventTable()
ev$add.sampling(seq(0,2000000,by=200000))
# Loop through each row of parameter values and simulate
for (i in 1:(dim(theta.all)[1]))
{
  try({
    print(i)
    inits=inits_orig
    theta = theta.all[i,]
        #Set initial levels of injury
        inits$disease_effect_on_nephrons = theta$disease_effect_on_nephrons
    inits$disease_effects_decreasing_Kf = theta$disease_effects_decreasing_Kf
    inits$disease_effect_losing_glomeruli = theta$disease_effect_losing_glomeruli
    inits$disease_effects_increasing_Kf = theta$disease_effects_increasing_Kf
        x <- data.frame(cvrsim$run(theta, ev, inits=inits))
        inits = as.list(x[dim(x)[1], names(x) %in% names(inits)])

    #Simulate
    x <- data.frame(cvrsim$run(theta, ev, inits=inits))
        #Store subject ID
    x$i=i
        #Store results
    VPdf_acei=rbind(VPdf_acei,x[dim(x)[1],])

  })
}
VPdfkeep = VPdf_acei
VPdf_acei$blood_glucose = theta.all$glucose_concentration
```

```
hist(VPdf_acei$mean_arterial_pressure_MAP)
summary(VPdf_acei$mean_arterial_pressure_MAP)
summary(VPdf_acei$GFR_ml_min)
summary(VPdf_acei$plasma_K)
view(VPdf_acei$mean_arterial_pressure_MAP)
```

C-8. Model validation codes

```
###To generate Figure 4.6
############################## Sodium-Potassium Homeostasis Model ##########################################
#Authors: Erfan Maddah, KM Hallow, University of Georgia
#September 2022
#This file simulates:
#Karagiannis, A., et al. (2008). "Spironolactone versus eplerenone for the treatment of idiopathic
#hyperaldosteronism." Expert opinion on pharmacotherapy 9(4): 509-515.
#Before running this file, run the file "runToEquilibrium.R"
#Used to produce mean arterial pressure
################################################################################################################
# Load Study data
dat = read.csv("Karagiannis2008.csv")
dat2=read.csv("Karagnis(BP).csv")
######################################## Simulate Spironolactone Arm ############################


#################### Make Hyperaldosteronism ################
inits=inits_orig

theta=theta.all[12,]
```

```
save(file ="HyperaldosteronismsVPs", theta.all, VPdf_acei)
load("HyperaldosteronismsVPs")

#set inital condition values equal to virtual patient parameters
inits$disease_effect_on_nephrons = theta$disease_effect_on_nephrons
inits$disease_effects_decreasing_Kf = theta$disease_effects_decreasing_Kf
inits$disease_effect_losing_glomeruli = theta$disease_effect_losing_glomeruli
inits$disease_effects_increasing_Kf = theta$disease_effects_increasing_Kf

theta$hyperaldo_effect =1.7#To produce hyperaldostronism

times=seq(0,28*60*24,1)
ev=eventTable(time.units = 'minutes')
ev$add.sampling(times)

#Simulate to new baseline
h <-data.frame(cvrsim$run(theta, ev, inits))
inits = as.list(h[dim(h)[1], names(h) %in% names(inits)])

#Store new starting parameters
theta_start = theta

### Simulate Spironolactone to week 4
times=seq(0, 4*7*24*60,1)
ev=eventTable(time.units = 'minutes')
ev$add.sampling(times)
ev$add.dosing(dose = 25*1000, nbr.doses = 4*7*2, dosing.interval = 12*60, dosing.to =
"spiro_depot")
```

```
SPR4W <-data.frame(cvrsim$run(theta, ev, inits))

SPR4W=data.frame(SPR4W)


#Define new starting point

inits = as.list(SPR4W[dim(SPR4W)[1], names(SPR4W) %in% names(inits)])


### Increase Dose, Simulate Spironolactone to week 8

ev=eventTable(time.units = 'minutes')

ev$add.sampling(times)

ev$add.dosing(dose = 50*1000, nbr.doses = 4*7*2, dosing.interval = 12*60, dosing.to =
"spiro_depot")

SPR8W <-data.frame(cvrsim$run(theta, ev, inits))

SPR8W=data.frame(SPR8W)

#Define new starting point

inits = as.list(SPR8W[dim(SPR8W)[1], names(SPR8W) %in% names(inits)])

### Increase Dose, Simulate Spironolactone to week 12

ev=eventTable(time.units = 'minutes')

ev$add.sampling(times)

ev$add.dosing(dose = 100*1000, nbr.doses = 4*7*2, dosing.interval = 12*60, dosing.to =
"spiro_depot")

SPR12W <-data.frame(cvrsim$run(theta, ev, inits))

SPR12W=data.frame(SPR12W)

#Define new starting point

inits = as.list(SPR12W[dim(SPR12W)[1], names(SPR12W) %in% names(inits)])

### Increase Dose, Simulate Spironolactone to week 16

ev=eventTable(time.units = 'minutes')

ev$add.sampling(times)

ev$add.dosing(dose = 200*1000, nbr.doses = 4*7*2, dosing.interval = 12*60, dosing.to =
"spiro_depot")
```

```
SPR16W <-data.frame(cvrsim$run(theta, ev, inits))

SPR16W=data.frame(SPR16W)

#make a new data frame includes the data and model response

modelsprS=c(h$plasma_K[40320],SPR4W$plasma_K[40320],SPR8W$plasma_K[40320],SPR12W$plasma_K[40320],SPR16W$plasma_K[40320])

modelsprs2=c(h$MAP_delayed[40320],SPR4W$MAP_delayed[40320],SPR8W$MAP_delayed[40320],SPR12W$MAP_delayed[40320],SPR16W$MAP_delayed[40320])

Data_modelS = cbind(dat[dat$Treat == "Spironolactone",],modelsprS*1000)

Data_modelS2 = cbind(dat2[dat2$Treat == "Spironolactone",],modelsprs2)

#plot

N=ggplot(Data_modelS, aes(x=Time, y=serum_potassium, colour="Karagiannis 2008")) +
  geom_errorbar(aes(ymin=PSD1, ymax=PSD2), width=.3) +
  geom_line(aes(y=modelsprS*1000,x=Time,colour="Model")) +
  geom_point(aes(x=Time, y=serum_potassium, colour="Karagiannis 2008")) +
  scale_colour_manual("",
                      breaks = c("Karagiannis 2008", "Model"),
                      values = c("Black", "Red"))+
  xlab("Week") +
  ylab("Plasma K+ (mEq/L)") +
  #ggtitle("Chronic changes of serum potassium after spironolactone administration") +
  #expand_limits(y=0) +                        # Expand y range
  scale_y_continuous() + scale_x_continuous(breaks=0:20*4)+        # Set tick every 4
  theme_bw() +theme(text=element_text(size=14,face = "bold" ))+
  annotate("rect", xmin = 0, xmax = 4, ymin = -Inf, ymax = Inf,
           alpha = 0.15, fill = "grey") +
  annotate("rect", xmin = 4, xmax = 8, ymin = -Inf, ymax = Inf,
           alpha = 0.3, fill = "grey") +
  annotate("rect", xmin = 8, xmax = 12, ymin = -Inf, ymax = Inf,
           alpha = 0.45, fill = "grey")+
```

```
  annotate("rect", xmin = 12, xmax = 16, ymin = -Inf, ymax = Inf,
           alpha = 0.6, fill = "grey")+
  annotate("text", x = 2, y = 2.9,
           label = " 50 mg") +
  annotate("text", x = 6, y = 2.9,
           label = " 100 mg")+
  annotate("text", x = 10, y = 2.9,
           label = " 200 mg")+
  annotate("text", x = 14, y = 2.9,
           label = " 400 mg")+
  theme(legend.position = "bottom", plot.title = element_text(hjust = 0.5))
```

N

```
J=ggplot(Data_modelS2, aes(x=Time, y=MAP, colour="Karagiannis 2008")) +
  geom_errorbar(aes(ymin=MAPSD1, ymax=MAPSD2), width=.3) +
  geom_line(aes(y=modelsprs2,x=Time,colour="Model")) +ylim(70,150)+
  geom_point(aes(x=Time, y=MAP, colour="Karagiannis 2008")) +
  scale_colour_manual("",
                      breaks = c("Karagiannis 2008", "Model"),
                      values = c("Black", "Red"))+
  xlab("Week") +
  ylab("MAP (mmHg)") +
                        # Expand y range
  scale_y_continuous() + scale_x_continuous(breaks=0:20*4)+        # Set tick every 4
  theme_bw() +theme(text=element_text(size=14,face = "bold" ))+
  annotate("rect", xmin = 0, xmax = 4, ymin = -Inf, ymax = Inf,
           alpha = 0.15, fill = "grey") +
```

```
  annotate("rect", xmin = 4, xmax = 8, ymin = -Inf, ymax = Inf,
           alpha = 0.3, fill = "grey") +
  annotate("rect", xmin = 8, xmax = 12, ymin = -Inf, ymax = Inf,
           alpha = 0.45, fill = "grey")+
  annotate("rect", xmin = 12, xmax = 16, ymin = -Inf, ymax = Inf,
           alpha = 0.6, fill = "grey")+
  annotate("text", x = 2, y = 95,
           label = " 50 mg") +
  annotate("text", x = 6, y = 95,
           label = " 100 mg")+
  annotate("text", x = 10, y = 95,
           label = " 200 mg")+
  annotate("text", x = 14, y = 95,
           label = " 400 mg")+
  theme(legend.position = "bottom", plot.title = element_text(hjust = 0.5))

J

grid.arrange(N,J, nrow = 2)


############################## Sodium-Potassium Homeostasis Model
########################################


###Use to generate fig 4.7

#Authors: Erfan Maddah, KM Hallow, University of Georgia

#November 2022

#This file simulates:


#Batterink, J., Stabler, S. N., Tejani, A. M., & Fowkes, C. T. (2010). Spironolactone for
hypertension. Cochrane database of systematic reviews, (8)..
```

```
#Before running this file, run the file "runToEquilibrium.R"

#Used to produce mean arterial pressure

################################################################################
#####################

# Load Study data
dat3 = read.csv("CochraneSpiro.csv")

dat3=data.frame(dat3)
######################################## Simulate Spironolactone Arm
############################

inits=inits_orig

theta=theta.all[74,]  #74

inits$disease_effect_on_nephrons = theta$disease_effect_on_nephrons
inits$disease_effects_decreasing_Kf = theta$disease_effects_decreasing_Kf
inits$disease_effect_losing_glomeruli = theta$disease_effect_losing_glomeruli
inits$disease_effects_increasing_Kf = theta$disease_effects_increasing_Kf
times=seq(0,28*60*24,1)
ev=eventTable(time.units = 'minutes')
ev$add.sampling(times)

#Simulate to new baseline
h <-data.frame(cvrsim$run(theta, ev, inits))
```

```
inits = as.list(h[dim(h)[1], names(h) %in% names(inits)])

SPR4W=NULL

for (i in 1:6) {

  #Store new starting parameters & inits
  inits = as.list(h[dim(h)[1], names(h) %in% names(inits)])

  theta_start = theta

  ### Simulate Spironolactone to week 4
  Doses=c(25,100,150,200,400,500)
  Cases=c("25 mg/day","100 mg/day","150 mg/day","200 mg/day","400 mg/day","500 mg/day")
  times=seq(0, 4*7*24*60,1)
  ev=eventTable(time.units = 'minutes')
  ev$add.sampling(times)
  ev$add.dosing(dose = (Doses[i]/2)*1000, nbr.doses = 4*7*2, dosing.interval = 12*60, dosing.to
= "spiro_depot")

  SPR4W <-data.frame(cvrsim$run(theta, ev, inits))
  SPR4W=data.frame(SPR4W)
  #Map=125.07 mmHg GFR=109.5 ml/min
  SPR4W$Case = Cases[i]
  eval(parse(text= paste0("SPR4W", i, " =SPR4W")))

  }
```

```
SPR4W_hyp =
c(h$mean_arterial_pressure_MAP[40320],SPR4W1$mean_arterial_pressure_MAP[40320],
SPR4W2$mean_arterial_pressure_MAP[40320],SPR4W3$mean_arterial_pressure_MAP[40320
],SPR4W4$mean_arterial_pressure_MAP[40320],SPR4W5$mean_arterial_pressure_MAP[4032
0],SPR4W6$mean_arterial_pressure_MAP[40320])


changes_MAP_output=c(SPR4W_hyp[1]-SPR4W_hyp[1],SPR4W_hyp[2]-
SPR4W_hyp[1],SPR4W_hyp[3]-SPR4W_hyp[1],SPR4W_hyp[4]-
SPR4W_hyp[1],SPR4W_hyp[5]-SPR4W_hyp[1],SPR4W_hyp[6]-
SPR4W_hyp[1],SPR4W_hyp[7]-SPR4W_hyp[1])


#data (calculate baseline alterations)

Change_MAPEs=(dat3$MAPEs-dat3$MAP)


Change_MAPEs_data=c(NA,Change_MAPEs[1],Change_MAPEs[2],Change_MAPEs[3],Chang
e_MAPEs[4],Change_MAPEs[5],Change_MAPEs[6])


#Lower band data


Change_MAPEsL=(dat3$MAPEsL-dat3$MAP)


Change_MAPEsL_data=c(NA,Change_MAPEsL[1],Change_MAPEsL[2],Change_MAPEsL[3],
Change_MAPEsL[4],Change_MAPEsL[5],Change_MAPEsL[6])


#upper band of the data

Change_MAPEsU=(dat3$MAPEsU-dat3$MAP)

Change_MAPEsU_data=c(NA,Change_MAPEsU[1],Change_MAPEsU[2],Change_MAPEsU[3]
,Change_MAPEsU[4],Change_MAPEsU[5],Change_MAPEsU[6])


Dose1=c(0,25,100,150,200,400,500)
```

```
AllResults=cbind(changes_MAP_output,Change_MAPEs_data,Change_MAPEsL_data,Change_
MAPEsU_data,Dose1)

AllResults=data.frame(AllResults)

W=ggplot(AllResults, aes(x=Dose1, y=Change_MAPEs_data, colour="Cochrane 2010")) +

  geom_errorbar(aes(ymin=Change_MAPEsL_data, ymax=Change_MAPEsU_data), width=28)
+

  geom_point(aes(y=changes_MAP_output,x=Dose1,colour="Model")) +ylim(-25,5)+

  geom_point(aes(x=Dose1, y=Change_MAPEs_data, colour="Cochrane 2010"))+

  geom_line(aes(y=changes_MAP_output,x=Dose1,colour="Model"))+

  geom_line(aes(x=Dose1, y=Change_MAPEs_data, colour="Cochrane 2010")) +

  geom_hline(yintercept = 0,linetype=2,colour="Black")+

  scale_colour_manual("",

                      breaks = c("Cochrane 2010", "Model"),

                      values = c("Black", "Red"))+theme_bw()+

  xlab("Dose (mg/day)") +

  ylab("MAP (mmHg)") +ggtitle("4 weeks")+scale_x_continuous(breaks=0:500*50)+

  theme(legend.position = "bottom", plot.title = element_text(hjust = .5))


W
```

C-9. Model Application

```
#### run the following codes after simulating the run file####

### SGLT2i administration and comparing plots in a healthy case

G=NULL

  for (i in 1:2) {

  inits = inits_orig

  theta_orig=theta

  inits_orig=as.list(inits)

  SGLT2in=c(1,0.22)
```

```
  Cases=c("Normal","SGLT2i")
  theta$SGLT2_inhibition=SGLT2in[i]
  #Run for 8 Weeks
  times=seq(0,8*7*24*60,1)
    ev=eventTable(time.units = 'minutes')
  ev$add.sampling(times)

  G <- cvrsim$run(theta, ev, inits=inits)
  G = data.frame(G)
  G$Case = Cases[i]
  eval(parse(text= paste0("G", i, " = G")))
}
#return(list(G=G))

G_t = rbind(G1, G2)

#####Plots#########
h1 = ggplot(G_t) + geom_path(aes(x=time/(7*24*60), y = CD_K_out, color = Case))+ylab("K+
excretion rate (mEq/min)")+xlab("Time(Weeks)")+theme_bw()+ theme(axis.text.x =
element_text(size = 9,face = "bold"),
  axis.title.x = element_text(size = 9,face = "bold"))+ theme(axis.text.y = element_text(size = 9,
face = "bold"),
axis.title.y = element_text(size = 9,face = "bold"))+scale_colour_manual("",
breaks = c(G_t$Case[1],G2$Case),
values = c("Blue","Red"))
h2 = ggplot(G_t) + geom_path(aes(x=time/(7*24*60), y = urine_flow_rate, color =
Case))+xlab("Time(Weeks)")+ylab(" Urine flow (ml/min)")+theme_bw()+ theme(axis.text.x =
element_text(size = 9,face = "bold"),
axis.title.x = element_text(size = 9,face = "bold"))+ theme(axis.text.y = element_text(size =
9,face = "bold"),
axis.title.y = element_text(size = 9,face = "bold"))+scale_colour_manual("",
breaks = c(G_t$Case[1],G2$Case),
values = c("Blue","Red")) #+ facet_wrap(~VP)
```

```
h3 = ggplot(G_t) + geom_path(aes(x=time/(7*24*60), y = CD_K_out/urine_flow_rate, color =
Case))+xlab("Time(Weeks)")+ylab("Urine K+ concentration")+theme_bw()+ theme(axis.text.x
= element_text(size = 9,face = "bold"),


axis.title.x = element_text(size = 9,face = "bold"))+ theme(axis.text.y = element_text(size =
9,face = "bold"),


axis.title.y = element_text(size = 9,face = "bold"))+theme(legend.text = element_text(face =
"bold"),


legend.title = element_text(face = "bold"))+scale_colour_manual("",


breaks = c(G_t$Case[1],G2$Case),


values = c("Blue","Red"))# + facet_wrap(~VP)

h4 = ggplot(G_t) + geom_path(aes(x=time/(7*24*60), y = plasma_K*1000, color =
Case))+xlab("Time(Weeks)")+ylab("Plasma K (mEq/l)")+theme_bw()+ theme(axis.text.x =
element_text(size = 9,face = "bold"),


axis.title.x = element_text(size = 9,face = "bold"))+ theme(axis.text.y = element_text(size =
9,face = "bold"),


axis.title.y = element_text(size = 9,face = "bold"))+scale_colour_manual("",


breaks = c(G_t$Case[1],G2$Case),


values = c("Blue","Red"))# + facet_wrap(~VP)


h5 = ggplot(G_t) + geom_path(aes(x=time/(7*24*60), y = intracellular_K, color =
Case))+xlab("Time(Weeks)")+ylab("Intracellular K conc (mEq/l)")+theme_bw()+
theme(axis.text.x = element_text(size = 9,face = "bold"),


axis.title.x = element_text(size = 9,face = "bold"))+ theme(axis.text.y = element_text(size =
9,face = "bold"),


axis.title.y = element_text(size = 9,face = "bold"))+scale_colour_manual("",


breaks = c(G_t$Case[1],G2$Case),
```

```
values = c("Blue","Red")) #+ facet_wrap(~VP)


h6 = ggplot(G_t) + geom_path(aes(x=time/(7*24*60), y = blood_volume_L, color =
Case))+xlab("Time(Weeks)")+ylab("Blood volume (L)")+theme_bw()+ theme(axis.text.x =
element_text(size = 9,face = "bold"),


axis.title.x = element_text(size = 9,face = "bold"))+ theme(axis.text.y = element_text(size =
9,face = "bold"),


axis.title.y = element_text(size = 9,face = "bold"))+scale_colour_manual("",


breaks = c(G_t$Case[1],G2$Case),


values = c("Blue","Red"))# + facet_wrap(~VP)

h7 = ggplot(G_t) + geom_path(aes(x=time/(7*24*60), y = K + intracellular_K + interstitial_K,
color = Case))+xlab("Time(Weeks)")+ylab("Total K (mEq)")+theme_bw()+ theme(axis.text.x =
element_text(size = 9,face = "bold"),


axis.title.x = element_text(size = 9,face = "bold"))+ theme(axis.text.y = element_text(size =
9,face = "bold"),


axis.title.y = element_text(size = 9,face = "bold"))+scale_colour_manual("",


breaks = c(G_t$Case[1],G2$Case),


values = c("Blue","Red"))# + facet_wrap(~VP)

h8 = ggplot(G_t) + geom_path(aes(x=time/(7*24*60), y = K, color =
Case))+xlab("Time(Weeks)")+ylab("K in blood (mEq)")+theme_bw()+ theme(axis.text.x =
element_text(size = 9,face = "bold"),

                                                                    axis.title.x =
element_text(size = 9,face = "bold"))+ theme(axis.text.y = element_text(size = 9,face = "bold"),


axis.title.y = element_text(size = 9,face = "bold"))+scale_colour_manual("",


breaks = c(G_t$Case[1],G2$Case),
```

```
values = c("Blue","Red"))# + facet_wrap(~VP)

h9 = ggplot(G_t) + geom_path(aes(x=time/(7*24*60), y = intracellular_fluid_volume, color =
Case))+xlab("Time(Weeks)")+ylab("Intracellular Volume (L)")+theme_bw()+ theme(axis.text.x
= element_text(size = 9,face = "bold"),


axis.title.x = element_text(size = 9,face = "bold"))+ theme(axis.text.y = element_text(size =
9,face = "bold"),


axis.title.y = element_text(size = 9,face = "bold"))+scale_colour_manual("",breaks =
c(G_t$Case[1],G2$Case),values = c("Blue","Red"))




h10= ggplot(G_t) + geom_path(aes(x=time/(7*24*60), y = plasma_renin_activity, color =
Case))+xlab("Time(Weeks)")+ylab("Renin (pmol/l-hr)")+theme_bw()+ theme(axis.text.x =
element_text(size = 9,face = "bold"),


axis.title.x = element_text(size = 9,face = "bold"))+ theme(axis.text.y = element_text(size =
9,face = "bold"),


axis.title.y = element_text(size = 9,face = "bold"))+scale_colour_manual("",


breaks = c(G_t$Case[1],G2$Case),


values = c("Blue","Red"))

h11= ggplot(G_t) + geom_path(aes(x=time/(7*24*60), y = Aldo, color =
Case))+xlab("Time(Weeks)")+ylab(" Aldo (nmol/l)")+theme_bw()+ theme(axis.text.x =
element_text(size = 9,face = "bold"),

                                                                    axis.title.x
= element_text(size = 9,face = "bold"))+ theme(axis.text.y = element_text(size = 9,face =
"bold"),


axis.title.y = element_text(size = 9,face = "bold"))+scale_colour_manual("",


breaks = c(G_t$Case[1],G2$Case),


values = c("Blue","Red"))# + facet_wrap(~VP)
```

```
h12= ggplot(G_t) + geom_path(aes(x=time/(7*24*60), y =
DCT1_K_passive_flux_lumenal+DCT2_K_passive_flux_lumenal+CNT_K_passive_flux_lumen
al+CCD_K_passive_flux_lumenal+MCD_K_passive_flux_lumenal, color =
Case))+xlab("Time(Weeks)")+ylab("Total K+ Secretion (mEq/min.cm2)")+theme_bw()+
theme(axis.text.x = element_text(size = 9,face = "bold"),


axis.title.x = element_text(size = 9,face = "bold"))+ theme(axis.text.y = element_text(size =
9,face = "bold"),axis.title.y = element_text(size = 9,face = "bold"))+scale_colour_manual("",


breaks = c(G_t$Case[1],G2$Case),


values = c("Blue","Red"))


h13=ggplot(G_t) + geom_path(aes(x=time/(7*24*60), y = intracellular_potassium_flux

                        , color = Case))+xlab("Time(Weeks)")+ylab("Intracellular K+ flux
(mEq/min.cm2)")+theme_bw()+theme(axis.text.x = element_text(size = 9,face = "bold"),

                                                                    axis.title.x =
element_text(size = 9,face = "bold"))+ theme(axis.text.y = element_text(size = 9,face =
"bold"),axis.title.y = element_text(size = 9,face = "bold"))+scale_colour_manual("",


breaks = c(G_t$Case[1],G2$Case),


values = c("Blue","Red"))




h14=ggplot(G_t) + geom_path(aes(x=time/(7*24*60), y = PT_K_out

                        , color = Case))+xlab("Time(Weeks)")+ylab(" K+ leaving PT
(mEq/min)")+theme_bw()+theme(axis.text.x = element_text(size = 9,face = "bold"),

  axis.title.x = element_text(size = 9,face = "bold"))+ theme(axis.text.y = element_text(size =
9,face = "bold"),

axis.title.y = element_text(size = 9,face = "bold"))+scale_colour_manual("",


breaks = c(G_t$Case[1],G2$Case),
values = c("Blue","Red"))

h15=ggplot(G_t) + geom_path(aes(x=time/(7*24*60), y = plasma_osmolality
```

```
                    , color = Case))+xlab("Time(Weeks)")+ylab("plasma osmolality
(mEq/L)")+theme_bw()+theme(axis.text.x = element_text(size = 9,face = "bold"),

  axis.title.x = element_text(size = 9,face = "bold"))+ theme(axis.text.y = element_text(size =
9,face = "bold"),axis.title.y = element_text(size = 9,face = "bold"))+scale_colour_manual("",
breaks = c(G_t$Case[1],G2$Case),
values = c("Blue","Red"))

h16=ggplot(G_t) + geom_path(aes(x=time/(7*24*60), y = Na_concentration

                    , color = Case))+xlab("Time(Weeks)")+ylab("Na+ concentration
(mEq/L)")+theme_bw()+theme(axis.text.x = element_text(size = 9,face = "bold"),

                                                    axis.title.x =
element_text(size = 9,face = "bold"))+ theme(axis.text.y = element_text(size = 9,face =
"bold"),axis.title.y = element_text(size = 9,face = "bold"))+scale_colour_manual("",


breaks = c(G_t$Case[1],G2$Case),


values = c("Blue","Red"))

h17=ggplot(G_t) + geom_path(aes(x=time/(7*24*60), y = LoH_K_out

  , color = Case))+xlab("Time(Weeks)")+ylab("K+ leaving LoH
(mEq/min)")+theme_bw()+theme(axis.text.x = element_text(size = 9,face = "bold"),    axis.title.x
= element_text(size = 9,face = "bold"))+ theme(axis.text.y = element_text(size = 9,face =
"bold"),axis.title.y = element_text(size =9,face = "bold"))+scale_colour_manual("",


breaks = c(G_t$Case[1],G2$Case),
values = c("Blue","Red"))

h18=ggplot(G_t) + geom_path(aes(x=time/(7*24*60), y = MCD_K_out

  , color = Case))+xlab("Time(Weeks)")+ylab("K+ leaving MCD
(mEq/min)")+theme_bw()+theme(axis.text.x = element_text(size = 9,face = "bold"),

     axis.title.x = element_text(size = 9,face = "bold"))+ theme(axis.text.y = element_text(size =
9,face = "bold"),axis.title.y = element_text(size = 9,face = "bold"))+scale_colour_manual("",

 breaks = c(G_t$Case[1],G2$Case), values = c("Blue","Red"))


ggarrange(h8,h6,h4,h14,h17,h18,h1,h2,h3,h13,h5,h9,h7, nrow=3, ncol=5,common.legend =
TRUE, labels = c("A","B","C","D","E","F","G","H","I","L","M","N","O"),hjust = -2,vjust = -
0.4)

##################################

#########################################333333
```

```
# Diabetic patient received SGLT2i compare with a healthy subject #
theta = theta_orig
inits = inits_orig
theta$glucose_concentration = 8.6  #Hba1c = 7
thetaDB = theta
h <-data.frame(cvrsim$run(theta, ev, inits))
initsDB = as.list(h[dim(h)[1], names(h) %in% names(inits)])
G=NULL
for (i in 1:2) {
  inits = initsDB#x[dim(x)[1], names(x) %in% names(inits)]
  theta = thetaDB

  SGLT2in=c(1,0.22)
  Cases=c("Normal","SGLT2i")
  theta$SGLT2_inhibition=SGLT2in[i]
  #Run for 8 weeks
  times=seq(0,8*7*24*60,24*60)
  ev=eventTable(time.units = 'minutes')
  ev$add.sampling(times)

  G <- cvrsim$run(theta, ev, inits=inits)
  G = data.frame(G)
  G$Case = Cases[i]
  eval(parse(text= paste0("G", i, " = G")))
}
G_t$VP = "Healthy"
DB = rbind(G1, G2)
DB$VP = "Diabetic"
dat = rbind(G_t, DB)
```

```
####Then run the plots######
##########################################
# Low GFR #
theta = theta_orig
inits = inits_orig
##applying renal dysfunciton
inits$disease_effect_losing_glomeruli = 0.65
inits$disease_effect_on_nephrons = 0.65
inits$disease_effects_decreasing_Kf = 0.5
#theta$glucose_concentration = 8.6  #Hba1c = 7
thetaCKD1 = theta
ev = eventTable()
ev$add.sampling(seq(0,24*60*365, by = 24*60))
h <-data.frame(cvrsim$run(theta, ev, inits))
initsCKD1 = as.list(h[dim(h)[1], names(h) %in% names(inits)])
G=NULL

for (i in 1:2) {

  inits = initsCKD1#x[dim(x)[1], names(x) %in% names(inits)]
  theta = thetaCKD1

  SGLT2in=c(1,0.22)
  Cases=c("Normal","SGLT2i")
  theta$SGLT2_inhibition=SGLT2in[i]
  #Run for 8 Weeks
  times=seq(0,24*60*7*8,60*24)
  ev=eventTable(time.units = 'minutes')
  ev$add.sampling(times)
  G <- cvrsim$run(theta, ev, inits=inits)
```

```
  G = data.frame(G)
  G$Case = Cases[i]
  eval(parse(text= paste0("G", i, " = G")))
}
G_t$VP = "Healthy"
lowGFR = rbind(G1, G2)
lowGFR$VP = "lowGFR"
dat = rbind(G_t, lowGFR)


###then we can have plots
h1= ggplot(dat,aes(x=time/(7*24*60), y = plasma_K*1000, color = Case)) +
geom_path()+geom_line(size=1.25) + facet_wrap(~VP)+theme(axis.text.x = element_text(face =
"bold"),
  axis.title.x = element_text(face = "bold"))+ theme(axis.text.y = element_text(face = "bold"),
  axis.title.y = element_text(face = "bold"))+theme_bw()+xlab("Time(Weeks)")+ylab("Plasma K
(mEq/l)")+theme(axis.text.x = element_text(face = "bold"),

axis.title.x = element_text(face = "bold"))+ theme(axis.text.y = element_text(face = "bold"),

axis.title.y = element_text(face = "bold"))+scale_colour_manual("",

breaks = c(G_t$Case[1],G2$Case),

values = c("Blue","Red"))+labs(color = "Legend Title") +
  theme(legend.key.size = unit(7, "lines"))+theme(strip.text.y = element_text(size = 16),
  strip.text.x = element_text(size = 16))+theme(strip.text.y = element_text(face = "bold"),
   strip.text.x = element_text(face = "bold"))+
  theme(axis.text.x = element_text(size = 15,face = "bold"),
       axis.title.x = element_text(size = 15,face = "bold"))+ theme(axis.text.y = element_text(size
= 15,face = "bold"),
axis.title.y = element_text(size = 15,face = "bold"))
```

```
h2 = ggplot(dat,aes(x=time/(7*24*60), y = blood_volume_L, color = Case)) + geom_path()
+geom_line(size=1.25)+ facet_wrap(~VP)+theme(axis.text.x = element_text(face = "bold"),
axis.title.x = element_text(face = "bold"))+ theme(axis.text.y = element_text(face =
"bold"),axis.title.y = element_text(face =
"bold"))+theme_bw()+xlab("Time(Weeks)")+ylab("Blood volume (L)")+theme(axis.text.x =
element_text(face = "bold"), axis.title.x = element_text(face = "bold"))+ theme(axis.text.y =
element_text(face = "bold"),
axis.title.y = element_text(face = "bold"))+scale_colour_manual("",
breaks = c(G_t$Case[1],G2$Case),
values = c("Blue","Red"))+labs(color = "Legend Title") +

  theme(legend.key.size = unit(7, "lines"))+theme(strip.text.y = element_text(size = 16),

  strip.text.x = element_text(size = 16))+theme(strip.text.y = element_text(face = "bold"),

      strip.text.x = element_text(face = "bold"))+

  theme(axis.text.x = element_text(size = 15,face = "bold"),

       axis.title.x = element_text(size = 15,face = "bold"))+ theme(axis.text.y = element_text(size
= 15,face = "bold"),

axis.title.y = element_text(size = 15,face = "bold"))

h3 = ggplot(dat,aes(x=time/(7*24*60), y = K, color = Case)) + geom_path()
+geom_line(size=1.25)+ facet_wrap(~VP)+theme(axis.text.x = element_text(face = "bold"),

                                                                    axis.title.x =
element_text(face = "bold"))+ theme(axis.text.y = element_text(face = "bold"),

   axis.title.y = element_text(face = "bold"))+theme_bw()+xlab("Time(Weeks)")+ylab("K in
blood (mEq)")+theme(axis.text.x = element_text(face = "bold"),


axis.title.x = element_text(face = "bold"))+ theme(axis.text.y = element_text(face = "bold"),


axis.title.y = element_text(face = "bold"))+scale_colour_manual("",


breaks = c(G_t$Case[1],G2$Case), values = c("Blue","Red"))+labs(color = "Legend Title") +

  theme(legend.key.size = unit(7, "lines"))+theme(strip.text.y = element_text(size = 16),

                                    strip.text.x = element_text(size = 16))+theme(strip.text.y =
element_text(face = "bold"),

   strip.text.x = element_text(face = "bold"))+

  theme(axis.text.x = element_text(size = 15,face = "bold"),

       axis.title.x = element_text(size = 15,face = "bold"))+ theme(axis.text.y = element_text(size
= 15,face = "bold"),
```

```
  axis.title.y = element_text(size = 15,face = "bold"))
ggarrange(h3,h2,h1, nrow=1, ncol=3,common.legend = TRUE, labels = c("A","B","C"))

##########
########MRA administration effect on a healthy subject
G=NULL
for (i in 1:2) {
  inits = inits_orig
    theta_orig=theta
  inits_orig=as.list(inits)
  EMRA=c(0,0.9)
  Cases=c("Normal","MRA")
  theta$E_esax=EMRA[i]
  #Run for 8 Weeks
  times=seq(0,8*7*24*60,24*60)
  ev=eventTable(time.units = 'minutes')
  ev$add.sampling(times)
  G <- cvrsim$run(theta, ev, inits=inits)
  G = data.frame(G)
  G$Case = Cases[i]
  eval(parse(text= paste0("G", i, " = G")))
}
G_t = rbind(G1, G2)

######plots (healthy

h1 = ggplot(G_t,aes(x=time/(7*24*60), y = plasma_K*1000, color = Case)) +
geom_path()+xlab("Time(Weeks)")+ylab("Plasma K (mEq/l)")+theme_bw()+ theme(axis.text.x
= element_text(face = "bold"),
  axis.title.x = element_text(face = "bold"))+ theme(axis.text.y = element_text(face = "bold"),
```

```
axis.title.y = element_text(face = "bold"))+scale_colour_manual("",
breaks = c(G_t$Case[1],G2$Case),

values = c("Blue","Red"))+theme(axis.text.x = element_text(face = "bold"),

axis.title.x = element_text(face = "bold"))+ theme(axis.text.y = element_text(face = "bold"),

axis.title.y = element_text(face = "bold"))+theme(legend.key.size = unit(7,
"lines"))+theme(strip.text.y = element_text(size = 16),

strip.text.x = element_text(size = 16))+theme(strip.text.y = element_text(face = "bold"),

strip.text.x = element_text(face = "bold"))+

  theme(axis.text.x = element_text(size = 15,face = "bold"),

      axis.title.x = element_text(size = 15,face = "bold"))+ theme(axis.text.y = element_text(size
= 15,face = "bold"),

                                                  axis.title.y = element_text(size = 15,face =
"bold"))+geom_line(size=1.25)

h2 = ggplot(G_t,aes(x=time/(7*24*60), y = blood_volume_L, color = Case)) +
geom_path()+xlab("Time(Weeks)")+ylab("Blood volume (L)")+theme_bw()+ theme(axis.text.x
= element_text(face = "bold"),

axis.title.x = element_text(face = "bold"))+ theme(axis.text.y = element_text(face = "bold"),
axis.title.y = element_text(face = "bold"))+scale_colour_manual("",

breaks = c(G_t$Case[1],G2$Case),

values = c("Blue","Red"))+scale_colour_manual("",

breaks = c(G_t$Case[1],G2$Case),

values = c("Blue","Red"))+theme(axis.text.x = element_text(face = "bold"),

axis.title.x = element_text(face = "bold"))+ theme(axis.text.y = element_text(face = "bold"),

axis.title.y = element_text(face = "bold"))+theme(legend.key.size = unit(7,
```

```
"lines"))+theme(strip.text.y = element_text(size = 16),
strip.text.x = element_text(size = 16))+theme(strip.text.y = element_text(face = "bold"),


strip.text.x = element_text(face = "bold"))+

  theme(axis.text.x = element_text(size = 15,face = "bold"),

      axis.title.x = element_text(size = 15,face = "bold"))+ theme(axis.text.y = element_text(size
= 15,face = "bold"),

                                                  axis.title.y = element_text(size = 15,face =
"bold"))+geom_line(size=1.25)


h3 = ggplot(G_t,aes(x=time/(7*24*60), y = K, color = Case)) +
geom_path()+xlab("Time(Weeks)")+ylab("K in blood (mEq)")+theme_bw()+ theme(axis.text.x
= element_text(face = "bold"),

axis.title.x = element_text(face = "bold"))+ theme(axis.text.y = element_text(face = "bold"),


axis.title.y = element_text(face = "bold"))+scale_colour_manual("",


breaks = c(G_t$Case[1],G2$Case),


values = c("Blue","Red"))+theme(axis.text.x = element_text(face = "bold"),


axis.title.x = element_text(face = "bold"))+ theme(axis.text.y = element_text(face = "bold"),


axis.title.y = element_text(face = "bold"))+theme(legend.key.size = unit(7,
"lines"))+theme(strip.text.y = element_text(size = 16),
strip.text.x = element_text(size = 16))+theme(strip.text.y = element_text(face = "bold"),
strip.text.x = element_text(face = "bold"))+

  theme(axis.text.x = element_text(size = 15,face = "bold"),

      axis.title.x = element_text(size = 15,face = "bold"))+ theme(axis.text.y = element_text(size
= 15,face = "bold"),

                                                  axis.title.y = element_text(size = 15,face =
"bold"))+geom_line(size=1.25)

ggarrange(h3,h2,h1, nrow=1, ncol=3,common.legend = TRUE, labels = c("A","B","C"))


####################################
```

```
####MRA administration effect on  a diabetic patient
##########################################
# Diabetic #
theta = theta_orig
inits = inits_orig
theta$glucose_concentration = 8.6  #Hba1c = 7
thetaDB = theta
h <-data.frame(cvrsim$run(theta, ev, inits))
initsDB = as.list(h[dim(h)[1], names(h) %in% names(inits)])
G=NULL
for (i in 1:2) {
  inits = initsDB#x[dim(x)[1], names(x) %in% names(inits)]
  theta = thetaDB
  EMRA=c(0,0.9)
  Cases=c("Normal","MRA")
  theta$E_esax=EMRA[i]
  #Run for a Month
  times=seq(0,8*7*24*60,4*60)
  ev=eventTable(time.units = 'minutes')
  ev$add.sampling(times)

  G <- cvrsim$run(theta, ev, inits=inits)
  G = data.frame(G)
  G$Case = Cases[i]
  eval(parse(text= paste0("G", i, " = G")))
}
G_t$VP = "Healthy"
DB = rbind(G1, G2)
```

```
DB$VP = "Diabetic"
dat = rbind(G_t, DB)
##########################################MRA effect on CKD patient
# Low GFR #
theta = theta_orig
inits = inits_orig
inits$disease_effect_losing_glomeruli = 0.65 #lowering the GFR
inits$disease_effect_on_nephrons = 0.65
inits$disease_effects_decreasing_Kf = 0.5
thetaCKD1 = theta
ev = eventTable()
ev$add.sampling(seq(0,24*60*365, by = 2000))
h <-data.frame(cvrsim$run(theta, ev, inits))
initsCKD1 = as.list(h[dim(h)[1], names(h) %in% names(inits)])
G=NULL
for (i in 1:2) {
  inits = initsCKD1#x[dim(x)[1], names(x) %in% names(inits)]
  theta = thetaCKD1
  EMRA=c(0,0.9)
  Cases=c("Normal","MRA")
  theta$E_esax=EMRA[i]
  #Run for 8 Weeks
  times=seq(0,24*60*7*8,60*4)
  ev=eventTable(time.units = 'minutes')
  ev$add.sampling(times)
    G <- cvrsim$run(theta, ev, inits=inits)
  G = data.frame(G)
  G$Case = Cases[i]
```

```
  eval(parse(text= paste0("G", i, " = G")))


}

G_t$VP = "Healthy"

lowGFR = rbind(G1, G2)

lowGFR$VP = "lowGFR"

dat = rbind(G_t, lowGFR)


##### Now we can run the plots to compare the effects in healthy and CKD patients

h1= ggplot(dat,aes(x=time/(7*24*60), y = plasma_K*1000, color = Case)) +
geom_path()+geom_line(size=1.25) + facet_wrap(~VP)+theme(axis.text.x = element_text(face =
"bold"),axis.title.x = element_text(face = "bold"))+ theme(axis.text.y = element_text(face =
"bold"),
axis.title.y = element_text(face = "bold"))+theme_bw()+xlab("Time(Weeks)")+ylab("Plasma K
(mEq/l)")+theme(axis.text.x = element_text(face = "bold"),
axis.title.x = element_text(face = "bold"))+ theme(axis.text.y = element_text(face = "bold"),
axis.title.y = element_text(face = "bold"))+scale_colour_manual("",
breaks = c(G_t$Case[1],G2$Case),
values = c("Blue","Red"))+labs(color = "Legend Title") +

  theme(legend.key.size = unit(7, "lines"))+theme(strip.text.y = element_text(size = 16),

    strip.text.x = element_text(size = 16))+theme(strip.text.y = element_text(face = "bold"),

  strip.text.x = element_text(face = "bold"))+  theme(axis.text.x = element_text(size = 15,face =
"bold"),

       axis.title.x = element_text(size = 15,face = "bold"))+ theme(axis.text.y = element_text(size
= 15,face = "bold"),

   axis.title.y = element_text(size = 15,face = "bold"))


h2 = ggplot(dat,aes(x=time/(7*24*60), y = blood_volume_L, color = Case)) + geom_path()
+geom_line(size=1.25)+ facet_wrap(~VP)+theme(axis.text.x = element_text(face =
"bold"),axis.title.x = element_text(face = "bold"))+ theme(axis.text.y = element_text(face =
"bold")
axis.title.y = element_text(face = "bold"))+theme_bw()+xlab("Time(Weeks)")+ylab("Blood
volume (L)")+theme(axis.text.x = element_text(face = "bold"),
axis.title.x = element_text(face = "bold"))+ theme(axis.text.y = element_text(face = "bold"),
```

```
axis.title.y = element_text(face = "bold"))+scale_colour_manual("",
breaks = c(G_t$Case[1],G2$Case),
values = c("Blue","Red"))+labs(color = "Legend Title") +

  theme(legend.key.size = unit(7, "lines"))+theme(strip.text.y = element_text(size = 16),

  strip.text.x = element_text(size = 16))+theme(strip.text.y = element_text(face = "bold"),

  strip.text.x = element_text(face = "bold"))+

  theme(axis.text.x = element_text(size = 15,face = "bold"),

      axis.title.x = element_text(size = 15,face = "bold"))+ theme(axis.text.y = element_text(size
= 15,face = "bold"),

  axis.title.y = element_text(size = 15,face = "bold"))

h3 = ggplot(dat,aes(x=time/(7*24*60), y = K, color = Case)) + geom_path()
+geom_line(size=1.25)+ facet_wrap(~VP)+theme(axis.text.x = element_text(face = "bold"),

axis.title.x = element_text(face = "bold"))+ theme(axis.text.y = element_text(face = "bold"),

element_text(face = "bold"))+theme_bw()+xlab("Time(Weeks)")+ylab("K in blood
(mEq)")+theme(axis.text.x = element_text(face = "bold"),
axis.title.x = element_text(face = "bold"))+ theme(axis.text.y = element_text(face = "bold"),
axis.title.y = element_text(face = "bold"))+scale_colour_manual("",
breaks = c(G_t$Case[1],G2$Case),
values = c("Blue","Red"))+labs(color = "Legend Title") +

  theme(legend.key.size = unit(7, "lines"))+theme(strip.text.y = element_text(size = 16),

strip.text.x = element_text(size = 16))+theme(strip.text.y = element_text(face = "bold"),

strip.text.x = element_text(face = "bold"))+

  theme(axis.text.x = element_text(size = 15,face = "bold"),

      axis.title.x = element_text(size = 15,face = "bold"))+ theme(axis.text.y = element_text(size
= 15,face = "bold"),

axis.title.y = element_text(size = 15,face = "bold"))

ggarrange(h3,h2,h1, nrow=1, ncol=3,common.legend = TRUE, labels = c("A","B","C"))
```

######Simulating the Drug effects (specific or in combination) SGLT2i & MRA on a healthy case

```
G=NULL
```

```
for (i in 1:4) {
  inits = inits_orig
    theta_orig=theta
  inits_orig=as.list(inits)
  SGLT2in=c(1,0.22,1,0.22)
  EMRA=c(0,0,0.9,0.9)
  Cases=c("Normal","SGLT2i","MRA","SGLT2i&MRA")
  theta$SGLT2_inhibition=SGLT2in[i]
  theta$E_esax=EMRA[i]
    #Run for 8 Weeks
  times=seq(0,8*7*24*60,4*60)
  ev=eventTable(time.units = 'minutes')
  ev$add.sampling(times)
  G <- cvrsim$run(theta, ev, inits=inits)
  G = data.frame(G)
  G$Case = Cases[i]
  eval(parse(text= paste0("G", i, " = G")))
}


G_t = rbind(G1, G2,G3,G4)

####plots for a healthy case affected by different drug administration

h1 = ggplot(G_t,aes(x=time/(7*24*60), y = plasma_K*1000, color = Case))+geom_path()
+geom_line(size=1.25)+xlab("Time(Weeks)")+ylab("Plasma K (mEq/l)")+theme_bw()+
theme(axis.text.x = element_text(size = 15,face = "bold"),axis.title.x = element_text(size =
15,face = "bold"))+ theme(axis.text.y = element_text(size = 15,face = "bold"),
axis.title.y = element_text(size = 15,face = "bold"))+scale_colour_manual("",
breaks = c(G_t$Case[1],G_t$Case[60*8],G_t$Case[60*8*2],G4$Case),
values = c("Black","green3","Blue","Red"))+ labs(color = "Legend Title") +
  theme(legend.key.size = unit(7, "lines"))

h2 =ggplot(G_t,aes(x=time/(7*24*60), y = blood_volume_L, color = Case)) +
geom_path()+geom_line(size=1.25)+xlab("Time(Weeks)")+ylab("Blood volume
```

```
(L)")+theme_bw()+ theme(axis.text.x = element_text(size = 15,face = "bold"),axis.title.x =
element_text(size = 15,face = "bold"))+ theme(axis.text.y = element_text(size = 15,face =
"bold"),axis.title.y = element_text(size = 15,face = "bold"))+scale_colour_manual("",

breaks = c(G_t$Case[1],G_t$Case[60*8],G_t$Case[60*8*2],G4$Case),
values = c("Black","green3","Blue","Red")) +

  labs(color = "Legend Title") +

theme(legend.key.size = unit(7, "lines"))

h3 =ggplot(G_t,aes(x=time/(7*24*60), y = K, color = Case)) +
geom_path()+geom_line(size=1.25)+xlab("Time(Weeks)")+ylab("K+ in blood
(mEq)")+theme_bw()+ theme(axis.text.x = element_text(size = 11,face = "bold"),axis.title.x =
element_text(size = 11,face = "bold"))+ theme(axis.text.y = element_text(size = 15,face =
"bold"),axis.title.y = element_text(size = 15,face = "bold"))+scale_colour_manual("",
breaks = c(G_t$Case[1],G_t$Case[60*8],G_t$Case[60*8*2],G4$Case)
values = c("Black","green3","Blue","Red"))+

  labs(color = "Legend Title") +

theme(legend.key.size = unit(7, "lines"))

ggarrange(h3,h2,h1, nrow=1, ncol=3,common.legend = TRUE, labels = c("A","B","C"),hjust = -
2,vjust = -0.4)


###############combination of drug effects on a diabetic patient

##########################################333333

# Diabetic #

theta = theta_orig

inits = inits_orig

theta$glucose_concentration = 8.6  #Hba1c = 7

thetaDB = theta

h <-data.frame(cvrsim$run(theta, ev, inits))

initsDB = as.list(h[dim(h)[1], names(h) %in% names(inits)])


G=NULL


for (i in 1:4) {
```

```
  inits = initsDB
  theta = thetaDB
  SGLT2in=c(1,0.22,1,0.22)
  EMRA=c(0,0,0.9,0.9)
  Cases=c("Normal","SGLT2i","MRA","SGLT2i&MRA")
  theta$SGLT2_inhibition=SGLT2in[i]
  theta$E_esax=EMRA[i]
  #Run for 8 Weeks
  times=seq(0,8*7*24*60,4*60)
  ev=eventTable(time.units = 'minutes')
  ev$add.sampling(times)
    G <- cvrsim$run(theta, ev, inits=inits)
  G = data.frame(G)
  G$Case = Cases[i]
  eval(parse(text= paste0("G", i, " = G")))
}
G_t$VP = "Healthy"
DB = rbind(G1, G2,G3,G4)
DB$VP = "Diabetic"

dat = rbind(G_t, DB)
##########################################combination of drug effects on CKD patients
# Low GFR #
theta = theta_orig
inits = inits_orig
inits$disease_effect_losing_glomeruli = 0.65 #lowering the GFR
inits$disease_effect_on_nephrons = 0.65
inits$disease_effects_decreasing_Kf = 0.
```

```
thetaCKD1 = theta
ev = eventTable()
ev$add.sampling(seq(0,24*60*365, by = 2000))
h<-data.frame(cvrsim$run(theta, ev, inits))
initsCKD1 = as.list(h[dim(h)[1], names(h) %in% names(inits)])
G=NULL
for (i in 1:4) {
  inits = initsCKD1#x[dim(x)[1], names(x) %in% names(inits)]
  theta = thetaCKD1
  SGLT2in=c(1,0.22,1,0.22)
  EMRA=c(0,0,0.9,0.9)
  Cases=c("Normal","SGLT2i","MRA","SGLT2i&MRA")
  theta$SGLT2_inhibition=SGLT2in[i]
  theta$E_esax=EMRA[i]
  #Run for 8 Weeks
  times=seq(0,8*7*24*60,4*60)
  ev=eventTable(time.units = 'minutes')
  ev$add.sampling(times)
    G <- cvrsim$run(theta, ev, inits=inits)
  G = data.frame(G)
  G$Case = Cases[i]
  eval(parse(text= paste0("G", i, " = G")))
}

G_t$VP = "Healthy"
lowGFR = rbind(G1, G2,G3,G4)
lowGFR$VP = "lowGFR"
dat = rbind(G_t, lowGFR)
```

```
####effect of drugs on a patient with diabetes and CKD (Both)
# Low GFR & diabetes #
theta = theta_orig
inits = inits_orig
inits$disease_effect_losing_glomeruli = 0.7 #Hba1c = 7
inits$disease_effect_on_nephrons = .7
inits$disease_effects_decreasing_Kf = 0.4
theta$glucose_concentration = 8.6  #Hba1c = 7
thetaDBCKD1 = theta
ev = eventTable()
ev$add.sampling(seq(0,24*60*365, by = 2000))
h <-data.frame(cvrsim$run(theta, ev, inits))
initsDBCKD1 = as.list(h[dim(h)[1], names(h) %in% names(inits)])
G=NULL
for (i in 1:4) {
  inits = initsDBCKD1
  theta = thetaDBCKD1
  SGLT2in=c(1,0.22,1,0.22)
  EMRA=c(0,0,0.9,0.9)
  Cases=c("Normal","SGLT2i","MRA","SGLT2i&MRA")
  theta$SGLT2_inhibition=SGLT2in[i]
  theta$E_esax=EMRA[i]
  #Run for 8 Weeks
  times=seq(0,8*7*24*60,4*60)
  ev=eventTable(time.units = 'minutes')
  ev$add.sampling(times)
  G <- cvrsim$run(theta, ev, inits=inits)
  G = data.frame(G)
```

```
  G$Case = Cases[i]
  eval(parse(text= paste0("G", i, " = G")))

}

G_t$VP = "Healthy"
lowGFR_diabetes = rbind(G1, G2,G3,G4)
lowGFR_diabetes$VP = "lowGFR&diabetes"
dat = rbind(G_t, lowGFR_diabetes)
####### now we can have plots

h1 = ggplot(dat,aes(x=time/(7*24*60), y = plasma_K*1000, color = Case)) +
geom_path()+geom_line(size=1.25) + facet_wrap(~VP)+theme(axis.text.x = element_text(face =
"bold"),axis.title.x = element_text(face = "bold"))+ theme(axis.text.y = element_text(face =
"bold"),axis.title.y = element_text(face =
"bold"))+theme_bw()+xlab("Time(Weeks)")+ylab("Plasma K (mEq/l)")+theme(axis.text.x =
element_text(face = "bold"),axis.title.x = element_text(face = "bold"))+ theme(axis.text.y =
element_text(face = "bold"),
axis.title.y = element_text(face = "bold"))+scale_colour_manual("",
breaks = c(G_t$Case[1],G_t$Case[60*8],G_t$Case[60*8*2],G4$Case), values =
c("Black","green3","Blue","Red"))+labs(color = "Legend Title") +
  theme(legend.key.size = unit(7, "lines"))+theme(strip.text.y = element_text(size = 16),
strip.text.x = element_text(size = 16))+theme(strip.text.y = element_text(face =
"bold"),strip.text.x = element_text(face = "bold"))+
  theme(axis.text.x = element_text(size = 15,face = "bold"),
       axis.title.x = element_text(size = 15,face = "bold"))+ theme(axis.text.y = element_text(size
= 15,face = "bold"),
   axis.title.y = element_text(size = 15,face = "bold"))

h2 = ggplot(dat,aes(x=time/(7*24*60), y = blood_volume_L, color = Case)) + geom_path()
+geom_line(size=1.25)+ facet_wrap(~VP)+theme(axis.text.x = element_text(face =
"bold"),axis.title.x = element_text(face = "bold"))+ theme(axis.text.y = element_text(face =
"bold"), axis.title.y = element_text(face =
"bold"))+theme_bw()+xlab("Time(Weeks)")+ylab("Blood volume (L)")+theme(axis.text.x =
```

```
element_text(face = "bold"),axis.title.x = element_text(face = "bold"))+ theme(axis.text.y =
element_text(face = "bold"),
axis.title.y = element_text(face = "bold"))+scale_colour_manual("",
breaks = c(G_t$Case[1],G_t$Case[60*8],G_t$Case[60*8*2],G4$Case),
values = c("Black","green3","Blue","Red"))+labs(color = "Legend Title") +

  theme(legend.key.size = unit(7, "lines"))+theme(strip.text.y = element_text(size = 16),
strip.text.x = element_text(size = 16))+theme(strip.text.y = element_text(face = "bold"),

  strip.text.x = element_text(face = "bold"))+ theme(axis.text.x = element_text(size = 15,face =
"bold"),

    axis.title.x = element_text(size = 15,face = "bold"))+ theme(axis.text.y = element_text(size =
15,face = "bold"),

axis.title.y = element_text(size = 15,face = "bold"))

h3 = ggplot(dat,aes(x=time/(7*24*60), y = K, color = Case)) + geom_path()
+geom_line(size=1.25)+ facet_wrap(~VP)+theme(axis.text.x = element_text(face =
"bold"),axis.title.x = element_text(face = "bold"))+ theme(axis.text.y = element_text(face =
"bold"),axis.title.y = element_text(face = "bold"))+theme_bw()+xlab("Time(Weeks)")+ylab("K
in blood (mEq)")+theme(axis.text.x = element_text(face = "bold"),axis.title.x =
element_text(face = "bold"))+ theme(axis.text.y = element_text(face = "bold"),
axis.title.y = element_text(face = "bold"))+scale_colour_manual("",
breaks = c(G_t$Case[1],G_t$Case[60*8],G_t$Case[60*8*2],G4$Case),
values = c("Black","green3","Blue","Red"))+labs(color = "Legend Title") +

  theme(legend.key.size = unit(7, "lines"))+theme(strip.text.y = element_text(size = 16),

strip.text.x = element_text(size = 16))+theme(strip.text.y = element_text(face = "bold"),

   strip.text.x = element_text(face = "bold"))+ theme(axis.text.x = element_text(size = 15,face =
"bold"),

  axis.title.x = element_text(size = 15,face = "bold"))+ theme(axis.text.y = element_text(size =
15,face = "bold"),
axis.title.y = element_text(size = 15,face = "bold"))

ggarrange(h3,h2,h1, nrow=1, ncol=3,common.legend = TRUE, labels = c("A","B","C"))
```

D-1. Renin angiotensin aldosterone system (RAAS) [54]

Renin is released at a basic pace of $SEC_{ren,0}$, which is influenced by the movement of sodium in the macula densa, and also by a potent negative feedback that is caused by the binding of Angiotensin to the AT1 receptor.

$$SEC_{renin} = \mu_{md-renin} * \mu_{AT1} * SEC_{renin,0} \quad \text{Eq. 1-D}$$

The macula densa responds to a decrease in sodium flow by signaling for the secretion of renin.

$$\mu_{md-renin} = e^{-A_{md-ren}(\phi_{Na,md} - \phi_{Na,md,0})} \quad \text{Eq. 2-D}$$

The inhibitory effect of AT1-bound AngII on renin secretion is described by the following equations:

$$\mu_{AT1} = 10^{A_{AT1,ren} * \log 10(AT1-bound\_AngII - AT1-bound\_AngII,0)} \quad \text{Eq. 3-D}$$

Also, plasma renin concentration (PRC) is given by:

$$\frac{d(PRC)}{dt} = SEC_{renin} - K_{d,renin} * PRC \quad \text{Eq. 4-D}$$

$K_{d,renin}$ is the renin degradation rate. Angiotensin I is produced by the activity of PRA, which is the step that determines the rate. Angiotensin I is then transformed into angiotensin II through the actions of the enzymes ACE and chymase, while its degradation occurs at a rate represented by $K_{d,AngI}$.

$$\frac{d(AngI)}{dt} = PRA - (ACE + Chymase) * AngI - K_{d,AngI} AngI \quad \text{Eq. 5-D}$$

Angiotensin II is formed from the action of ACE and chymase on AngI, can be eliminated by binding to either the AT1 or AT2 receptors at the rate C_{AT1} and C_{AT2} respective, and is degraded at a rate of $K_{d,AngII}$.

$$\frac{d(AngII)}{dt} = (ACE + Chymase) * AngI - (C_{AT1} + C_{AT2}) * AngII - K_{d,AngII} AngII \quad \text{Eq. 6-D}$$

The complex of Angiotensin II bound to the AT1 receptor is the physiologically active entity within the pathway, and is given by:

$$\frac{d\left(AT1_{bound_{AngII}}\right)}{dt} = (C_{AT1}) * AngII - K_{d,AT1}AT1\_bound\_AngII \qquad \text{Eq. 7-D}$$

When AngII is bound to AT1, it produces various physiological effects. These include constriction of the efferent and preglomerular afferent arterioles, as well as the systemic vasculature, retention of sodium in the PT, and secretion of aldosterone. Each of these effects can be represented by the following relationship.

$$\mu_{AT1,i} = 1 + S_{AT1,i} * \left(\frac{1}{1+\exp\left(\frac{\text{AT1-bound}_{\text{AngII}_0} - \text{AT1-bound\_AngII}}{m_{AT1,i}}\right)} - 0.5 \right) \qquad \text{Eq. 8-D}$$

i represents the impact on efferent, afferent, preafferent, or systemic resistance, PT sodium reabsorption, or aldosterone secretion.

The RAAS pathway involves the activity of aldosterone as the second physiologically active agent, which acts by attaching to MR present in the CNT/CD and DCT regions to encourage the reabsorption of sodium. The concentration of aldosterone bound to MR is represented by the nominal concentration $Aldo_0$, which is affected by the presence of AT1-bound AngII and the normalized availability of MR receptors (which is 1 in the absence of an MR antagonist)

$$MR - bound\_Aldo = Aldo_0 * \mu_{AT1} * MR \qquad \text{Eq. 9-D}$$

The impacts of MR-bound aldosterone on CNT/CD and DCT sodium reabsorption are described as:

$$\mu_{aldo,i} = 1 + S_{aldo,i} * \left(\frac{1}{1+exp\left(\frac{MR-bound\ Aldo_0 - MR-bound\ Aldo}{m_{aldo,i}}\right)} - 0.5 \right) \qquad \text{Eq. 10-D}$$

i is the CNT/CD or DCT.

E-1. Sobol sensitivity analysis for plasma potassium and aldosterone

Perturbation: Potassium infusion (0.1 mEq/min)

Output Response: Plasma potassium and plasma aldosterone

Procedure:

- The Sobol method can be used for both global sensitivity analysis (when all inputs are varied simultaneously) and local sensitivity analysis (when only one input is varied at a time).
- Sensitivity Indices calculated:
    - First-order indices, which measure the main effect of each input variable on the model output
    - Total-order indices, which measure the total effect of an input variable, including its interactions with other variables.

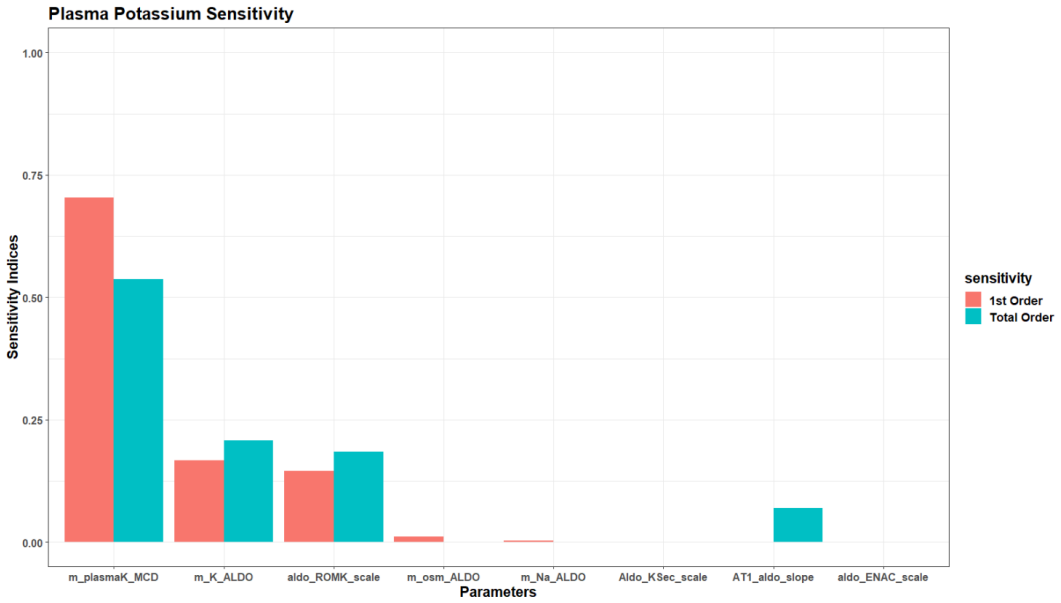

Fig E.1. Sensitivity results for plasma K^+ indicate that the output is most sensitive to parameters related to the effect of plasma K^+ on the K^+ reabsorption in MCD and the effect of plasma K^+ on plasma ALDO levels.

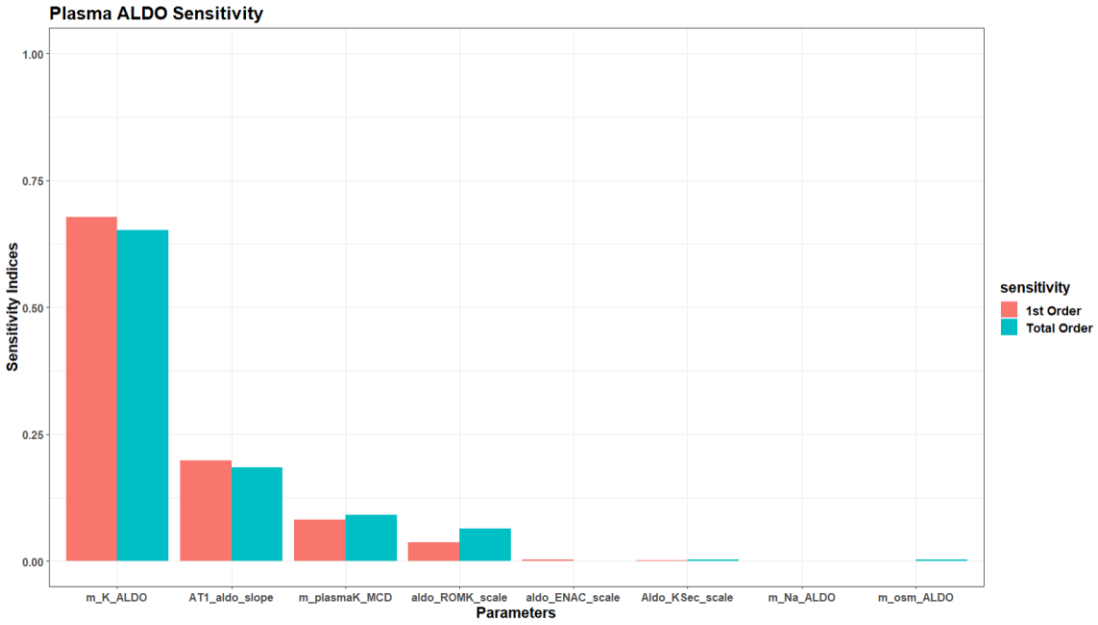

Fig E.2. Sensitivity results for plasma ALDO indicate that the output is most sensitive to parameters related to the effect of plasma K^+ on plasma ALDO levels and angiotensin effect on ALDO.

E-2. Sobol sensitivity analysis codes

```
library(randtoolbox)
library(MESS)
library(sensobol)
library(RxODE)
library(foreach)
library(doParallel)
library(tidyverse)
library(ggh4x)
library(ggpubr)
################## Load model, parameters, and initial conditions

#Load model
source("modelfile.R")
#calibrated parameters
theta$Aldo_KSec_scale =0.01042546*100
theta$m_plasmaK_MCD = 2.06075080*1e-7
theta$aldo_ROMK_scale = 0.84620333
theta$m_osm_ALDO = 0.30819925
theta$m_K_ALDO = 14.03830959*100
theta$E_MAX_spiro = 1
theta$EC50_spiro = 0.57593467*10
theta$AT1_NCC_scale = 0.33904493
theta$md_renin_tau = 6.48999762
theta$Q_K_intracellular = 0.19164617
theta$aldo_ENAC_scale  = 0.15190523
theta$AT1_NKCC_scale = 0.00100000
#theta$Kin=0.12
```

```
theta$AT1_aldo_slope =0.04340511
theta$Q_Na = 17.49701249
theta$m_Na_ALDO=0.0001*10
theta$C_aldo_on_ENAC=0.34352298
#Load other parameters
source("calcNomParams.R")
eta = theta
eta["m_K_ALDO"] = theta$m_K_ALDO#theta["m_K_ALDO"]
eta["m_plasmaK_MCD"] = theta$m_plasmaK_MCD
eta["Aldo_KSec_scale"] =theta$Aldo_KSec_scale#theta["Aldo_KSec_scale"]
eta["aldo_ENAC_scale"]=theta$aldo_ENAC_scale
eta["m_Na_ALDO"] =theta$m_Na_ALDO#theta["m_Na_ALDO"]
eta["m_osm_ALDO"]=theta$m_osm_ALDO
eta["aldo_ROMK_scale"]=theta$aldo_ROMK_scale
eta["AT1_aldo_slope"]=theta$AT1_aldo_slope
inits = x[dim(x)[1], names(x) %in% names(inits)]

#########################################################################3333
#Sobol Sensitivity
#Define Output of interest that you are trying determine sensitivity
#Define list of parameters that may influence output of interest
#Generate sobol sequence
N = 2^10
source("pars_test1.R") #This file as minimum and maximum parameter values calculated
parsmax1=do.call("rbind", replicate(N*(length(names(parsmax))+2), parsmax, simplify = FALSE))
parsmin1=do.call("rbind", replicate(N*(length(names(parsmax))+2), parsmin, simplify = FALSE))
parsmax1=data.frame(parsmax1)
parsmin1=data.frame(parsmin1)
mat <- sobol_matrices(N = N, params = names(parsmax))
```

```
mat=data.frame(mat)
pars = (parsmax1-parsmin1)*mat + parsmin1
outdf = NULL
ev = eventTable()
ev$add.sampling(seq(0,60,by=1))
outdf = NULL
parsdf = data.frame(pars)  #make dataframe
for (i in 1:nrow(pars)) {
 tryCatch({
  print(i)
  thispars = parsdf[i,]
    theta[intersect(names(theta), names(thispars))] = thispars[intersect(names(theta),
names(thispars))]
  theta$Kinfusion = 0.1
  x = data.frame(cvrsim$run(theta, ev, inits))
  x = tail(x,n=1)
    outdf = rbind(outdf, x)
 },
 error =  function(err) {
  return(NULL)
 })
 }
pars = data.frame(pars)
outdf=data.frame(outdf)
outdfkeep = outdf
plot_scatter(data = mat, N = N, Y = outdf$plasma_K, params = names(pars), method = "bin")

plot_scatter(data = pars, N = N, Y = outdf$Aldo, params = names(pars))
plot_multiscatter(data = pars, N = N, Y = outdf$objval, params = names(pars)) + theme_bw()+
```

```
  xlab("test")# + scale_color_continuous(type = "viridis")

################## Calculate Indices ######################

#Calculate indices

indEDP <- sobol_indices(Y = outdf$plasma_K, N = N, params = names(pars), boot = TRUE, R = 100)

indEDP <- sobol_indices(Y = outdf$Na_concentration, N = N, params = names(pars), boot = TRUE, R =
100)

indEDP <- sobol_indices(Y = outdf$Aldo, N = N, params = names(pars), boot = TRUE, R = 100)

indEDP <- sobol_indices(Y = outdf$mean_arterial_pressure_MAP, N = N, params = names(pars), boot =
TRUE, R = 100)

indEDP <- sobol_indices(Y = outdf$GFR_ml_min, N = N, params = names(pars), boot = TRUE, R = 100)

resultsEDP = indEDP$results

resultsEDP = resultsEDP[order(original, decreasing = T),]

Si = resultsEDP[resultsEDP$sensitivity == "Si",]

resultsEDP$parameters = factor(resultsEDP$parameters, levels = Si$parameters)

resultsEDP$original[resultsEDP$sensitivity == "Si"] = resultsEDP$original[resultsEDP$sensitivity ==
"Si"]/sum(resultsEDP$original[resultsEDP$sensitivity == "Si"])

resultsEDP$std.error[resultsEDP$sensitivity == "Si"] = resultsEDP$std.error[resultsEDP$sensitivity ==
"Si"]/sum(resultsEDP$original[resultsEDP$sensitivity == "Si"])

resultsEDP$original[resultsEDP$sensitivity == "Ti"] = resultsEDP$original[resultsEDP$sensitivity ==
"Ti"]/sum(resultsEDP$original[resultsEDP$sensitivity == "Ti"])

resultsEDP$std.error[resultsEDP$sensitivity == "Ti"] = resultsEDP$std.error[resultsEDP$sensitivity ==
"Ti"]/sum(resultsEDP$original[resultsEDP$sensitivity == "Ti"])

plotIndicesEDP = ggplot(resultsEDP) + geom_bar(aes(x=parameters, y = original, fill = sensitivity),stat =
"identity",  position=position_dodge()) +

  theme(plot.title = element_text(hjust = 0.5),

        legend.title = element_blank()) +

  ylab("Sensitivity Indices") +

  scale_fill_discrete(labels = c("1st Order","Total Order"))+ylim(0,1)+

  theme_bw()+ggtitle("Plasma Potassium Sensitivity")+

  xlab("Parameters")+ylab("Sensitivity Indices")+theme(legend.title = element_text(size = 15, face =
"bold"),

                                        legend.text = element_text(size = 12, face = "bold"),
```

```
                                axis.title = element_text(size = 15, face = "bold"),
                                axis.text = element_text(size = 11, face = "bold"),
                                title =element_text(size = 15, face = "bold") )
############ minimum and maximum parameter values calculated
parsmin = c(
  m_K_ALDO=600,
  m_osm_ALDO = 0.1,
  Aldo_KSec_scale = 0,
  m_plasmaK_MCD = 0,
  aldo_ROMK_scale = 0.0001,
  aldo_ENAC_scale =0.0001,
  AT1_aldo_slope =0.001,
  m_Na_ALDO=0
)
parsmax = c(m_K_ALDO=3000,#1500,#15,
          m_osm_ALDO = .8,
          Aldo_KSec_scale = 9,
          m_plasmaK_MCD = 5e-7,
          aldo_ROMK_scale = 4,
          aldo_ENAC_scale =0.2,
          AT1_aldo_slope =1,
          m_Na_ALDO=0.2
)
pars = names(parsmin)
```