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Tissue Sodium in CKD Patients

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A thesis submitted in partial fulfillment of the requirements for the Master of Science degree in Medical Biophysics

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Abstract

Introduction: Sodium hemostasis is altered in patients with chronic kidney disease (CKD) due to long term loading, and sodium (^{23}Na) can be deposited in the skin, muscle and skeleton. We measured ^{23}Na content in these tissues of CKD patients, using ^{23}Na magnetic resonance (MR) imaging.

Methods: This is a pilot cross-sectional study of CKD stage 4-5D patients and controls with normal kidney function. Subjects underwent a ^{23}Na MR study of their right lower leg using a multinuclear-capable 3.0-T MRI. An axial proton T1-weighted fast-low-angle-shot sequence was acquired to delineate the anatomy; followed by a ^{23}Na MR image obtained with a custom-made ^{23}Na coil and sodium-optimized pulse sequences. Concentration maps were generated using saline solutions at different ^{23}Na concentrations as calibration vials. Four regions of interest (ROIs) were drawn: 1) pre-tibial skin, 2) posterior leg skin, 3) tibia, and 4) soleus muscle. Baseline characteristics and blood samples were also collected.

Results: 10 controls, 12 CKD and 23 dialysis (10 peritoneal dialysis (PD) and 13 hemodialysis (HD)) subjects participated in the study. ^{23}Na concentration in all four tissues was significantly higher in dialysis patients compared to controls (p -value <0.05). No significant difference was found in the tissue ^{23}Na of HD, PD and CKD groups. Tissue sodium levels correlated strongly with markers of mineral bone disease and inflammation. Associated factors appear to be tissue specific.

Conclusion: Dialysis patients were found to have significantly higher ^{23}Na level in their tissues compared to controls with normal kidney function. The effect and clinical utility of tissue ^{23}Na remains to be studied.

Keywords

Sodium MRI, Non-osmotic Sodium, Tissue sodium concentration, Chronic Kidney Disease (CKD), Dialysis, Hemodialysis, Peritoneal dialysis

Lay Summary

Introduction: Introduction: When the kidney malfunctions, individuals cannot excrete sodium. This results in the deposition of sodium in the skin, muscle and bones, especially in patients who require dialysis because of minimal kidney function. We used magnetic resonance technology to study the amount of sodium in these tissues of patients with kidney disease.

Methods: We studied 10 healthy individuals, 12 patients with kidney disease not on dialysis and 23 dialysis patients (13 on hemodialysis and 10 on peritoneal dialysis - the two most common types of dialysis). Each subject was brought in for a study visit, where we collected their medical history and blood samples, and obtained a cross-sectional image of their R leg. The image was taken with a modified magnetic resonance scanner which detects sodium content. We had vials with known sodium concentration included in the image, to calibrate the level of sodium in the different regions. Four regions of interest were drawn over the image: over the soleus muscle, the bone, and two areas of the skin (the skin over the shin and from the back of the calf) - to highlight the different tissues.

Results: Sodium levels in all four areas (three tissues) were higher in dialysis patients compared to healthy individuals. We did not find a difference between patients on peritoneal dialysis, hemodialysis, or not on dialysis yet. Higher sodium levels in the muscle and bone were associated with increased inflammation, as well as other markers of kidney disease. The sodium levels in the different tissues were associated with different factors.

Conclusions: Dialysis patients have higher sodium levels in their skin, muscle and bone, compared to healthy individuals. The mechanism of sodium storage and its effect on individuals needs to be studied further.

Co-Authorship Statement (where applicable)

Material from this thesis has been/will be submitted for publication and has been presented at the American Society of Nephrology – Kidney Week, San Diego, USA in October 2018. Chapter 2 contains a review paper, co-authored with Christopher W. McIntyre, which will be adapted and shortly submitted for publication. Chapter 3 consists of a manuscript. The data from this manuscript was re-analyzed and has been successfully published in *Nephrology Dialysis Transplant* (April 6, 2020; epub ahead of print): “*Tissue Sodium Concentration in Chronic Kidney Disease and Dialysis Patients by Lower Leg Sodium-23 Magnetic Resonance Imaging*”; with Fabio R Salerno, Alireza Akbari, Lisa Hur, Jarrin Penny, Timothy J. Scholl and Christopher W. McIntyre as co-authors.

The initial study was designed by Elena Qirjazi, Timothy J. Scholl and Christopher W. McIntyre. Elena Qirjazi was responsible for drafting a research proposal and applying for the institutional Research Ethics Board approval. Alireza Akbari was responsible for the custom-made 12-rung, 18-cm-diameter sodium birdcage radiofrequency coil, and the density-adapted projection reconstruction pulse sequence optimized for sodium acquisition. Elena Qirjazi helped with subject recruitment, and in collaboration with Alireza Akbari, was responsible for carrying out the study sessions and data acquisition. Alireza Akbari also developed sodium concentration maps of the MRI images acquired, mentored the image analysis process and contributed to the methods section.

For the above mentioned published article, Lisa Hur analyzed the images and contributed to the statistical analysis. Jarrin Penny also contributed to image analysis. Elena Qirjazi and Fabio R Salerno contributed equally to the writing the final manuscript and performing the statistical analysis

For the purpose of the following thesis, Elena Qirjazi was responsible for drawing the regions of interest and acquiring sodium concentrations in the tissues. Furthermore, she was responsible for the statistical analyzing of the data, data interpretation, and clinical significance.

Dr. Timothy J. Scholl provided mentoring and technical insight for the sodium MRI development and image acquisition. Dr. Christopher W. McIntyre conceived the original study idea, was medically responsible for the study, provided funding, and supervised the full data acquisition and statistical analysis, and approved the manuscripts.

*To my sister Eftila... I don't tell you often (maybe ever...), but
you are quite the role model!*

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List of Abbreviations

- ^1H – Hydrogen
 ^{13}C – Carbon
 ^{15}N – Nitrogen
 ^{17}O – Oxygen
 ^{19}F – Fluorine
 ^{22}Na – Sodium Radio isotope
 ^{23}Na – Sodium
 ^{31}P – Phosphorus
 α - Flip angle
 γ – Gyromagnetic ratio
 μ – Magnetic dipole moment
 ω_0 - Larmor frequency
ACEI – Angiotensin converting enzyme inhibitor
ADH – Antidiuretic Hormone
ALP – Alkaline phosphatase
ANP – Atrial Natriuretic Peptide
ARB – Angiotensin II receptor blocker
 \vec{B}_0 - External magnetic field
 \vec{B}_1 – External time-varying magnetic field generated by radiofrequency pulse
Beta coeff. – standardized coefficient
BMI – Body mass index
BNP – Brain Natriuretic Peptide
CAD – Coronary Artery Disease
CHF – Congestive Heart Failure
CI – Confidence Intervals
CKD – Chronic Kidney Disease
CKD 5D – Chronic Kidney Disease Stage 5 on Dialysis
COPD – Chronic Obstructive Pulmonary Disease
Corr. Coeff. – Correlation coefficient
CRP – C-Reactive Protein

ECF – Extracellular Fluid
eGFR- estimated Glomerular Filtration Rate
ENaC- Epithelial Sodium Channel
ESA – Erythropoietin stimulating agent
 $G(t)$ – Gradient applied over time
GFR - Glomerular Filtration Rate
hsTroponin T – High sensitivity troponin T
 I – nuclear spin
ICF – Intracellular Fluid
INR – International normalized ratio
ISF – Interstitial Fluid
HD – Hemodialysis
KDIGO – Kidney Disease Improving Global Outcomes
 \vec{M}_0 - Magnetization of protons in the external magnetic field B_0
 \vec{M}_t - Transverse magnetization
 \vec{M}_z – Longitudinal magnetization
MBD – Mineral Bone Disease
MR – Magnetic Resonance
MRI – Magnetic Resonance Imaging
Na-K-ATPase – Sodium Potassium ATPase
NEX – Number of excitations
NFAT5 – another name for Tonicity-Responsive Enhancer-Binding Protein
NO-Na – Non-osmotic sodium
O-Na – Osmotic sodium
PD – Peritoneal Dialysis
PF – Plasma Fluid
PTH – Parathyroid Hormone
RF – Radiofrequency
SD- standard deviation
SNR – Signal to Noise Ratio
SNS – Sympathetic Nervous System
 T_1 – Time constant for longitudinal recovery of the magnetic signal

T_2 – Time constant for transverse decay of the magnetic signal

T_2^* - Effective relaxation time T_2

TBW – Total Body Water

TE – Echo Delay Time

TONEBP – Tonicity-Responsive Enhancer-Binding Protein

TR – Repetition Time

VEGF-C – Vascular Endothelial Growth Factor C

Chapter 1

1 Background

This chapter provides some background information about the work described in the following chapters. This includes descriptions of the Kidney (in Health and Disease) (Section 1.1), Current Knowledge of Sodium Metabolism (Section 1.2), ^1H Magnetic Resonance Imaging (Section 1.3), and ^{23}Na Magnetic Resonance Imaging (Section 1.4).

1.1 The Kidney – in Health and Disease

The kidney is a vital organ with several key functionalities. This section highlights the anatomy and physiological function of the kidney, as well as definitions of CKD and renal replacement therapies. A further in-depth description of the kidney and its role can be found in multiple textbooks. [1-5]

1.1.1 Kidney Anatomy

The kidneys are bean shaped organs, weighting on average about 150 g each, and located in the retroperitoneum. In total, they receive approximately 20% of the cardiac output, which is distributed through the ~2 millions of nephrons – the functional units of the kidneys (Fig. 1-1). [1-5] The nephron is composed of two main parts: the glomerulus and the tubules, and is surrounded by a set of arterioles, capillaries and venules. Blood is initially filtered through the glomerulus, producing ultra-filtrate - an acellular aqueous solution with plasma-like contents (except for macromolecules). The contents of this ultra-filtrate are then modified by the absorption and secretory function of the different parts of the tubule to produce the end-result: urine.

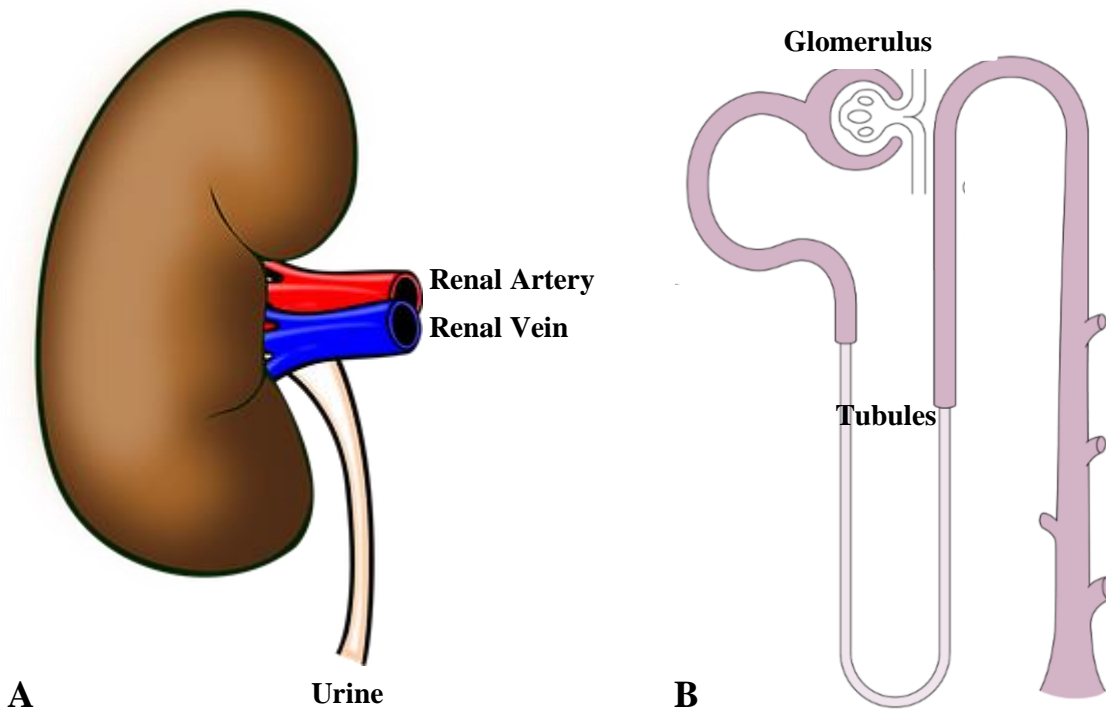


Figure 1-1: A) The kidney with its renal artery and vein, and ureter for urine output; B) The nephron – the functional unit of the kidney. It has two main sections: the glomerulus and the tubule. Adapted from [5]

1.1.2 Kidney Function

The functions of the kidney include:

- *Maintaining the body composition of water and electrolytes:* The kidney regulates the composition of the fluid in the body and its osmolytes. Using a series of feedback mechanisms, the amount of water and other electrolytes that the kidneys excrete in the urine are highly adaptable. As such, the kidney plays an important role in regulating the body content of water, sodium, potassium, chloride, bicarbonate, calcium, magnesium and phosphate. This role of the kidney is quite crucial since most cellular enzymatic processes and electrochemical gradients rely on the tight regulation of these ionic concentrations.
- *Excreting metabolic waste products and foreign substances:* A number of end-products and toxins are produced during physiological and pathological processes in the body, which are cleared by the kidneys. This includes urea, creatinine, acids and drugs.

- *Production of hormones:*

- Renin: In response to renal blood flow, the kidneys will release renin – a hormone which catalyzes the formation of Angiotensin II – a potent vasoconstrictor. Angiotensin II not only causes significant changes in peripheral vascular resistance, but also has direct and indirect effects in the tubular reabsorption of sodium and water through its effect on renal vasculature and aldosterone production by the adrenal glands. Through these mechanisms, the kidneys play a key role in blood pressure regulation and hemodynamic stability.
- Erythropoietin: The kidney is the main site for production of erythropoietin – a hormone that is key in stimulating the production of erythrocytes (or red blood cells) in the bone marrow.
- 1,25-Dihydroxyvitamin D: Kidneys are needed to activate vitamin D into its active metabolite 1,25-dihydroxyvitamin D. This active metabolite then helps regulate body calcium and phosphate levels, as well as having feedback roles on parathyroid hormone and bone metabolism.

1.1.3 Chronic Kidney Disease

CKD is defined by the Kidney Disease Improving Global Outcomes (KDIGO) guideline as “abnormalities of kidney structure or function, present for >3months, with implication for health”. [6] Even so, this condition describes a wide range of diseases that can affect the kidney and has significant variations in severity. As such, KDIGO further classifies CKD in stages 1-5 (Table 1-1), based on the glomerular filtration rate (GFR). [6] Patients who are on dialysis are often classified as CKD 5D, to differentiate them from CKD 5 – not on dialysis. GFR is estimated based on serum creatinine (a molecule readily filtered in the glomerulus). [7]

The prevalence of CKD stages 1-5 is approximately 13.4% of the general population, while stages 3-5 is 10.6%. [8] Patients with CKD are at increased risk of morbidity and mortality – particularly cardiovascular mortality. The risk of hospitalization and cardiovascular events progressively increases as GFR declines. [9] Survival rates for dialysis patients at one, two and five years are approximately 81, 65, and 34%, respectively. [10] As such, due to its high prevalence and significant disease burden, CKD is a global health concern. [11]

Table 1-1: Description of the Stages of CKD, from mild (Stage 1) to severe (Stage 5) [6]

Stage	GFR (ml/min/1.73 m²)	Description
Stage 1	>89	Normal or high
Stage 2	60-89	Mildly decreased
Stage 3a	45-59	Mild-moderately decreased
Stage 3b	30-44	Moderate-severely decreased
Stage 4	15-29	Severely decreased
Stage 5	<15	Kidney Failure

1.1.4 Renal Replacement Therapies

When the kidney function has decreased to a level incompatible with a functional life, patients tend to have two main options with regards to renal replacement therapies: dialysis – either hemodialysis or peritoneal dialysis, or kidney transplantation. Furthermore, hemodialysis is sub-categorized into in-center hemodialysis – where patients come to a medical facility three times a week for 3-4 hours to have their treatments, or home hemodialysis – where the patients are trained to do their own treatment in a home setting.

For the purpose of this thesis, the following sections provide information on the two renal replacement therapies relevant to this study: in-center hemodialysis and peritoneal dialysis. More in-depth discussion can be found in the references. [2-4,12-14]

Dialysis is a poor approximation of the kidneys – in that it helps remove particles (for e.g. electrolytes, end products of metabolism, and drugs) and excess water from the body, but its performance falls short of the full functionality of the kidneys. The dialysis apparatus requires three main components: access to blood or fluid compartment in the body, a semi-permeable membrane and dialysate fluid. Particles (or solutes) and water mostly cross the semi-permeable membrane into the dialysate fluid (with few exceptions – e.g. bases like bicarbonate or lactate –

which diffuse into the blood). In general, two different processes occur simultaneously during dialysis: 1) solute clearance and 2) ultrafiltration. Solute clearance refers to the clearance of solutes via diffusion, while ultrafiltration is the removal of fluid – via hydrostatic or osmotic pressures. Ultrafiltration does result in some removal of solutes, since water will often drag solutes via solvent drag across the semi-permeable membrane.

1.1.4.1 In-Center Hemodialysis

Hemodialysis is a process where blood is taken from a patient's vessels, run through a hemodialysis filter (Fig. 1-2) to adjust its composition, before it is returned back to the patient's vasculature. Inside the filter, blood runs counter-parallel to the dialysate fluid, separated through a polymeric membrane. The counter-parallel flow is integrated to ensure maximal solute clearance by maintaining concentration gradients throughout the filter length. Ultrafiltration rates can be adjusted by applying negative hydrostatic pressure to the dialysate compartment of the HD filter.

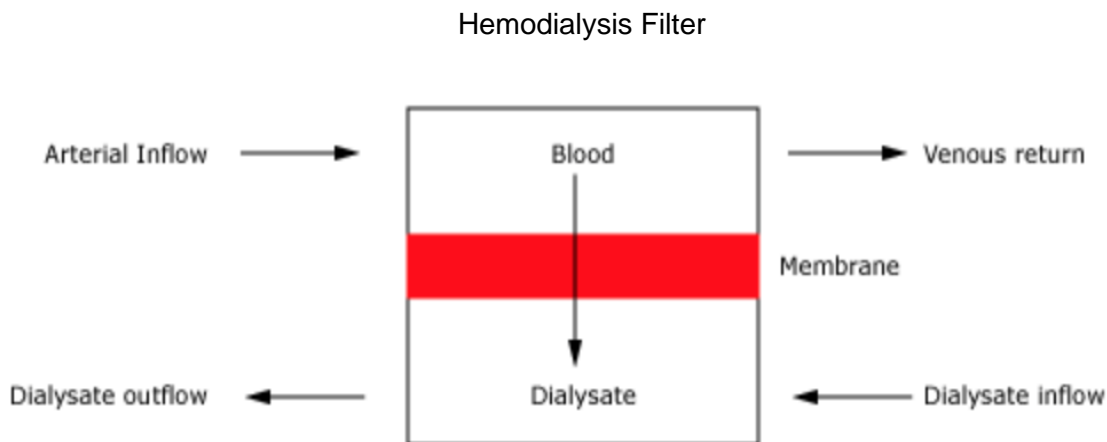


Figure 1-2: Schematic representation of the hemodialysis apparatus: depicting blood and dialysate counter current flows within the hemodialysis filter, the membrane (in red) and the movement of solutes mostly from blood to dialysate. [13]

In-center HD is the outpatient delivery of hemodialysis treatments in hemodialysis units. Patients come to these units usually three times a week, for 3 to 4 hour-long hemodialysis treatments. The HD machine is set up, monitored and adjusted by trained health care

professionals, who also closely observe the patients for complications. Since this is the only dialysis modality that has trained professionals available for the entire treatment, in-center HD patients tend to have lower health status and higher levels of comorbidities compared to those on other dialysis modalities.

1.1.4.2 Peritoneal Dialysis

Peritoneal dialysis involves the transport of solutes and water across the peritoneal membrane, which serves as the semipermeable membrane between the blood in the peritoneal capillaries and the dialysis fluid in the peritoneal cavity. Dialysate solution is infused into the abdomen of patients and allowed to dwell there for a set period of time (varies from 1 to over 16 hours). The transport of solutes and water between the dialysate solution and blood occurs during this dwelling time. For solutes, this transport is dependent on concentration gradients, osmotic and oncotic pressures (since water transports affects solute movements through convection or solvent drag), as well as the pore sizes in the peritoneal membrane (which acts as a filter). Water movement depends on the oncotic and osmotic pressures. Initially these pressures are set by adding dextrose or other polymers to the dialysate fluid, but with time they adapt with the diffusion of solutes (in and out of the peritoneal space). Overall, with longer dwell times the concentration of solutes (like toxins, urea, phosphate etc.) in the dialysate fluid equilibrates with that of blood. Generally, patients undergo PD for at least 8 hours, with anywhere from 1-7 dwells a day. As such, PD prescriptions are highly adjustable.

In contrast to HD, PD tends to be less efficient with respect to solute clearances. Even so, PD does have some advantages. First, it is less expensive than traditional in-center HD. [15-17] Second, PD is associated with better health related quality of life. [18,19] This dialysis is done at home by the patients or their caregivers, allowing patients the freedom, flexibility and control to perform their own treatment regimen and facilitate their return to everyday activities. Lastly, PD has been found to have an early survival advantage compared to in-center HD – attributed to preserved residual renal function. [20,21]

1.1.5 Complications of CKD

CKD results in a number of biochemical abnormalities and complications. [1-4,6,12] These complications become more severe as CKD progresses to later stages (stage 5). Only some of them are treated with dialysis.

- Uremia: As kidney function deteriorates, the ability of the kidney to excrete metabolic end-products and toxins decreases. This results in an accumulation of these compounds – including creatinine and urea (readily measured in plasma in clinical practice) and other unmeasured non-protein nitrogen compounds. Build-up of these toxins past a critical threshold (which is patient-specific) results in a series of non-specific symptoms collectively known as “uremic symptoms”. Uremic symptoms include nausea and vomiting, decrease appetite, pruritus (itch), lethargy, confusion and decreased level of consciousness, and chest pain from uremic pericarditis. The presence of these symptoms indicate the need to initiate renal replacement therapy, otherwise the individual will die as a result of their kidney disease.
- Hyperkalemia: As CKD progresses, the excretion of potassium in the urine is impaired, resulting in hyperkalemia, a dangerous condition which can result in fatal cardiac arrhythmias. Hyperkalemia is treated with two main approaches: 1) Decreased intake through low-potassium diet and potassium-binding resins to reduce potassium absorption in the gastrointestinal tract; 2) Increased excretion through diuretics (to increase excretion from the native kidneys) and renal replacement therapies – the latter as a last resort.
- Acidosis: The kidneys ability to re-absorb and produce bicarbonate is compromised in CKD, resulting in the build-up of acid components produced by metabolism. Treatment of acidosis involves supplementation with sodium bicarbonate or renal replacement therapy.
- Anemia: CKD (usually stages 4-5) often results in anemia. The mechanism through which CKD causes anemia is multifactorial. First, as its function deteriorates, the kidney releases less erythropoietin into the blood stream – a hormone that is needed for erythrocyte production in the bone marrow. Second, absorption of iron (a key element in hemoglobin) from the gastrointestinal tract is diminished in these patients. Third, the increased toxins result in a pro-inflammatory milieu in the bone marrow –

- which diminishes its normal activity. Anemia is often treated in CKD by supplemental iron (oral or intravenous), erythropoietin stimulating agents, and (if needed) dialysis to increase clearance of toxins.
- Mineral Bone Disease (MBD)– As kidney function diminishes, the kidneys are unable to re-absorb calcium, secrete phosphate and activate Vitamin D to 1,25-dihydroxy-Vitamin D. These changes in calcium/phosphate homeostasis result in secondary and eventually tertiary hyperparathyroidism (increased parathyroid hormone- PTH), and osteomalacia and other metabolic bone diseases. Dysregulation of the mineral bone disease markers has also been associated with increased mortality, and vascular disease from extra-skeletal calcification in vessels. Current treatment includes adhering to a low-phosphate diet, phosphate binders to decrease phosphate absorption, calcimimetic agents – to trick the parathyroid gland that the body is hypercalcemic, administration of active vitamin D compounds, and renal replacement therapies. With regards to the latter, aside for transplantation, dialysis is limited in phosphate removal – since the majority of this anion is intracellularly stored, and thus, not readily accessible with dialysis.
 - Water and Sodium Retention, and Blood Pressure: As CKD progresses, the residual renal function decreases, and thus, smaller volumes of urine are excreted. These smaller urine volumes result in less water and sodium excretion, and consequently, increased total body fluid and total body sodium content. Clinically, this manifests as signs and symptoms of volume overload and hypertension. Patients are advised to decrease their sodium and water intake. In addition, attempts are made to increase residual renal function with the use of diuretics, and once medical therapy fails, with renal replacement therapy. Lastly, sometimes paradoxically, as CKD progresses the kidney releases more renin, causing significant vaso-constriction and hypertension through activation of Angiotensin II.
 - Cardiovascular: CKD patients are at increased risk for cardiovascular disease and mortality. [9, 22,23] This risk has been attributed to a higher prevalence of traditional risk factors (e.g. hypertension, diabetes, left ventricular hypertrophy, smoking, sedentary lifestyle, etc.), as well as non-traditional factors like chronic volume overload, anemia, oxidative stress, and MBD. Troponin, a serological marker of

cardiac injury, is often elevated in CKD patients – partially due to its decreased clearance. Even so, chronically elevated troponin levels are associated with worse prognosis in CKD. [24,25]

- Inflammation: CKD and dialysis are also associated with recurrent and chronic inflammatory processes. [26-31] These patients often have low albumin and hemoglobin, and high C-reactive protein (CRP) levels, indicative of inflammation. The etiology of inflammation is believed to be multifactorial including elevated pro-inflammatory cytokines, oxidative stress, increased infections, and the uremic milieu.

1.2 Current Knowledge of Sodium Metabolism

This section provides a brief overview of current teaching on sodium handling by the body. Sodium metabolism has been previously described in multiple other sources. [1-5] The main principle in sodium balance physiology postulates that sodium content is conserved in steady-state:

$$\text{Sodium Intake} = \text{Sodium Excretion} \quad [1.1]$$

Since a large portion of the total sodium content is in aqueous solution, this section also contains a brief explanation of the fluid compartments in the body.

1.2.1 Fluid Compartments

The total body water (TBW) is compartmentalized into intracellular fluid (ICF = ~2/3 of TBW) and extracellular fluid (ECF = ~1/3 of TBW) (Fig. 1-3). These two compartments are separated by the cell membrane. ECF is further divided into interstitial fluid (ISF), which bathes all the cellular components in tissues, and plasma fluid (PF) – separated by the capillary wall. The concentrations of the main cations and anions in the PF and ICF are highlighted in Table 1-2. Since these electrolytes freely permeate the capillary wall, PF concentrations are generally assumed to be representative of ECF content. This assumption is not completely accurate, since the protein composition (specifically albumin) of PF and interstitial fluid are different. Albumin is negatively charged and attracts cations, thus distorting their concentration slightly in its vicinity – a phenomenon called the Gibbs-Donnan effect. Since albumin levels are higher intravascularly, the cation concentration is also a bit higher in the PF compartment.

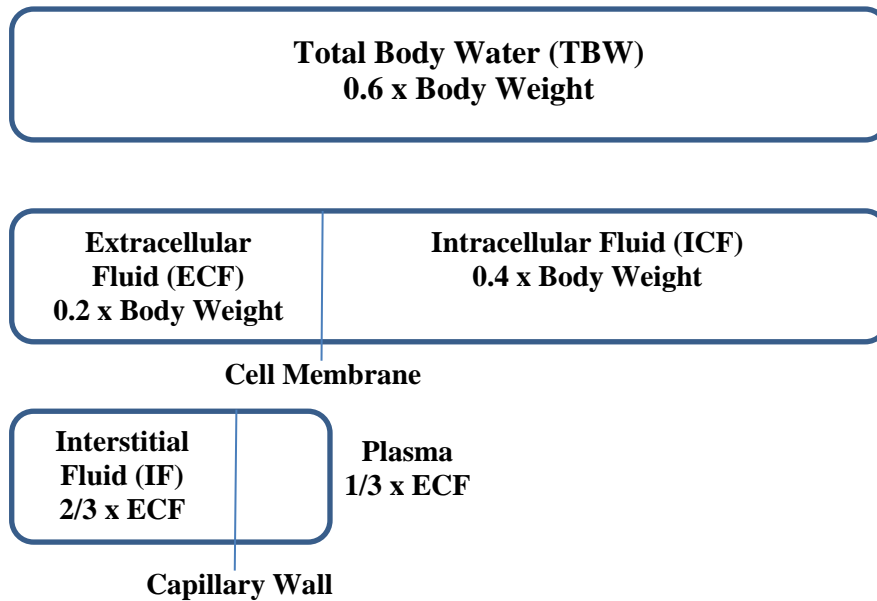


Figure 1-3: Estimates of major body fluid compartments with respect to body weight and the membranes that separate these compartments. Adapted from [5]

Table 1-2: Typical ionic composition of plasma and intracellular fluid (adapted from [1,4])

Constituent	Blood Plasma (mmol/L)	Intracellular fluid (mmol/L)
Sodium (Na ⁺)	143	12
Potassium (K ⁺)	4	150
Chloride (Cl ⁻)	104	4
Bicarbonate (HCO ₃ ⁻)	24	10

1.2.2 Sodium Intake

In industrialized countries, average sodium intake is 100-200 mmol/day (~2-4 grams/day) [29,30]. Since humans can survive and function normally on 10-20 mmol/day, current sodium intake is almost always greater than the amount necessary for homeostasis. Yet, salt appetite is often independent of salt-repletion. However, sodium-deplete states – often perceived as

decreased blood volume, low blood pressure, or decreased extracellular fluid sodium concentration – are significant stimuli for both sodium appetite and water thirst.

1.2.3 Sodium Excretion

The sodium balance equation [1.1] translates a precisely balanced sodium and water intake with their excretion, in order to keep the size of the ECF constant. Sodium loss is dependent on three main mechanisms: losses through sweat and the gastrointestinal tract – which are not regulated, and renal losses which are. Sodium excretion by the kidney is subject to 1) ultrafiltration in the glomerulus, and 2) reabsorption in the tubule. Sodium is not secreted in urine. Since sweat and gastrointestinal losses are minimal (less than 5mmol/day and approximately 10mmol/day, respectively) in regular conditions (room air, regular activity and normal bowel movements), [32] the following explanation on the regulatory mechanism of sodium excretion is focused on renal losses.

Since sodium is the most abundant extracellular cation, it is a key osmolyte regulating the ECF volume, and thus, effective circulatory volume (through PF) and blood pressure. As such, any fluctuations in total body sodium results in concurrent fluctuations in ECF volume. In response, a number of regulatory feedback mechanisms are activated (Fig. 1-4, described in multiple textbooks [1-5]):

- Sympathetic Nervous System (SNS): fluctuations in the ECF volume are readily detected by stretch and baro-receptors in the blood vessels. If ECF volume is low, these receptors will activate the sympathetic nervous system. Increased sympathetic activity increases renal arteriole vasoconstriction – decreasing GFR and ultrafiltration, and enhances renal salt reabsorption in the tubule.
- Renin-Angiotensin-Aldosterone System: Renin release by the kidneys is triggered by low intravascular fluid volumes (detected as decreased tubular flows), baroreceptor stimulation and direct adrenergic activity (sympathetic nervous system). Renin catalyzes the activation of Angiotensin II, a potent vasoconstrictor. Similar to the sympathetic activity, Angiotensin II decreases sodium excretion by affecting both ultrafiltration (by decreasing GFR) and directly enhancing sodium reabsorption in the proximal tubule. In addition, Angiotensin II stimulates the production and release of aldosterone from the adrenal glands. Aldosterone, in turn, acts at the collecting tubule (the most distal part of

the nephron) to increase the activity of epithelial sodium channel, ENaC, to reabsorb more sodium and excrete potassium.

- Urodilatin, Uroguanylin/guanylin, Atrial Natriuretic Peptide (ANP) and Brain Natriuretic Peptide (BNP): Multiple organs have their own hormone peptides to help adjust sodium (and subsequently water) content in the body. ANP and BNP are peptides produced by atrial and ventricular myocytes, respectively, in conditions of increased cardiac stretching – or volume expansion. Urodilatin is produced by the kidneys, and uroguanylin/guanylin are produced by the gastrointestinal tract in response to oral ingestion of sodium. All these peptides induce natriuresis (or excretion of sodium) along the nephron tubule.
- Increased Cardiac Output: Increased cardiac output results in vasodilation of the renal arterioles, increasing GFR and thus ultrafiltration and ultimately loss of sodium.
- Tubulo-glomerular Feedback: Changes in the GFR can be induced in the kidney by local feedback mechanism from the flow rate and content of ultrafiltrate in the tubules. These ultrafiltrate factors result in changes in the Starling forces via hydrostatic and oncotic pressures that are detected by the tubules. Changes in these factors initiate a negative feedback that serves to restore normal Starling forces in the tubule.
- Water Reabsorption and Antidiuretic Hormone (ADH): Since sodium and water balances are quite closely interconnected, water reabsorption in the kidneys also plays a role. Water reabsorption is mostly controlled by osmoreceptors in the brain which detect changes in plasma osmolality. When this osmolality is increased, ADH is produced, a hormone that stimulates free water absorption in the kidney. Furthermore, ADH is stimulated by decreased circulatory volume, with the aim of restoring it. Thus, ADH indirectly affects sodium metabolism, by its effect on the circulatory volume.

Through these mechanisms, sodium excretion by the kidney can fluctuate from virtually zero, to several hundreds of millimoles per day [33]

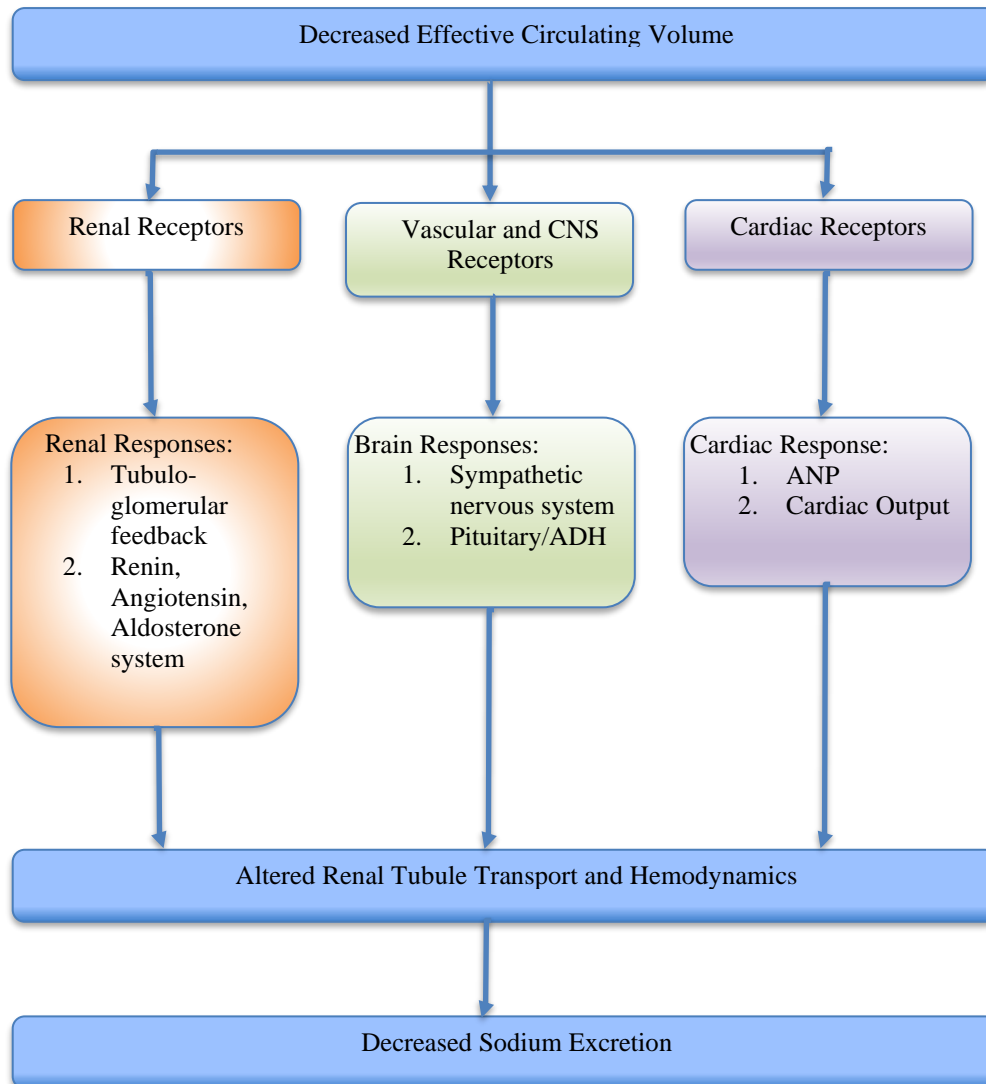


Figure 1-4: Key players in regulating effective circulating volume and sodium excretion by the kidneys. CNS- Central nervous system, ADH – anti-diuretic hormone, ANP – Atrial natriuretic peptide. Adapted from [34]

1.2.4 Sodium in Disease

A high salt diet has been implicated in a number of diseases including hypertension, glucose intolerance, cardiovascular disease, progression of CKD, autoimmune conditions and even cancer. [34-43] Such diets also interfere with treatments – for example high salt diets make anti-hypertensive drugs less effective, and trigger episodes of congestive heart failure (CHF).

Given that sodium excretion is mostly a renal process, it is believed that dysregulation in the kidney function contributes to sodium retention and consequently disease. More specifically, in hypertension, the kidney is believed to have an inherent inclination toward sodium and water retention, which reset the pressure natriuresis (i.e. higher blood pressures are needed to excrete the steady state sodium intake).

In CKD and dialysis patients, sodium excretion is impaired. 24-hour urine collections of CKD patients show decreasing sodium excretion with advancing CKD. [43] This is particularly important for dialysis patients, who often have negligible urine outputs. As such, these patients are at increased risk of sodium loading – especially in between their dialysis sessions (usually hours for PD, and up to 3 days for HD). Dialysis does remove sodium, yet sodium removal patterns are hardly physiological due to their intermittent pattern, especially for hemodialysis (due to its short intra-dialytic and long inter-dialytic times). [42-44] Thus, this population may be quite vulnerable to salt-related pathophysiological changes.

1.2.5 Sodium in CKD

CKD is comprised of a large group of heterogeneous pathophysiological conditions. As such, depending on the exact mechanism and severity, the effect on sodium hemostasis can be variable. The mechanism of CKD progression and its effect on sodium balance has been discussed in detail in previous literature. [1-5,12] This section provides a brief summary.

Patients with CKD can experience both sodium retention and sodium depletion. The latter is less common and often a consequence of tubulo-interstitial disorders (affecting the tubules and interstitial space). In these conditions, the tubule loses its ability to re-absorb sodium, which results in high sodium losses in the urine. Glomerular diseases are more common and result in increased sodium retention.

If a critical number of nephrons is damaged, the remaining nephrons will be exposed to increased blood flow and compensate by hypertrophying and increasing their GFR. While these adaptive changes are beneficial in the short term, in the long term they result in glomerular injury and increase proteinuria (leaking of protein into the urine). Furthermore, increased Angiotensin II local cytokine activity cause inflammation and fibrosis of the glomerulus and the vessels. Glomerular scarring decreases the ultrafiltration in the glomeruli, while Angiotensin II increases sodium reabsorption (as explained above), thus increasing sodium retention.

In CKD, in order to try to maintain a neutral sodium balance, patients are often prescribed low salt diets (less than 2g of sodium a day) and diuretics. These medications work by decreasing sodium re-absorption in different parts of the tubule, and thus increasing the sodium loss from the functioning nephrons.

1.2.5.1 Sodium in Dialysis

Sodium excretion in dialysis patients consist of the cumulative losses from dialysis and residual renal function (equation [1.2]). These patients often have minimal residual renal function, and thus need to rely on the clearance and ultrafiltration processes of dialysis to excrete most of their sodium intake (equation [1.3]). Sodium handling during dialysis has been previously outlined in the literature [12,34,46,47]

$$\text{Sodium Losses} = \text{Renal Sodium Excretion} + \text{Dialysis Sodium Removal} \quad [1.2]$$

$$\text{Dialysis Sodium Removal} = \text{Diffusive Removal} + \text{Ultrafiltration Removal} \quad [1.3]$$

For in-center HD, sodium removal is dependent primary on ultrafiltration through convective losses (~78%), rather than clearance through diffusive losses (~22%). [48] As fluid is pulled from the body, it drags with it sodium – a phenomenon called “solvent drag”. The sodium concentration in the ultrafiltrate is slightly lower than that of plasma due to the Gibbs-Donnan effect of proteins (i.e. the effect of the negatively charged proteins on this positive cation, as explained in Section 1.2.1). Thus, sodium removal with ultrafiltration depends primarily on the amount of fluid removal (i.e. if HD removes 2L of fluid with a plasma concentration of 140mmol/L, this results in a bit less than 280mmol of sodium removal with ultrafiltration). Diffusive losses of sodium serve to fine tune the sodium balance in these patients. Yet, not all plasma sodium is available for diffusion. The easily diffusible sodium is dissolved in the aqueous component of plasma, unbound to other molecules. The diffusion process is dependent on the concentration gradient between plasma sodium and dialysate sodium. This latter parameter can be adjusted in the HD machine (in the range of 132-155mmol/L) for each patient. Thus, diffusive losses are variable and depend on the difference between the set dialysate sodium concentration and the pre-HD plasma sodium levels for each specific patient. [49]

Since the 1980s, dialysis units adopted a higher sodium concentration in the dialysate, since it reduces intra-dialytic hypotension and increases patient tolerance of the HD process. [50] This often results in dialysate sodium concentration which are higher than plasma sodium ones; and thus, a diffusive influx of sodium into the body. In general, post HD plasma sodium exceeds pre-HD levels by 2-4mmol/L – implying that total losses during dialysis are hyponatremic.(i.e. the concentration of sodium in the fluid losses during dialysis is lower than that of plasma) [50] Unfortunately, this approach can result in lower sodium removal compared to intake, and thus sodium accumulation in the body. Higher dialysate sodium is associated with increased inter-dialytic weight gains and higher blood pressures in patients. [51] In addition, while sodium removal in HD can be increased by rising ultrafiltration rates, there are clinical limitations to the amount of fluid removed due to patient symptoms. Furthermore, increased ultrafiltration rates are associated with hemodynamic disequilibrium and organ dysfunction. [52-58]

Equations [1.2] and [1.3] also apply to peritoneal dialysis. In contrast, the dialysate sodium concentration in PD is usually set at 132mmol/L (dialysate fluid bags used in North America come pre-packaged at this concentration). When dialysate is infused into the abdomen, the high osmolality from its carbohydrate content causes an influx of water. This water influx drags along some sodium cations through solvent drag. This ultrafiltrate fluid has slightly lower sodium concentration than plasma (due to the Gibbs-Donnan effect) and results in an initial decrease in the sodium concentration in the peritoneal fluid. [59] While the sodium concentration is lower, total sodium content is higher due to the increased volume of fluid. As the fluid is left to dwell in the peritoneal space, the sodium concentration rises toward plasma sodium levels due to diffusion. Yet, the osmotic gradient is usually slowly lost when the dialysate contains simple carbohydrates which are easily absorbed. The loss of the osmotic gradient decreases the dialysate volume. As such, depending on the initial osmotic gradient, dwell time and the rate of osmotic gradient loss (dependent on the properties of the peritoneal membrane), the amount of sodium and fluid removal is variable. In order to adjust sodium and fluid removal several parameters can be adjusted: the initial osmotic gradient, dwell time and number of cycles. Even so, increasing osmotic gradient by adding more carbohydrate results in significant metabolic consequences (e.g. insulin resistance) and damage to the peritoneal membrane. Lastly, dialysis solutions exist containing complex carbohydrates which are not readily absorbed, and thus maintain osmotic

and oncotic pressures, but these solutions have only been studied as single dwell solutions over 24 hours (i.e. they are only used for one dwell a day).

The majority of sodium removal in PD, similar to HD, occurs due to ultrafiltration (removal of sodium from fluid removal), as opposed to diffusion. [60] Attempts have been made to increase sodium removal via lowering dialysate sodium concentration in this modality to increase sodium removal with mixed results. [61-64]

Thus, both dialysis modalities remove sodium mostly through ultrafiltration, with some contribution from diffusion. Different parameters can be adapted to increase sodium removal, but these are limited due to hemodynamic and metabolic consequences.

1.3 ¹H Magnetic Resonance Imaging

This section provides general information about the principles of magnetic resonance imaging (MRI). Further details about this technology can be found in several references. [65-68]

MRI is an imaging modality that utilizes the magnetic properties of nuclei to develop images of tissues and structures. This technology is used extensively in the medical field to form 3-dimensional images of the anatomy and the physiological processes of the body with unparalleled soft tissue contrast. Furthermore, it can detect pathological changes in tissues and thus, help diagnose medical conditions.

In this modality, patients are placed in a strong homogenous magnetic field (1.5-3.0 T in clinical systems) generated by superconducting magnets. The temperature of these magnets is maintained near absolute zero (4.2 K) by liquid helium to eliminate electrical resistance in their windings. This sustains a large persistent electrical current in the magnet and a commensurate magnetic field within the MRI system.

1.3.1 Nuclear Spin and the Effect of External Magnetic Fields

In principle, any nucleus with an odd number of protons or neutrons can be detected with magnetic resonance. Biologically relevant nuclei include ¹H, ¹³C, ¹⁵N, ¹⁷O, ¹⁹F, ²³Na and ³¹P. Compared with other imaging modalities MRI has an intrinsically limited sensitivity and *in-vivo* MRI is typically restricted to ¹H imaging due to its high tissue concentration and high sensitivity for MR. Protons (¹H) have a nuclear spin, $I = \frac{1}{2}$. The strength of their interaction with magnetic fields is determined by their magnetic dipole moment, μ given by

$$\mu = \gamma I \quad [1.4]$$

where γ is known as the gyromagnetic ratio. The signal in MRI scales as γ^3 and the gyromagnetic ratio for protons is 42.576 MHz/T, which is the largest value for any nucleus. When water-associated protons in tissue are placed in a magnetic field, B_0 , their magnetic dipole moments precess around the magnetic field direction (Fig. 1-5) at the Larmor frequency, ω_0 given by:

$$\omega_0 = \gamma B_0. \quad [1.5]$$

Under normal conditions at body temperature there is a *small* preference for the cone of precession to be oriented in the direction of the magnetic field. This leads to a net magnetization, (\vec{M}_0) of the protons and hence the tissue. The amount of magnetization, M_0 is dependent on the concentration and magnetic dipole moment of the protons as well as the temperature of the sample (body temperature for tissue).

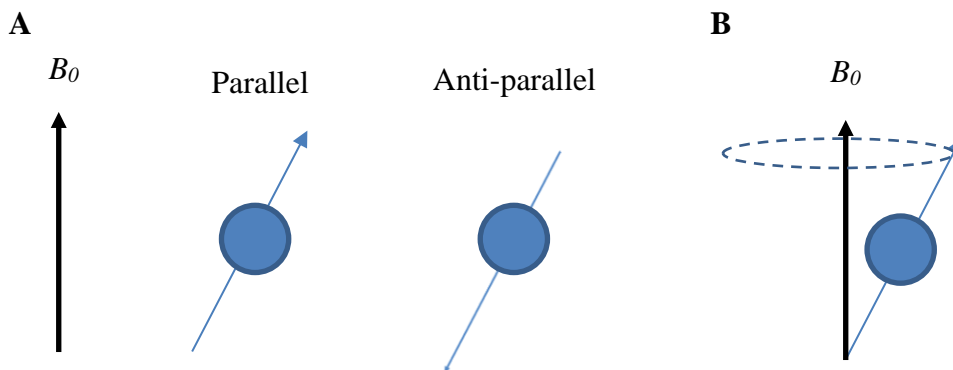


Figure 1-5: Dipoles in an external magnetic field B_0 : A) Alignment of the dipoles with the magnetic field in the parallel or anti-parallel directions, and B) Processing of the dipole at Larmor frequency around the axis of the external magnetic field B_0 . Adapted from [65]

MRI imaging works by perturbing the direction of the \vec{M}_0 vector away from \vec{B}_0 by applying a brief radiofrequency (RF) pulse (creating a time-varying \vec{B}_1 magnetic field at the Larmor frequency in a direction perpendicular to \vec{B}_0). The magnitude of \vec{B}_1 is much weaker than that of \vec{B}_0 , on the order of 50 μT . This produces a transverse magnetization, \vec{M}_t , which precesses around the magnetic field, \vec{B}_0 , (whose direction is conventionally assumed to be along the z -axis) in the xy plane at the Larmor frequency. This is depicted in Fig. 1-6. The initial amplitude of \vec{M}_t is determined by the duration and amplitude of the RF pulse quantified by a quantity known as the flip angle, α , which can range from a few to 90 degrees. After the RF pulse is turned off, the amplitude of the transverse magnetization, \vec{M}_t decays as the z -component of the longitudinal magnetization, M_z regrows. This asymptotic regrowth of the longitudinal magnetization is known as spin-lattice relaxation, which is governed by the spin-lattice relaxation time constant T_1 as described by equation [1.6]:

$$M_z(t) = M_0(1 - e^{-t/T_1}) \quad [1.6]$$

T_1 is known as the spin-lattice relaxation time because it is indicative of the time needed for the nuclei to lose the energy absorbed from the RF pulse into the surrounding lattice to produce longitudinal relaxation. T_1 is dependent on tissue type, temperature and magnetic field strength.

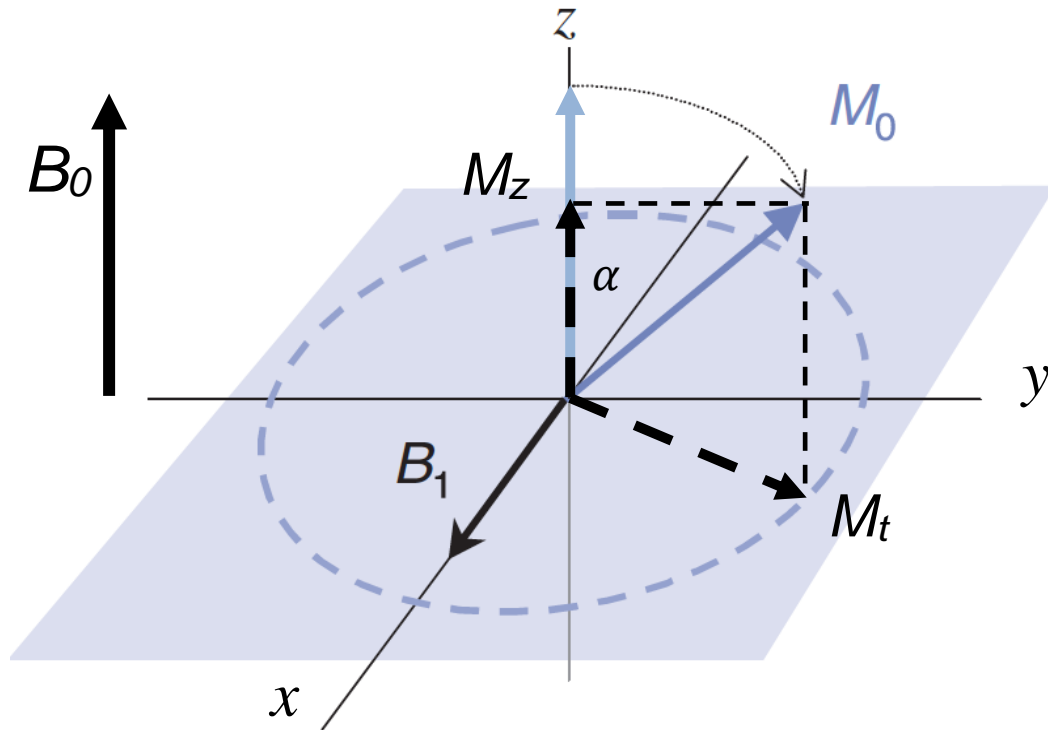


Figure 1-6: The initial effect of the RF pulse B_1 on the magnetization vector of the tissues M_0 . Initially M_0 is flipped from the z-axis, with a flip angle α , and has two components: longitudinal component (M_z) and transverse component (M_t). Adapted from [66]

Prior to the RF pulse, the magnitude of the transverse magnetization, M_t is zero, and after the pulse, its magnitude exponentially decays back to zero, via the following equation:

$$M_t(t) = M_0 e^{-t/T_2}. \quad [1.7]$$

T_2 is the transverse relaxation time (also known as the spin-spin relaxation time) and is due to dephasing of individual nuclear spins while precessing in the xy plane. This dephasing is a result of the interactions of the spins with each other. In practice, the effective relaxation time (T_2^*) is actually shorter than that predicted by natural atomic and molecular mechanisms due to external field inhomogeneities. The external magnetic field \vec{B}_0 produced by the superconducting magnet is not strictly uniform, and even though shim coils are employed to increase uniformity, small inhomogeneities still exist (usually more significant the farthest from the central bore of the magnet). In addition, certain tissues induce magnetic changes – depending on their magnetic susceptibility and add to these imperfections. T_2 decay tends to be 5-10 times faster than T_1 recovery for any given tissue type.

1.3.2 MRI Signal Detection

After excitation of the longitudinal magnetization, the resulting transverse magnetization will induce a sinusoidal voltage in an RF receive coil, which is the source of the MR signal. This signal needs to be significantly larger than any random or spurious fluctuations known as noise for good imaging. Signal-to-noise (SNR) levels in MRI imaging depend on the magnitude of the \vec{M}_0 , which in clinical medicine – at body temperature, tends to be relatively small. Since M_0 also depends on nuclear density, water protons in tissue are the best option for *in-vivo* imaging since they have the highest concentration (~88 mol/L). M_0 also increases with the magnitude of the external magnetic field B_0 (for e.g. from 1.5 to 3 T).

For effective excitation and detection of the transverse magnetization, the RF coils are resonant antennas tuned to the Larmor frequency of a specific nucleus such as protons. Often a separate larger tuned transmit RF coil is used for uniform excitation of the protons and a smaller receive coil or array of coils are employed as close as possible to the region-of-interest for maximum signal reception since the receive signal decreases rapidly with distance.

1.3.3 Pulse Sequences

In order to acquire MR images, the excitation of the longitudinal magnetization and detection of the resulting transverse magnetization needs to be repeated multiple times. The set of instructions defining RF excitation, gradient fields for signal location and signal reception is

called a pulse sequence. Key pulse sequence parameters, which control the acquisition of MR images include:

- 1) Time-to-repetition (TR) or “repetition time”, which governs how often the pulse sequence is repeated;
- 2) Time-to-echo (TE) or “echo time”, which determines the time interval between signal measurements within a single TR;
- 3) Flip angle (α), which determines how much of the longitudinal magnetization is “tipped” into the transverse (xy) plane
- 4) Number of excitations (NEX), which specifies the number of signal averages to improve SNR.

These pulse sequence parameters are specified by the operator to obtain MR images with specific information and SNR.

Since the transverse magnetization (M_t) decays quicker than the recovery of the longitudinal magnetization (M_z) after the application of the initial RF pulse, several techniques are used to reverse this decay and create an “echo” signal. Two common techniques are used in spin-echo and gradient-echo sequences. In spin-echo sequences, the dephasing of the spin is refocused by applying a 180° RF pulse (a certain time after the initial 90° pulse). This refocusing of the spins also reverses the effects of field inhomogeneities. On the other hand, in gradient-echo sequences, the flip angle (α) is chosen to be less than 90° , to ensure the longitudinal component (M_z) has an adequate magnitude, and a gradient magnetic pulse is utilized to refocus the dephasing (Fig. 1-7). Typically, with gradient-echo sequences, slice images are acquired one slice at a time.

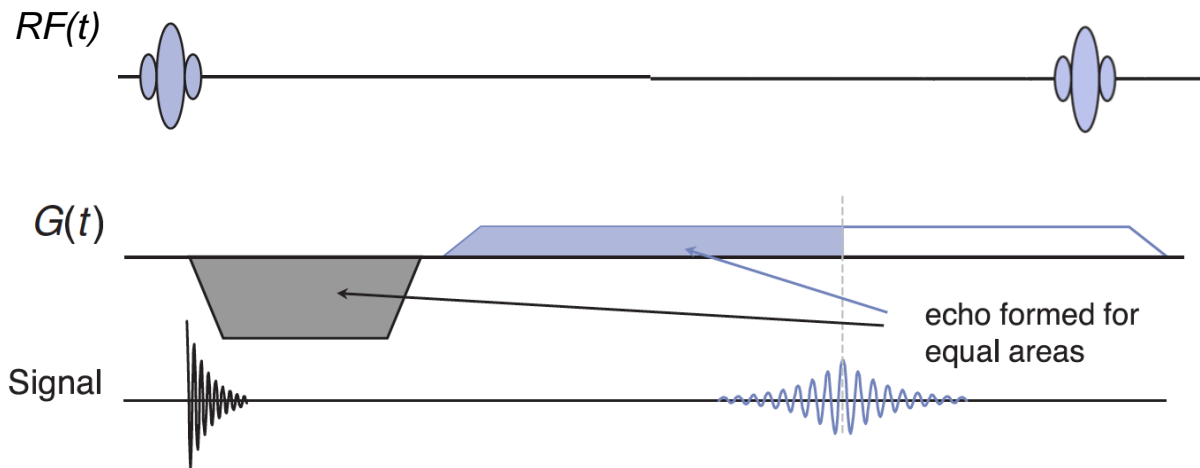


Figure 1-7: Gradient-echo sequence – showing the exciting RF pulses, the gradient ($G(t)$) magnetic field applied and the signal detected (initial and the echo formed by $G(t)$) over time. Adapted from [66]

1.3.4 Proton Density, T_1 and T_2 Imaging

Images in MRI can be T_1 -weighted, T_2 (or T_2^*)-weighted or proton density weighted, where the contrast between different tissues are enhanced based on their intrinsic T_1 , T_2 and proton densities, respectively. Depending on the pulse sequence utilized, TE and TR (and even the flip angle) can be optimized to get any of the three types of images. Tables 1-3 and 1-4 demonstrate the type of images obtained for standard spin-echo and gradient-echo sequences at different parameters.

	Short TE	Long TE
Short TR	T_1 - weighted	mixed
Long TR	Proton-density weighted	T_2 weighted

Table 1-3: Type of Images obtained depending with ^1H MRI depending on TE and TR with spin-echo sequences

	Small	Large
Flip Angle (θ)	Proton-density weighted	T_1 -weighted
TR	T_2^* -weighted	T_1 -weighted
TE	Proton-density weighted	T_2^* -weighted

Table 1-4: Type of images obtained with ^1H MRI depending on the flip angle, TE and TR with gradient-echo sequences

Note, that for spin-echo sequences T_2 -weighted images can be obtained since field inhomogeneities have been removed, while for gradient-echo sequences only T_2^* -weighting can be obtained. In general, solid tissues with rigid lattices have increased spin-spin interactions and thus shorter T_2 , while in water these interactions are less, resulting in longer T_2 values. As such, in T_2 -weighted images fluids tend to be bright due to their higher transverse signal.

1.4 ²³Na Magnetic Resonance Imaging

MRI requires a non-zero magnetic dipole moment to detect a nucleus. While current clinical MRI focuses on detecting hydrogen (due to its abundance in tissues and large gyromagnetic ratio), this technology can be adapted to detect other nuclei. In biological tissues, sodium yields the strongest MR signal after ¹H. [69] The detection of sodium is possible since ²³Na nuclei possess a nuclear spin of 3/2. ²³Na MRI has been utilized since the 1980s [70-76]; even so, this technology does have significant challenges and limitations.

1.4.1 Technical Adaptation for ²³Na MRI

Since ²³Na has different nuclear properties compared to ¹H (Table 1-5), ²³Na MRI requires specific technical adaptation – in terms of both RF hardware and pulse sequences. Custom RF coils are needed for the excitation and detection of the ²³Na nuclei at their specific Larmour frequency. [77] These RF coils are highly optimized and can be paired with short echo times in pulse sequences to maximize possible SNR. Furthermore, these RF coils should not interfere with proton imaging, which are also needed for anatomical context. Lastly, since ²³Na has specific excitation and relaxation properties, the pulse sequences used also need to be adapted (in terms of TR, TE and flip angle).

	Spin Quantum Number	Gyromagnetic Ratio (MHz/T)
¹H	1/2	42.6
²³Na	3/2	11.3

Table 1-5: Difference between hydrogen and sodium nuclei in terms of their spin quantum number and gyromagnetic ratio. [66]

1.4.2 Limitations and Challenges with ^{23}Na MRI

The main challenge with ^{23}Na MRI is that relative to ^1H , ^{23}Na concentration in tissues is much lower. As shown in Table 1-2, ^{23}Na concentration in the ICF and ECF is 143 and 12 mmol/L, respectively. These values are approximately 1000 times smaller than the hydrogen concentration ($\sim 80\text{mol/L}$), limiting the signal that can be acquired within a reasonable amount of time. In addition, since the signal for MRI scales as γ^3 , the lower gyromagnetic ratio for ^{23}Na also contributes to lower SNR.

Furthermore, due to its $3/2$ spin, ^{23}Na exhibits a quadrupolar moment. This additional moment results in bi-exponential T_2 relaxation with a short and a fast T_2 component, which is dependent on the molecular environment. The short component generally contributes $\sim 60\%$ of the signal, while the long one corresponds to the remaining $\sim 40\%$ [78]. In order for both components to be detected, ultrashort TE (less than 0.5ms) and short T_1 are required. [78]

1.4.3 Quantifying Sodium Concentration with ^{23}Na MRI

In order to quantify absolute *in-vivo* sodium concentrations with MRI, the sensitivity profile of the RF system needs to be measured. This is typically undertaken in a separate experiment using an imaging phantom containing a uniform known concentration of sodium such as saline solution. Sodium MRI images are acquired for these phantoms, which are assessed for regional variation in signal. These relative signal maps are then used to normalize *in-vivo* sodium images. [79] In addition, for *in-vivo* ^{23}Na MRI studies, three or four calibration vials with known sodium concentrations (spanning the expected range of sodium concentrations to be observed) are incorporated within the field of view of the receiving RF coil. Linear trend analysis is then used to quantify the tissue sodium concentration in the voxel of interest.

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Chapter 2

2 Non-Osmotic Sodium: What do we know? (A review)

This chapter provides a review of the current knowledge of non-osmotic sodium: the rationale behind this hypothesis (Section 2.1), our current knowledge (Section 2.2), and its clinical significance (Section 2.3).

2.1 Rationale behind Non-Osmotic Sodium Hypothesis

Sodium is a key element that plays important biological roles in several physiological processes in the human body. While it is known that significant amounts of sodium are stored in the matrix of osseous tissue, mixed connective tissue and cartilage, [1-5] this stored sodium has been assumed to be relatively biologically quiescent and impervious to short term fluctuations in sodium intake and excretion. The biologically active sodium is believed to be the osmotically active one (O-Na), found primarily in the extracellular fluid compartment. This O-Na is thought to be strictly regulated primarily by the neurological osmostat and baroreceptors, and the kidneys. Furthermore, due to its aqueous state, it is assumed to be directly proportional to water balance. [6,7] For the purpose of this review, we refer to the sodium stored in tissues without a corresponding increase in water as non-osmotically active sodium (NO-Na). At steady state, humans are believed to have net neutral sodium and water balances [7]; thus, implying that sodium does not move freely between the osmotically active and non-osmotically active compartments. In fact, the NO-Na has largely been ignored in clinical medicine; for example, it is omitted from consideration in the treatment of patients with dysnatremias. [8-10] The corrective equations are based on water equilibrium and active osmolytes such as sodium and potassium. [8-10]

Yet, follow up studies evaluating the accuracy of these equations and models have shown significant disparities between the expected and observed sodium corrections [10-15]. In a short term experiment, Engberink *et al.* [11] studied the effect of infusing 5mmol of sodium per L of total body water over 30min in 12 healthy individuals during the first four hours post infusion. They found that these models overestimated the change in plasma sodium - even when accounting for urinary sodium losses. In an observational study, Hessels *et al.* [13]

retrospectively assessed sodium balance in 38 critically ill patients during their first four days in an ICU setting, and found that while the plasma sodium did not change significantly, patients had a positive sodium balance of 1441 ± 75 mmol (unaccounted by weight changes). A second observational study in the ICU setting found that the development of hypernatremia could not be explained by sodium and water balances. [14]

Heer *et al.* [12] performed a meticulously controlled, randomized study in 32 healthy males in a metabolic ward, where subjects were given diets with a set of different sodium quantities for at least 7 consecutive days. Sodium and water balances were calculated based on intake and excretion from the kidneys, gastrointestinal tract, sweat and insensible losses. Body fluid compartments were analyzed using inulin-dilution methods, mean corpuscular volume of erythrocytes and red cell mass. This group found that while higher sodium diets did result in larger plasma volumes, there was no significant change in the extracellular volume, intracellular volume or total body mass - despite positive sodium balances.

Lastly, in a set of controlled simulated space-flight experiments lasting anywhere from 105 to 205 days, [16-18] the sodium balance of healthy men was evaluated during longer periods of time. These experiments revealed that sodium accumulation was unrelated to changes in body weight (and thus extracellular compartment) and that in humans, regulation of sodium and water is not circadian. [16-18]

A recent systematic meta-analysis of the current literature found that only 93% of the ingested sodium is accounted for in 24-hour urine collections; and when looking at studies in controlled environments, this amount dropped to 84%. [19]

All these studies suggest an uncoupling of sodium and water homeostasis that could only be explained via a more fluid equilibrium between the osmotically active and non-osmotically active sodium storage compartments. This review focuses on our current knowledge of this non-osmotically active sodium buffering system and its biological implications.

2.2 Current Knowledge of Non-Osmotic Sodium

A more fluid equilibrium between the O-Na and NO-Na stores has been hypothesized for more than 50 years. One of the first studies to notice an exchangeable reservoir of sodium in healthy individuals was completed in the 1960s. [20] In their study, Streeten *et al.* administered radiosodium to 7 normal young men and noticed sodium exchange with an enlarging pool for up

to 28 days – suggesting that the two sodium reservoirs equilibrate over time. [20] With the development of new technological advances, our current knowledge of this non-osmotic sodium buffering system has increased significantly.

2.2.1 Measuring Techniques

Several methodologies have been applied to quantify NO-Na stores. Initial studies [1-4] analyzed cadaveric human and animal bones by chemical and caloric removal of adherent tissues, water and fat. The left-over tissues were dissolved by HNO₂ digestion in platinum crucibles and sodium content was determined with a lithium internal standard Barclay flame photometer. [21] Furthermore, several early studies utilized radio-active ²²NaCl to analyze *in-vitro*, [22] *ex-vivo* [3, 23] and *in-vivo* [20] sodium content of tissues. In these experiments, ²³Na in the tissues was allowed to equilibrate with known concentrations of its radio-isotope ²²Na, and radioactivity levels were measured to quantify sodium levels. In addition, in 1988 Warner *et al.* utilized electron probe analysis and analytical electron microscopy to detect *ex-vivo* sodium content in the layers of human skin. [24]

More recently, in several *ex-vivo* studies, sodium content of tissues from animal or human sources was determined using ashing. [25-30] With this technique, tissues are initially desiccated and ashed, before sodium atomic levels could be measured with flame photometry [25,26] or atomic absorption spectrometry [27-30]. Alternatively, frozen tissue sections have been analyzed with inductively coupled plasma-optical emission spectrometry. [31]

While the above methodologies are relatively accurate, they tend to be cumbersome (partly due to their invasive nature) and unsuited for repeat sampling attempts or *in-vivo* clinical settings. [26] Even the previous *in-vivo* assessments done by Streeten *et al.* [20] are limited due to radiation exposure. These limitations were eliminated with the advent of ²³Na MRI. Since sodium is an ion with an odd atomic number – it is detectable by magnetic resonance detection and imaging. This relatively novel technology was first utilized in the 1980s and 1990s to image normal and ischemic canine and rabbit hearts. [32-34] Even so, Kopp *at al.* were the first to utilize ²³Na MRI in assessing NO-Na content of tissues. [26] The accuracy of sodium quantification with ²³Na MRI has been previously validated against ashing techniques in both animal and human studies. [26, 34, 35] In addition, Dyke *et al.* recently assessed its reliability in skin and muscle tissues of healthy individuals. [36] Thus, ²³Na MRI technology allowed for *in-*

vivo, accurate, non-invasive measurements of sodium content in tissues of human beings, suitable for repetitive and dynamic quantification.

2.2.2 Mechanism Non-Osmotic Sodium Storage

Two main mechanisms for NO-Na storage in tissues have been proposed – with variable levels of supporting evidence: glycosaminoglycan (GAG)-bound and intracellularly-stored.

2.2.2.1 Glycosaminoglycan-Bound Sodium

One of the key components of the extracellular matrix are proteoglycans. These molecules consist of a core protein with one or more covalently-bonded long unbranched polymer of disaccharide units, or glycosaminoglycan. GAGs can be sulfated, affecting their negative charge density. [37] This negatively charged GAGs are believed to recruit sodium atoms and render them non-osmotically active. [38,39]

The association between increased tissue sodium and GAGs was first noticed by Ivanova *et al.* in the 1970s. [5] This Russian group demonstrated that in Wistar albino rats fed different sodium content, increased storage of sodium in the skin correlated with an increase in negatively charged sulfated GAGs. Similarly, in a series of experiments in Sprague-Dawley rats, higher skin NO-Na was associated with higher GAG content in skin, while sodium deprivation correlated with reduced sulfated GAGs and increased hyaluronan (non-sulfated GAG). [39,40] Direct correlations between tissue sodium and GAGs have also been demonstrated in cartilage tissue – where sodium concentrations can be as high as 250-300 mmol/L. [23,41-43] Lastly, Fischereder *et al.* [31] studied the tissue sodium content of skin, muscle and arteries in 27 dialysis patients and 21 healthy controls and found a significant association with GAG content. All these studies suggest that NO-Na storage in tissues may be dependent on the negatively-charged density of sulfated GAGs, an adaptable component of the extracellular matrix.

2.2.2.2 Intracellularly Stored Sodium

Bhave *et al.* speculated that excess sodium can also be stored intracellularly in highly cellular tissues like muscle. [44] In this hypothesis, sodium is exchanged for potassium and other intracellular osmolytes. To validate this theory, electrolyte balance studies in dogs revealed that

changes in total body sodium often were accompanied by alterations in total body potassium, and once both these cations were accounted, total body water could be closely predicted. [45] Furthermore, in bovine cartilage, higher tissue sodium has been associated with increased density of the Na-K-ATPase pump (the main exchanger responsible for preserving gradients for sodium and potassium across membranes). [23] This suggests that cells may respond to the extracellular sodium levels by altering the density of this key exchanger. Furthermore, excess sodium could be stored in segregated intracellular compartments, while maintaining a relatively constant sodium concentration in the main cell cytoplasm. More studies are needed to delineate the exact storage mechanism of NO-Na.

2.2.3 Current Knowledge

Sodium concentration has been studied in several different tissues including skin, muscle, bone and cartilage. [26,39,44,46] Table 2-1 contains approximate ranges of sodium concentration in these tissues.

Tissue	Sodium Concentration (mmol/L)
Skin	10-60
Muscle	20-60
Bone	400
Cartilage	250-350

Table 2-1: Approximate sodium concentration in different tissues, based on previous literature [26,39,44,46]

Several groups have studied whether this detected tissue sodium is truly osmotically inactive, by trying to assess concentrations in the interstitial fluid of skin tissue compared to plasma levels. Unfortunately, since the interstitial compartment consists of a GAG and collagen-rich gel-like matrix - with pockets of entrapped free fluid, this fluid is not easily accessible for analysis. [30,49] In a number of animal studies, Haljamäe and his co-workers used the liquid-

paraffin cavity technique and micropipettes to sample nanoliters of this fluid and found higher concentrations of sodium and potassium but lower concentrations of chloride, relative to plasma. [50-51] Higher cation concentrations, which cannot be explained by the Gibbs-Donnan effect, have also been found using the wick technique. [52] However, when Gilányi *et al.* [49] tried replicating these results, they found that the ion distribution in the interstitium and plasma corresponded to the Gibbs-Donnan equilibrium.

Similar contradictory findings are seen in high sodium states. Nikpey *et al.* looked at the osmotic properties of interstitial fluid, lymph (derived from the interstitial fluid) and plasma in rats on a low salt diet (controls), compared to rats with increased sodium retention - induced by either high salt diet or deoxycorticosterone pellets. [30] These latter two groups had increased skin sodium accumulation (higher than water accumulation), and increased skin osmolality. Furthermore, using micropipettes, the authors found that, in all three groups, interstitial fluid, lymph and plasma were iso-osmolar. Yet, Wiig *et al.* [29] used micro-dialysis and energy-dispersive x-ray electron microprobe analysis to find that sodium concentrations and osmolality were higher in the rat skin tissue and lymphatic fluid compared to plasma.

There may be a number of reasons behind these discrepancies. First, the measuring techniques are prone to experimental error due to evaporation or intracellular fluid contamination – especially given the small sample sizes. [30,49,52] Second, the Gibbs-Donnan effect derived from the GAGs in the interstitial space is difficult to quantify, since the GAG concentration is dynamic. Third, multiple studies have demonstrated gradients in the osmolality and sodium content in the skin, [24,30] suggesting that depending on the exact area sampled, different concentrations can be obtained. More studies are needed to elucidate the exact nature of this stored sodium in the interstitial space.

2.3 Clinical Significance of Non-Osmotic Sodium

2.3.1 Non-Osmotic Sodium and the Immune System

The link between sodium and activation of the immune system has been known since the 1990s, when hypertonic saline was noticed to enhance cellular immune function. [53-54] Recently, evidence has emerged that local sodium levels impact innate and adaptive immune cell function and vice versa. [55-56]

Sodium has been shown to have a chemotaxis effect on macrophages, where higher sodium concentrations draw these cells into the tissues. [57] Furthermore, a series of *in-vitro* and *in-vivo* animal experiments have shown that the mononuclear phagocyte system (which includes macrophages, monocytes and dendritic cells) respond to tissue osmotic stress by increasing the transcription factor tonicity-responsive enhancer-binding protein (TONEBP, also known as NFAT5), and inducing on-site vascular endothelial growth factor C (VEGF-C). [29,58-59] This latter growth factor stimulates lymphatic hyperplasia, necessary for draining the interstitial fluid and electrolytes back into the intravascular compartment. Lymphatic hyperplasia has been observed with high-salt diets and sodium retention states. [29, 58-60] These lymphatic changes were prevented by blocking the pathway at the cellular and VEGF-C levels. Furthermore, depleting the mononuclear phagocytic system resulted in higher skin NO-Na levels in rats. [59] Thus, it is believed that by increasing lymphatic drainage, the mononuclear phagocyte system can influence the NO-Na levels in the tissues.

On the other hand, sodium has also been shown to have direct and indirect effects on the immune system. *In-vitro* studies have shown that high sodium cultures induce pro-inflammatory changes in T-helper and T-regulatory cells, macrophages, and dendritic cells. Furthermore, high salt diets have been associated with increased inflammatory cytokines, and several auto-immune conditions and infections in both animal and human studies. Lastly, sodium also affects the intestinal microbiome, which plays a significant role in immune activation. These findings have been previously summarized in the literature. [55-56,61-62] For the purpose of this review, we will focus on the effect of NO-Na on the immune system from a clinical perspective.

Multiple studies have demonstrated increased levels of NO-Na – as measured by sodium MRI technology, in infectious, inflammatory and ischemic conditions. Jantsch *et al.* studied patients with lower leg bacterial cellulitis, before and after treatment, and found that the infected

skin had higher NO-Na levels, and that antibiotic treatment decreased these levels toward those of un-infected controls. [63] Furthermore, they looked at *in-vitro* macrophage elimination of intracellular *Escherichia coli* and *Leishmania major* infections in high and low sodium chloride media and discovered that high sodium boosted clearance of these infections through NFAT5 (or TONEBP) activation and increased nitric oxide production. Lastly, they infected mice (fed high and low salt diet) with *Leishmania major* in their footpads and found that higher tissue sodium levels were associated with faster healing. They concluded that sodium accumulation in the skin microenvironment may strengthen host defenses against infection.

In addition, NO-Na has been associated with ischemic conditions in the heart and the brain. Animal studies with induced coronary ischemia and reperfusion demonstrated increased NO-Na in the affected myocardium, corresponding to non-viable tissue, but normal values in the hibernating tissue (penumbra). [33-35,64] Similar findings were seen in humans studied with ²³Na MRI post myocardial infarction. [65-66] Sodium levels reached a peak 4-days post infarct, then trended down and stabilized by 90-days post. [65] After 90 days, the NO-Na level in the damaged myocardium did not correlate with infarct age, or functional or morphological indices. [66] Analogously, in the brain, NO-Na levels in stroke areas are elevated; yet, sodium signal in the penumbra (tissue amenable to salvage with reperfusion therapy) is preserved. [48] These findings suggest that ischemia may result in altered sodium content – potentially through inflammatory effects or altered trans-cellular sodium gradients. Furthermore, in ovariectomized rats, high salt diet and higher tissue sodium was associated with increased ischemic neurological damage. [67]

Lastly, two main inflammatory conditions have been studied with regards to their association with NO-Na: multiple sclerosis and scleroderma. Multiple sclerosis has been studied by several groups, who have demonstrated increased sodium levels, not only in the demyelinated lesions of the brain, but also in the total gray and white matter. [68-73] In addition, higher NO-Na levels were associated with increased disease severity and clinical disability. The increased NO-Na in this disease may result from a combination of neuro-axonal metabolic dysfunction (resulting in increased intracellular sodium) and interstitial inflammation. [72] Similarly, systemic scleroderma lesions in the skin were also associated with increased NO-Na levels, and were predictive of higher progression of disease in one year. [74]

NO-Na and the immune system appear to be significantly interdependent based on current evidence, and further investigations will be crucial in elucidating this relationship. Yet, clinically, ^{23}Na MRI technology and the relative changes in NO-Na levels already show significant promise as diagnostic and prognostic tools in inflammatory conditions.

2.3.2 Non-Osmotic Sodium, Hypertension and the Endocrine System

The link between sodium and hypertension is long-recognized, and sodium restriction is a common recommendation for hypertensive patients. The advent of a non-osmotic buffering system for sodium opened up a new area of research in this field. In both animal and human studies, hypertension (particularly refractory hypertension) has been associated with increased skin NO-Na levels. [26,28,75] The relationship between NO-Na and blood pressure has been extensively reviewed in the literature. [38,76-77] We will review some of the key players affecting this relationship.

The first key player is the immune system. As discussed above, the immune system plays an important role in regulating NO-Na by the activation of the mononuclear phagocytic system, production of VEGF-C and lymphatic hyperplasia. Blocking this inflammatory response results in salt-sensitive hypertension in mice and rats. [29,58-59] This suggests that the immune system plays an important role in blood pressure regulation by altering the non-osmotic buffering system of sodium. [26]

The second key player is the endocrine system. In the simulated space-flight experiments looking at sodium balance in humans, 24-hour urine sodium excretion exhibited a circaseptan (7 days) and circalunar (approximately monthly) rhythmic pattern, which appeared to be under the control of aldosterone, cortisol and cortisone levels. [16,78] Furthermore, skin sodium levels are particularly higher in patients with hyperaldosteronism, and improve with surgical or spironolactone treatments. [26] Aldosterone may also influence the immune response to sodium. Dendritic cells have been found to express epithelial sodium channel (ENaC), a membrane transporter typically under aldosterone control. [79] These findings would suggest that aldosterone and corticosteroids impact NO-Na storage; whether directly or indirectly through renal excretion remains to be studied.

Two other hormonal axes that have been partially studied with regards to their relation to NO-Na include sex hormones and insulin. Multiple studies have demonstrated that sex affects

the amount of sodium stored in skin and muscle. [26,75,80-81] Furthermore, ovariectomy resulted in decreased sodium storing capacity in the tissues of female rats, and greater fluid retention. [28] In addition, insulin resistance has been shown to be associated with higher mean arterial blood pressure in diabetics and exogenous insulin decreases the urine fractional excretion of sodium.[82] In two studies of hemodialysis patients, increased muscle sodium levels was associated with insulin resistance, while diabetes was associated with higher NO-Na deposition in both skin and muscle.[83-84] These findings suggest roles for insulin, estrogen, progesterone and testosterone that need to be further elucidated.

Thirdly, the existence of osmolyte gradients in the skin has given rise to a theory of extrarenal countercurrent system in the skin that has direct effects on blood pressure. [38,77,85] The skin has been shown to have osmolality, urea and sodium gradients. [24,30] The similarity of these gradients to those found in the kidney, make them particularly interesting as a novel mechanism of hemodynamic control. Thus, blood pressure and NO-Na likely have some direct correlation based on these osmolyte gradients in the skin, and indirect ones through multiple hormonal axis and the immune system.

2.3.3 Non-Osmotic Sodium and Malignancy

Increased tissue sodium has been detected in neoplastic lesions (compared to normal tissue) in the breast, prostate and brain. [86-91] In addition, pathologically more aggressive lesions had the highest sodium concentration in prostate and brain, indicating that this index may be of use clinically for prognostication. [88,91] In malignant lesions, the increased sodium signal detected with ²³Na MRI has been attributed to a breakdown of local cellular energy-based sodium transporters (sodium-proton antiport and Na-K-ATPase) and sustained cell depolarization – resulting in higher intracellular sodium levels, and increased extracellular sodium from disruption of the necrotic tumor cells. [86,88,90-91] Unfortunately, current knowledge is still quite limited.

2.3.4 Non-Osmotic Sodium and Degenerative Diseases

NO-Na has been associated with several degenerative conditions and structural changes in tissues.

In skeletal muscle, ^{23}Na MRI was utilized to assess sodium levels in aerobic and anaerobic exercise. [92] An increase in NO-Na was observed with anaerobic exercise only. The authors speculated that this increase could be from higher intracellular sodium concentration due to depletion of the Na-K-ATPase. The lack of response with aerobic exercise remains a puzzle. Yet, increased NO-Na attributed to intracellular shifts has also been recorded in other channelopathies, namely during cold and exercise-induced weakness in severe hyperkalemic periodic paralysis. [93] In addition, two patients with myotonic dystrophy also had increased sodium signal in their skeletal muscles, compared to healthy controls. [94]

In the brain, in a study of 10 patients with drug-resistant epilepsy and 27 healthy controls, increased sodium levels were found in the epileptogenic zones (compared to irritated or non-involved regions) in the inter-ictal state. [47] Since sodium ions are key for the maintenance of the transmembrane gradient crucial to normal neuronal action potential, this change (in the absence of seizure activity) was attributed to ongoing modification of the processes governing transmembrane sodium concentrations. In fact, alterations in voltage-gated sodium channels have been shown to be triggers in epileptic neuronal tissue. [95-97] Furthermore, increased tissue sodium has been seen in patients with Alzheimer's and Huntington's degenerative disorders. [98-99]

Unfortunately, the main limitation of these studies is their relatively small sample sizes (usually less than 13 patients per group). Even so, current findings suggest that NO-Na may serve as a diagnostic tool to identify regions of interest with structural or functional anomalies in tissues.

2.3.5 Non-Osmotic Sodium and Sodium Surplus States: Heart and Kidney

NO-Na has also been studied in conditions traditionally associated with increased-sodium retention: specifically heart failure and kidney disease. Hammon *et al.* (2015) studied 9 patients with acute exacerbation of congestive heart failure (CHF), before and after diuretic therapy, compared to healthy control. [100] They found that patients had higher levels of sodium in both skin and muscle, and that diuretic therapy significantly reduced sodium content in both these tissues. Furthermore, water content was not reduced with treatment in muscle, but did show a trend ($p = 0.06$) toward decreased amount in the skin. These findings suggest that in CHF, there is

significant NO-Na in the muscle and potentially a combination of osmotic and non-osmotic sodium storage in the skin.

NO-Na has also been studied in renal conditions – where the ability of the kidneys to excrete sodium may be compromised. Hammon *et al.* (2017) studied patients with acute kidney injury. [101] They found that these patients did have higher NO-Na in both skin and muscle, compared to healthy control. Yet, hemodialysis treatment did not significantly change these levels, despite lowering blood pressure and weight. Dahlman *et al.* studied 33 patients on chronic hemodialysis, and discovered that only patients older than 60years had increased skin and muscle sodium compared to healthy controls. [102] Higher sodium levels were associated with decreased VEGF-C. Furthermore, sodium stores in these tissues was successfully mobilized with hemodialysis treatments.

Olde Engberink *et al.* have proposed that the endothelial surface layer, which tends to be quite rich in glycosaminoglycans, may play an important role in adjusting NO-Na. [103] This endothelial layer is itself composed of a combination of GAGs – a microenvironment highly regulated by endothelial cells. [104] As such, this group proposed that this layer may serve as the first layer in regulating the passage of sodium between the osmotic and non-osmotic compartments. Furthermore, this endothelial layer is often damaged in renal disorders, [105,106] which may explain the altered sodium handling by the kidneys in diseased conditions. Lastly, NO-Na accumulation in CKD has been associated with end-organ damage; specifically, increased skin NO-Na in mild to moderate CKD patients was associated with left ventricular mass. [107]

Thus, the relationship between the kidney and sodium is likely more complicated than previously appreciated. Sodium handling by the non-osmotic compartment and the endothelial layer could result in renal injury, and diminished renal function may increase NO-Na – though likely affected by other factors, like age. More studies are needed to shed more light on these interactions and also on the effect of renal replacement therapies on this non-osmotic component.

2.4 Conclusion

The discovery of a non-osmotic sodium buffering mechanism has resulted in a better understanding of sodium hemostasis. Previously, sodium was believed to have a role restricted to that of an effective osmolyte necessary for hemodynamic stability and for maintaining the transmembrane electric potential. With the above studies, the role of non-osmotic sodium as a key player in creating tissue micro-environments and affecting multiple signaling pathways and biological process is becoming more obvious. More studies are needed to elucidate this compartment further (e.g. in terms of its location – intracellular or extracellular) and reveal its full potential. Furthermore, the use of ^{23}Na MRI opens a door of possibilities to study this non-osmotic sodium compartment and use it in clinical medicine for diagnostic, and prognostic purposes. Lastly, a better understanding of sodium's multitude of roles and effects, can even lead to new targets for therapeutic uses.

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Chapter 3

3 Non-Osmotic Sodium in CKD

This chapter contains a condensed introduction (background and the study objective and hypothesis), as well as the main methodology, results and discussion of this thesis.

3.1 Introduction

3.1.1 Background

Sodium is a key element that plays important biological roles in several physiological processes. For years, the paradigm of ^{23}Na homeostasis in humans relied on the assumption that the majority of the biologically active sodium is stored osmotically solvent in the extra-cellular volume, and that body sodium content is regulated primarily by the kidneys. ^{23}Na storage independent of water retention was often neglected. Even so, multiple short and long-term studies in humans have shown that ^{23}Na mass balance cannot be maintained without the existence of a non-osmotic ^{23}Na compartment. [1-4] This compartment has further been studied and quantified, [5-7] especially with the advent of ^{23}Na MRI technology. [8-16] Current evidence suggests that this non-osmotic ^{23}Na is bound to glycosamino-glycans (GAGs) in the extracellular matrix of tissues. [17-21] Sodium deposition has been linked to multiple physiological and pathological processes including: exercise, hypertension and hyperaldosteronism, congestive heart failure, malignancy, inflammation, and ischemia. [6-7,9-11,13,21-23]

Sodium homeostasis is fundamentally altered in patients with kidney disease – due to the lack of renal sodium excretion. [30-33] As such, patients with chronic kidney disease may have a higher utilization of this non-osmotic ^{23}Na buffering system. Previous studies have demonstrated increased skin and muscle ^{23}Na in patients undergoing acute hemodialysis treatment and those older than 60 years on chronic HD – relative to healthy controls. [10,12] Even so, in a cohort of patients with stage 1-3 of kidney disease, Schneider *et al.* did not find a correlation between estimated glomerular filtration rate (eGFR) and skin ^{23}Na . [14] The effect of the severity of kidney disease on the non-osmotic ^{23}Na accumulation in tissues of patients has yet to be determined.

3.1.2 Study Hypothesis and Objective

The objectives of this thesis are:

- 1) To use ^{23}Na MRI technology to examine the sodium deposition in skin, muscle and skeleton tissues of two groups of CKD patients: 1) CKD stage 4-5 (not dialysis) patients, and 2) dialysis patients – sub categorized into PD and in-center HD, and compare this deposition with controls with normal kidney function.

Hypothesis: Since the kidney plays a key role in regulating sodium content in the body, and since patients with CKD have altered sodium excretion, we hypothesized that CKD patients would have higher levels of non-osmotic sodium in their tissues. In addition, we hypothesized that the degree of non-osmotic sodium would be dependent on the stage of kidney function, i.e. patients with end-stage kidney function on renal replacement therapy would have more sodium in their tissues than patients with stage 4-5 CKD (not on dialysis). Also, since peritoneal dialysis patients tend to have higher residual renal function, we speculated that they would have lower sodium levels than patients on in-center hemodialysis.

- 2) To explore associations between sodium deposition in these tissues with baseline characteristics and biochemical markers, to explore specific correlations.

Hypothesis: Given the many associations between non-osmotic sodium and physiological functions (discussed in Chapter 2), we suspected that sodium accumulation may be associated with some of the pathophysiological changes observed in the CKD patients.

3.2 Methods

3.2.1 Study Design

This is a pilot cross-sectional cohort study of patients with different levels of kidney disease: CKD 4-5 not on dialysis (CKD group), and dialysis - peritoneal dialysis (PD) and hemodialysis (HD); compared to controls with normal kidney function (Control group). During the study sessions, we obtained baseline characteristics (including age, sex, past medical history, medications, and renal history), blood work for biochemical analysis, and MRI study of the right lower leg, for each participant.

This study was approved by the University of Western Ontario Health Sciences Research Ethics Board and was conducted in compliance with the approved protocol, Good Clinical Practice Guidelines and all applicable regulatory requirements.

3.2.2 Subjects

Controls from London, Ontario, and CKD and dialysis (both PD and HD) patients from London Health Sciences Centre – Regional Renal Program participated in the study. All participants had to be 18 years or older and provided written informed consent prior to the study. Subjects were considered controls if they lacked any history of kidney disease, heart failure, liver cirrhosis or peripheral edema. This group was recruited through posters advertising the study in the London community (hospitals and churches). CKD patients had to have CKD Stage 4-5 disease (as defined by Kidney Disease: Improving Global Outcomes 2012 Guideline [34]) and no indications to start dialysis. Dialysis patients included PD and in-center thrice-weekly HD patients who had been established on their respective dialysis modality for at least 3 months. Subjects were excluded if they were pregnant, breast-feeding, intending pregnancy or unable to provide consent, or if they had any contraindication to MRI studies.

3.2.3 Biochemical Measurements

Plasma, serum and blood specimens from each subject were collected, processed and analyzed by a central laboratory (London Health Sciences Centre, London, Canada) for routine clinical chemistry of a core set of parameters. eGFR values were estimated using the Chronic Kidney Disease Epidemiology Collaboration formula. [35]

3.2.4 Sodium MRI Quantification of Tissue Sodium

Tissue ^{23}Na concentration in the lower leg tissues was assessed non-invasively using MRI technology, similarly to previously described methods. [8-9] This technology has been previously validated against sodium quantification with chemical analysis by ashing, [8] and has been found to be reliable. [36] A multinuclear-capable 3.0-T MRI (Discovery MR750, General Electric Healthcare, Milwaukee, WI) was used to acquire proton and sodium images. Subjects were positioned supine in the magnet bore with the right lower leg (10-15 cm below the knee) centered in a custom-made 12-rung 18-cm-diameter ^{23}Na birdcage radiofrequency (RF) coil (Fig. 3-1). Calibration vials with 10, 20 and 40 mmol/L of saline were placed in the RF coil, over the subjects' shins. Axial proton T1-weighted images with a fast-low-angle-shot (FLASH) sequence were acquired to delineate the anatomy of the lower leg. A single-slice ^{23}Na MR image was obtained with a density-adapted three-dimensional radial projection reconstruction pulse sequence optimized for sodium acquisition, [37] with the following parameters: slice-selective RF pulse with a 90° flip angle, TR/TE: 100/1.5ms, total acquisition time: 30min, number of signal averages: 100, slice-thickness: 30mm, and isotropic field of view/resolution: 18/0.3 cm^2 .



Figure 3-1: Custom-made RF coil and position of the right leg in the MRI.

Maps of tissue ^{23}Na concentration were generated with Matlab (Mathworks, Natick, USA, R2018a), using the calibration vials relating signal intensity to ^{23}Na concentration using linear trend analysis. ^{23}Na concentration maps were superimposed with the proton anatomical images to delineate the regions of interest. Four regions of interest (ROIs) were drawn, using OsiriX Lite (Version 9.5.2) software, highlighting different tissues: 1) pre-tibial skin, 2) posterior leg skin, 3) soleus muscle, and 4) tibia (including bone marrow) (Fig. 3-2). Two skin regions were included - based on reproducibility, because ^{23}Na distribution in this tissue was relatively inhomogeneous. This inhomogeneity in sodium content could make one of these areas more clinically relevant. In addition, the bone marrow was included in the tibia ROI because the sodium signal from the bone marrow and the osseous tissues were comparable in our preliminary analysis (data not shown). ^{23}Na concentration in these four regions was recorded for analysis.

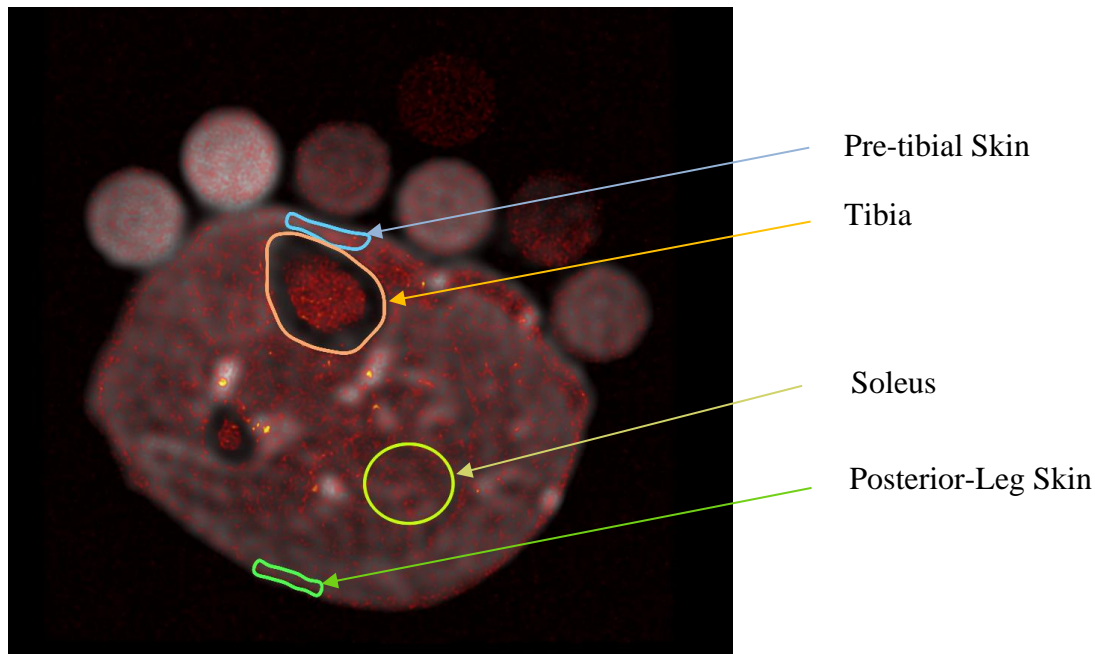


Figure 3-2: Sample overlap image with sodium MRI image (gray scale) and proton image (red scale) superimposed together and the four regions of interest (ROIs) highlighted.

3.2.5 Data Analysis

Statistical analysis was performed using SPSS Statistics, Version 23 (IBM, Chicago). Data were analyzed using descriptive statistics (means with standard deviation (SD) or 95% confidence intervals (CI) for continuous variables; percentages for categorical variables). Kruskal-Wallis, Anova and Fisher's exact tests were used to compare groups across baseline characteristics. Mann-Whitney and t-tests were applied to unpaired observations. Correlations were assessed by calculating Pearson (r) and Spearman (ρ) correlation coefficients. We then performed some exploratory multiple regression analysis to examine the parameters that independently influence tissue ^{23}Na concentration. The basic regression models were generated using age, sex, and the parameter with the strongest correlation (r or ρ) from each of the main areas: inflammation, bone mineral disease, and blood pressure. Other variables with strong correlation ($p < 0.01$) were then added to the basic model sequentially. F-statistics were used to remove factors who did not significantly contribute to the model. The models were compared using corrected R^2 coefficient. Due to the exploratory nature of these models, interactions between the factors were not assessed. Furthermore, these parameters were not adjusted for renal function. Missing data were managed by removing that subject from that particular analysis. Two-tailed p -values < 0.05 were considered statistically significant.

3.3 Results

In total, 10 Control, 12 CKD, and 23 dialysis (10 PD and 13 HD) subjects participated in the study (Fig. 3-3). 56% of all participants were male and 84% were Caucasian. Table 2-1 highlights the baseline characteristics of these groups. CKD and dialysis subjects were older and had higher weights and BMIs. As expected, they also had higher rates of comorbidities including: hypertension, atherosclerosis, and congestive heart failure. Correspondingly, subjects with kidney disease had significantly higher use of anti-hypertensive medication and other renal-specific medications (erythropoietin-stimulating agents and phosphate binders). The main etiologies of renal disease included hypertension, diabetes mellitus, reno-vascular conditions, glomerulonephritis and other (some subjects had multiple etiologies). The three groups also varied across multiple serum biomarkers – most of which are explained by the presence of kidney disease. Notably, serum sodium was different among the groups, with means of 141mmol/L, 139.9mmol/L and 138.3mmol/L in the Control, CKD and dialysis groups (PD and HD) respectively (p -value 0.01).

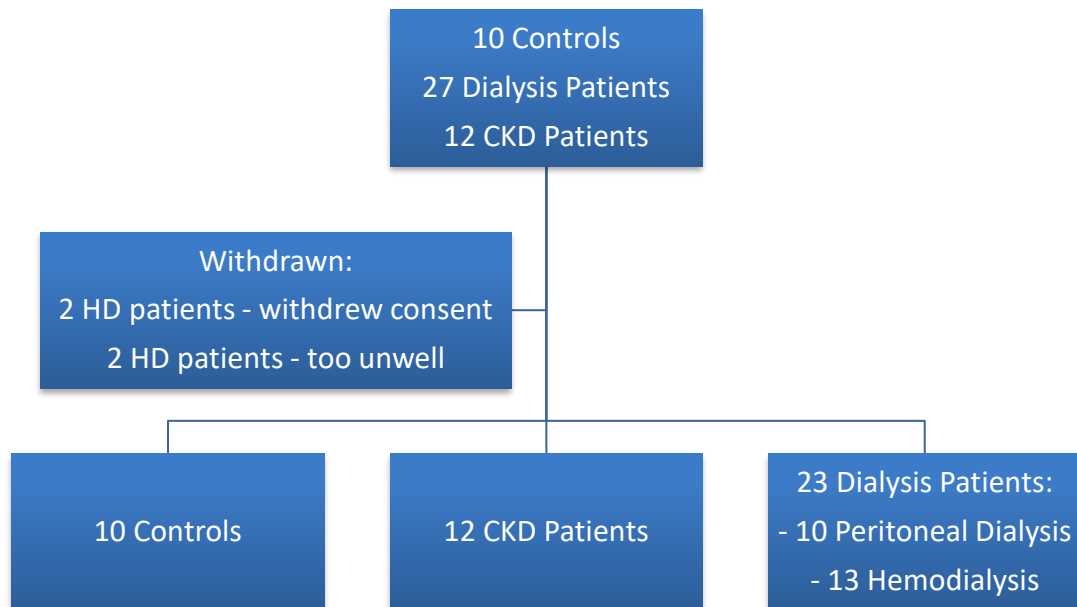


Figure 3-3: Study Population: including recruited participants who were withdrawn, and the ones included in the study.

When comparing the PD and HD subgroups (Table 3-1), PD subjects had higher residual renal function (means of 1175ml vs 220ml, p -value 0.003), and higher use of diuretic therapy (100% vs 23.1%, p -value <0.001). These two subgroups also varied in a number of serum biomarkers, consistent with the different clearance-characteristics of these dialysis modalities. PD subjects had statistically significant higher creatinine and lower albumin concentrations. Lastly, HD participants had higher hemoglobin levels compared to PD ones, 120.3 g/L vs 104.0 g/L respectively (p -value 0.01). High sensitivity C-reactive protein (CRP) was not statistically different between the two.

Table 3-1: Baseline characteristics for the three study groups, and for the two subgroups (HD and PD).

Parameter	Control (n=10)	CKD (n=12)	Dialysis (n=23)	p-value	HD (n=13)	PD (n=10)	p-value*
Age, year, mean (SD)	53.3 (19.4)	66.3 (6.7)	61.6 (9.4)	0.04	62.5 (9.1)	60.3 (10.2)	0.59
Sex, men/women	4/6	8/4	14/9	0.52	9/4	5/5	0.42
Race, Caucasian/other	8/2	12/0	18/5	0.228	8/5	10/0	0.046
Height, cm, mean (SD)	167.3 (7.0)	166.4 (9.8)	170.8 (7.7)	0.24	171.2 (7.5)	170.3 (8.4)	0.78
Weight, kg, mean (SD)	70.5 (8.2)	88.5 (18.6)	83.5 (18.7)	0.047	83.9 (22.6)	83.1 (13.0)	0.92
BMI, kg/m ² , mean (SD)	25.2 (2.7)	31.7 (4.5)	28.6 (6.1)	0.02	28.4 (7.0)	28.8 (5.0)	0.89
<i>Comorbidities</i>							
Hypertension, %	10.0	91.7	87.0	<0.001	100.0	70.0	0.07
Atherosclerosis, %	0	50.0	43.5	0.02	46.2	40.0	1.00
Coronary artery disease, %	0	16.7	21.7	0.36	30.8	10.0	0.34
Cerebrovascular disease, %	0	16.7	13.0	0.58	15.4	10.0	1.00
Peripheral vascular disease, %	0	8.3	13.0	0.80	15.4	10.0	1.00

Congestive heart failure, %	0	0	26.1	0.04	30.8	20.0	0.66
Diabetes Mellitus, %	10.0	58.3	43.5	0.053	46.2	40.0	1.00
COPD, %	0	0	17.4	0.16	23.1	10.0	0.60
<i>Etiology of Kidney Disease</i>							
<i>Hypertensive nephropathy, %</i>	-	41.7	44.5	0.04	38.5	50.0	0.69
<i>Diabetic nephropathy, %</i>	-	33.3	39.1	0.06	38.5	40.0	1.00
<i>Glomerulonephritis, %</i>	-	16.7	8.7	0.54	15.4	0	0.49
<i>Vascular nephropathy</i>	-	25.0	39.1	0.053	46.2	30.0	0.67
<i>Other</i>	-	41.7	30.4	0.06	30.8	30.0	1.00
Dialysis vintage, months, mean (SD)	-	-	37.1 (34.3)	-	47.1 (41.7)	24.2 (14.8)	0.09
Residual renal function, ml, mean (SD)	-	-	635 (721)	-	220 (326)	1175 (746)	0.003
<i>Medications</i>							

Anti-hypertensives, %	10.0	91.7	78.3	<0.001	84.6	70.0	0.62
ACEI/ARB, %	10.0	58.3	30.4	0.053	30.8	30.0	1.00
Diuretic, %	10.0	58.3	56.5	0.03	23.1	100.0	<0.001
Beta blocker, %	0	66.7	65.2	0.001	76.9	50.0	0.22
Calcium channel blocker, %	0	41.7	52.2	0.01	46.2	60.0	0.68
ESA, %	0	25.0	60.9	0.001	69.2	50.0	0.42
Phosphate binders, %	0	33.3	87.0	<0.001	84.6	90.0	1.00
Calcium Binders, %	0	33.3	82.6	<0.001	76.9	90.0	0.60
Aspirin, %	10.0	66.7	39.1	0.003	38.5	40.0	1.00
<i>Serum Biomarkers</i>							
Sodium, mmol/L, mean (SD)	141 (2.2)	139.9 (1.8)	138.3 (2.8)	0.01	138.4 (2.6)	138.1 (3.2)	0.81
eGFR, ml/min/1.73m ² , mean (SD)	88.1 (16.1)	23.1 (11.8)	-	<0.001	-	-	-
Creatinine, µm/L, mean (SD)	77.0 (12.2)	257.3 (83.5)	630.7 (242.3)	<0.001	557.7 (215.3)	718.3 (254.3)	0.13
Albumin, g/L, mean (SD)	43.7 (2.5)	43.3 (2.4)	39.0 (4.4)	0.002	41.2 (3.6)	36.7 (4.0)	0.02
Hemoglobin, g/L, mean (SD)	142.9 (12.8)	117.3 (22.7)	112.9 (16.1)	0.001	120.3 (15.2)	104.0 (12.6)	0.01

CRP, mg/L, mean (SD)	2.2 (2.6)	2.9 (2.4)	13.7 (16.9)	<0.001	15.8 (22.1)	10.6 (7.3)	0.46
PTH, pmol/L, mean (SD)	4.5 (1.2)	18.8 (16.9)	40.7 (40.3)	0.001	44.8 (42.9)	35.9 (38.7)	0.62
hsTroponin T, ng/L, mean (SD)	5.5 (3.2)	27.8 (16.9)	130.1 (157.4)	<0.001	165.2 (196.7)	88.0 (83.3)	0.24

p-value* - comparing HD and PD; *p*-values calculated with Fisher's exact test.

ACEI – Angiotensin converting enzyme inhibitor, ARB – Angiotensin II receptor blocker, BMI – Body mass index, COPD – Chronic obstructive pulmonary disease, CRP – high sensitivity C-Reactive protein, eGFR- Estimated glomerular filtration rate – using CKD-EPI equation, [35] ESA – Erythropoietin stimulating agent, hsTroponin T – High sensitivity troponin T, PTH – Parathyroid hormone, SD- standard deviation

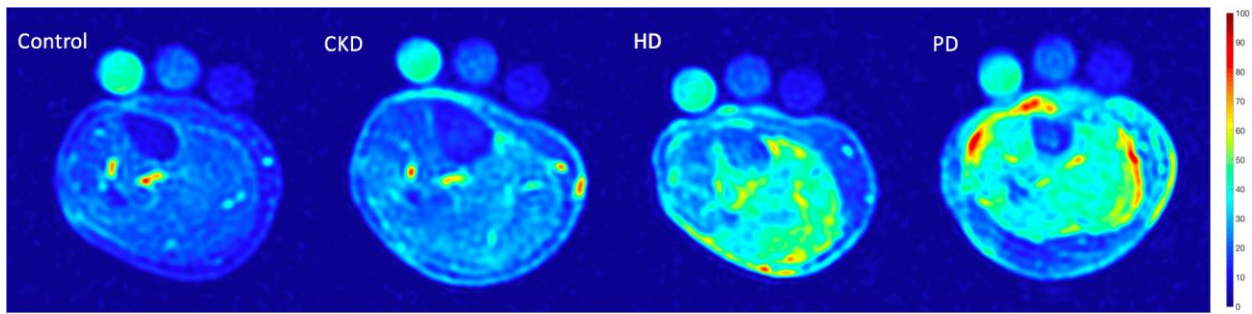
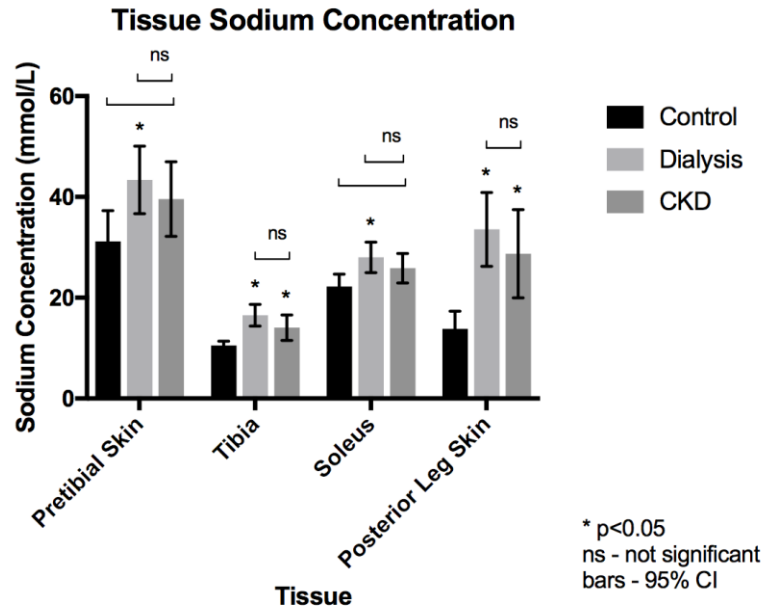


Figure 3-4: Examples of sodium concentration maps for the different study groups: control, CKD, HD and PD. (Colour map on the side indicates the sodium concentration in mmol/L)

Four representative examples of tissue ^{23}Na concentration maps are shown in Fig. 3-4. The ^{23}Na concentration in the study groups for the four regions of interest is shown in Fig. 3-5. ^{23}Na levels were statistically higher in dialysis subjects compared to healthy controls ($p < 0.05$) across all tissues. No statistically significant difference was found between ^{23}Na levels in dialysis and CKD subjects. CKD participants had statistically significant higher ^{23}Na levels in their tibia and posterior leg skin compared to controls. Furthermore, when the dialysis group was split into HD and PD subgroups, no difference was found between PD, HD and CKD. Yet, all three had higher ^{23}Na in the tibia and posterior leg skin compared to the control group. Only PD subjects had statistically higher ^{23}Na than controls in the soleus muscle and pretibial skin.

A



B

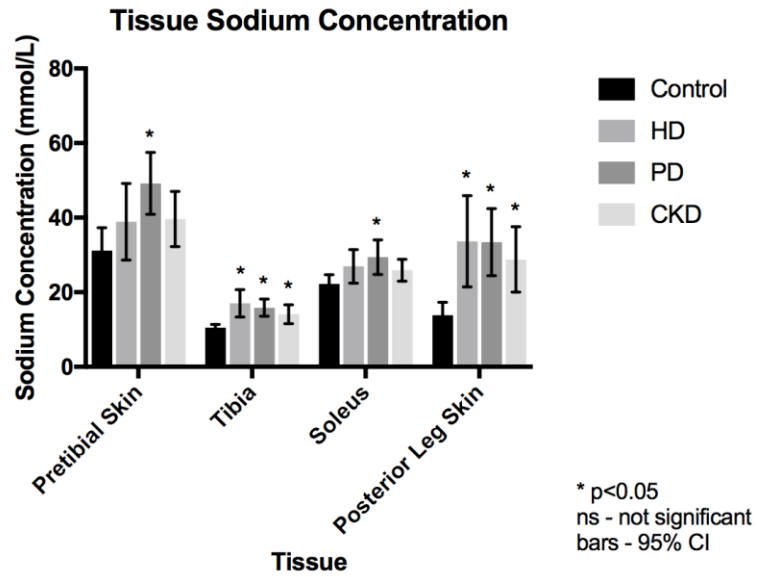


Figure 3-5: Tissue Sodium Concentration in the study groups. A – results for the main three groups (Control, Dialysis and CKD), and B – results when PD and HD patients are separated.

Correlations between ^{23}Na levels in the four tissues, and different subject parameters were also explored (Table 3-2). Multiple statistically significant correlations were discovered between ^{23}Na levels and baseline characteristics, medications, and renal, inflammatory, mineral bone disease and end-organ markers. Some correlations were persistent across the four tissues studied (e.g. correlations with parathyroid hormone, high sensitivity troponin T and inflammatory markers). Conversely, some parameters were associated with ^{23}Na levels only in specific tissues (e.g. increased age was associated with higher ^{23}Na in soleus and posterior leg skin only). Serum sodium levels did not correlate with ^{23}Na concentrations of any of the tissues studied.

Multi-variable regression linear models are shown in Table 2-3. Tibia and soleus ^{23}Na levels were explained relatively well by the third models (R^2 0.713 and 0.603, respectively). The models for pretibial and posterior leg skin were weaker (models 2 had R^2 0.274 and 0.456, respectively). These models were not improved by the addition of other parameters (including age (when not already included), sex, hypertension, and eGFR; data not shown). Each tissue had its own set of parameters contributing to the models.

Table 3-2: Correlations between Sodium deposition in the tissues and subjects' baseline characteristics, medications and serum markers.

	Pre-Tibial Skin		Post-Leg Skin		Tibia		Soleus	
	Corr. Coeff.	P-value	Corr. Coeff.	P-value	Corr. Coeff.	P-value	Corr. Coeff.	P-value
<i>Baseline Characteristic</i>								
Age	0.202	0.180	0.441	0.002	0.16	0.294	0.386	0.009
Sex	-0.163	0.285	-0.222	0.143	0.298	0.047	0.083	0.587
Atherosclerosis	0.272	0.071	0.332	0.026	0.329	0.027	0.104	0.498
CHF	0.262	0.082	0.302	0.044	0.383	0.010	0.249	0.099
Hypertension	0.219	0.148	0.547	<0.001	0.449	0.002	0.355	0.017
Diabetes Mellitus	0.346	0.020	0.318	0.029	0.049	0.750	0.143	0.348
COPD	0.271	0.072	0.403	0.006	0.307	0.054	0.054	0.724
Gout	-0.049	0.748	0.125	0.412	0.179	0.239	0.342	0.021
Dialysis Vintage (months)	0.144	0.346	0.234	0.122	0.610	<0.001	0.360	0.015
<i>Medications</i>								

Calcium Binder	0.298	0.047	0.366	0.013	0.455	0.002	0.317	0.034
Phosphate Binder	0.360	0.015	0.442	0.002	0.490	0.001	0.328	0.028
ESA	0.166	0.276	0.431	0.003	0.508	<0.001	0.286	0.057
Diuretics	0.394	0.007	0.295	0.049	0.329	0.027	0.196	0.198
Beta blocker	0.110	0.474	0.438	0.002	0.335	0.024	0.315	0.035
Anti-hypertensives	0.250	0.097	0.505	<0.001	0.377	0.011	0.301	0.044
Aspirin	0.300	0.045	0.448	0.002	0.180	0.237	0.056	0.713
<i>Renal Markers</i>								
Serum Sodium	-0.038	0.806	-0.057	0.712	-0.082	0.598	0.021	0.817
eGFR	-0.348	0.021	-0.450	0.002	-0.511	<0.001	-0.467	0.001
Creatinine	0.351	0.019	0.416	0.005	0.447	0.002	0.375	0.012
<i>Inflammatory Markers</i>								
Albumin	-0.254	0.100	-0.377	0.013	-0.577	<0.001	-0.613	<0.001

Hemoglobin	-0.387	0.009	-0.458	0.002	-0.741	<0.001	-0.522	<0.001
CRP	0.374	0.015	0.315	0.042	0.511	0.001	0.215	0.171
<i>Mineral Bone Disease Markers</i>								
1,25(OH) ₂ Vitamin D	-0.413	0.009	-0.444	0.005	-0.540	<0.001	-0.395	0.013
PTH	0.345	0.024	0.312	0.042	0.622	<0.001	0.408	0.007
ALP	0.238	0.119	0.091	0.557	0.452	0.002	0.194	0.207
<i>End-Organ Markers</i>								
INR	0.198	0.203	0.176	0.258	0.431	0.004	0.242	0.117
<u>hsTroponin T</u>	0.358	0.017	0.563	<0.001	0.585	<0.001	0.478	0.001

Corr. Coeff. – Correlation coefficient, ALP – Alkaline phosphatase, CHF – Congestive heart failure, COPD – Chronic obstructive pulmonary disease, CRP – high sensitivity C-Reactive protein, eGFR- Estimated glomerular filtration rate – using CKD-EPI equation, [35] ESA – Erythropoietin stimulating agent, hsTroponin T – High sensitivity troponin T, INR – International normalized ratio, PTH – Parathyroid hormone.

Table 3-3: Linear regression models for explanation of ²³Na in the different tissues studied.

Tibia						
		Model 1		Model 2		Model 3
		R ² 0.474		R ² 0.670		R ² 0.713
Parameter	Beta Coeff.	p-value	Beta Coeff.	p-value	Beta Coeff.	p-value
Hemoglobin	-0.689	<0.001	-0.504	<0.001	-0.367	0.003
PTH			0.479	<0.001	0.479	<0.001
Albumin					-0.249	0.030
Soleus						
		Model 1		Model 2		Model 3
		R ² 0.450		R ² 0.528		R ² 0.603
Parameter	Beta Coeff.	p-value	Beta Coeff.	p-value	Beta Coeff.	p-value
Albumin	-0.677	<0.001	-0.620	<0.001	-0.547	<0.001
PTH			0.270	0.021	0.291	0.008
Age					0.283	0.011
Post-Leg						
Skin						
		Model 1		Model 2		
		R ² 0.304		R ² 0.456		
Parameter	Beta Coeff.	p-value	Beta Coeff.	p-value		
Hypertension	0.551	<0.001	0.455	0.001		
COPD			0.402	0.003		
Pretibial Skin						
		Model 1		Model 2		
		R ² 0.178		R ² 0.274		
Parameter	Beta Coeff.	p-value	Beta Coeff.	p-value		
Hemoglobin	-0.422	0.010	-0.356	0.025		
Age			0.317	0.045		

Beta coeff. – standardized coefficient

PTH – Parathyroid hormone, eGFR- Estimated glomerular filtration rate – using CKD-EPI equation,³⁵ COPD - Chronic obstructive pulmonary disease.

3.4 Discussion and Conclusions

In our study we found that there was a statistically significant increase in ^{23}Na accumulated in the tissues of dialysis patients compared to controls with normal kidney function. Evidence of sodium accumulation was observed even in CKD patients (even if not always statistically significant). No statistically significant difference was found in the ^{23}Na levels among CKD, PD and HD participants. In addition, we found significant associations between ^{23}Na levels and markers for mineral bone disease and inflammation. Even so, the major contributing parameters were tissue specific.

3.4.1 Age and Sex, and Sodium

In healthy controls, sex and age have been correlated to skin and muscle ^{23}Na levels. [9,38] Age has also been associated with higher ^{23}Na deposits in the skin of patients with mild CKD. [14] The effect of age on hemodialysis patients has been inconsistent. Dahlmann *et al.* [10] showed increased skin ^{23}Na in older subjects, while Fischereder *et al.* [20] did not observe an age-related effect. As demonstrated in Tables 3-2 and 3-3, age did correlate with ^{23}Na deposition in the posterior-leg skin, soleus and tibia, however this effect lost its statistical significance in the multivariable regression models. An opposite phenomenon was observed between age and pretibial skin. These inconsistencies suggest a need for larger in-depth studies.

There were no observed changes in ^{23}Na deposition in the tissues based on sex. Similarly, Fischereder *et al.* did not detect a difference in ^{23}Na deposition based on sex, in hemodialysis patients. [20] Even so, Schneider *et al.* showed that in patients with mild CKD, male sex was associated with significantly higher levels of skin ^{23}Na . [14] We postulate that the sex-effect may be overshadowed by the altered pathophysiology in severe CKD. Since the majority of our participants had CKD - Stages 4-5D, we speculate that in this population, CKD-related factors may supersede the effect of sex on non-osmotic ^{23}Na deposition in the tissues.

3.4.2 CKD and Sodium

Our study found an increase in ^{23}Na accumulation in dialysis and CKD patients relative to controls in all tissue types, not just skin and muscle. This is the first study that has reported on

measurement of sodium levels in osseous tissue with ^{23}Na MRI, and found increased sodium deposition even in this compartment.

While the groups were statistically different with regards to their measured serum sodium (Table 3-2), controls had the lowest tissue ^{23}Na concentrations despite having the highest serum sodium levels. This confirms that tissue and serum sodium are not correlated. Our skin and muscle ^{23}Na concentrations were similar in range to previously reported values (range of 10-50 mmol/L).[10,12,14]

Hammon *et al.* found similarly higher sodium accumulation in the skin and muscle of new-hemodialysis-start patients compared to healthy controls. [12] However, two other groups did not show a difference between ^{23}Na deposition in the skin and muscle of HD patients versus controls. [10,20] This discrepancy may be due to the different populations studied, with previous studies focusing solely on HD patients, as opposed to both HD and PD patients. Our study is the first one to include PD and CKD stage 4-5 patients. Yet, even when we compare the HD subgroup and controls, ^{23}Na in the soleus muscle and posterior leg skin is still significantly higher. All these studies are relatively small, involving less than 30 HD patients. Larger studies are needed to resolve the different findings.

Of note, while eGFR and creatinine had statistically significant correlations with tissue ^{23}Na (Table 3-2), when these parameters were added to the regression models, the *b*-coefficients were not statistically significant (data not shown). Furthermore, both of these parameters are difficult to interpret in the setting of dialysis, since both PD and HD will affect these values. As such, these data should be interpreted carefully. It is difficult to determine from our data if kidney function has a direct or indirect (by altering inflammation and other parameters) effect on sodium levels, or if these results are due to confounding factors.

3.4.3 Inflammation and Sodium

Our study did demonstrate significant correlations between the measured inflammatory markers (CRP, albumin and hemoglobin) and tissue ^{23}Na deposition – and all but two of these associations were statistically significant (Table 3-2). The correlations of inflammatory markers with muscle and bone and posterior leg skin ^{23}Na appear to be more robust – persisting even in the linear regression models. This finding is consistent with pre-existing literature, where non-osmotic sodium is closely related to inflammatory states and immune activity. [7,24,27,39-43]

Even so, these correlations were not adjusted for renal function, and their interpretation is limited.

3.4.4 Mineral Bone Disease and Sodium

The existence of non-osmotic sodium storage in osseous tissue has been known since the 1950s. [44-47] Even so, previous studies of ^{23}Na in humans have not looked at osseous tissue - due to the assumption that this ^{23}Na reservoir is less responsive to acute physiological and pathological changes. [48-49] Yet, CKD is a chronic process, allowing for ^{23}Na equilibration across the multiple ^{23}Na compartments. Furthermore, CKD patients often have altered mineral bone physiology due to mineral bone disease and increased bone turnover (except in patients with adynamic bone disease). [50-51] The presence of mineral bone disease (MBD) (and its variations) make bone ^{23}Na levels in this population a clinically relevant outcome.

In our study, we found that biomarkers of MBD had significant associations, not only with ^{23}Na deposition in the tibia, but also with non-osmotic ^{23}Na in the skin and muscle (Table 3-2). These associations persisted for soleus and tibia ^{23}Na even after adjusting for other confounders (Table 3-3). These associations can be explained by the existence of multiple sodium-dependent phosphate transporters in bone, renal and intestinal tissues, which are regulated by the parathyroid hormone, 1,25-dihydroxy-Vitamin D and fibroblast growth factor 23. [52-53] Thus, sodium and phosphate homeostasis are closely intertwined, and MBD markers may have a role in ^{23}Na deposition in tissues. Whether sodium levels in the bones is affected by the degree of mineral bone disease, or CKD level is still unclear.

3.4.5 Cardiopulmonary System and Sodium

CKD patients have high rates of cardiovascular mortality. [54-55] The correlation of skin ^{23}Na and cardiac findings in patients with CKD has been previously looked at by Schneider *et al.* [14] This group demonstrated that ^{23}Na in the skin of 99 patients with CKD stages 1-3 was associated with MRI evidence of left ventricular hypertrophy. [14] In our study, we used troponin T as a surrogate marker of cardiovascular health, since troponitis has often been associated with worse prognosis in CKD. [56-60] Increased troponin T levels were associated with higher non-osmotic ^{23}Na in all regions studied (Table 3-2), suggesting that ^{23}Na

accumulation may play a role in end-organ damage; potentially via its immune-modulating effects. [24,39-43] However, these associations were not statistically significant in our regression models. As such, based on current evidence, no clear conclusions can be derived on the link of tissue ^{23}Na and serum troponin T levels.

Primary and secondary hypertension (another cardiovascular risk factor) has often been associated with increased skin and muscle ^{23}Na in non-CKD patients. [8,9,26,61] In contrast, in patients with mild CKD (Stage 1-3), Schneider *et al.* only demonstrated a trend of higher skin ^{23}Na and hypertension, which did not reach statistical significance (p -value 0.07). [14] Our data shows a similar (albeit statistically significant) association between the clinical diagnosis of hypertension in CKD and ^{23}Na levels in the posterior-leg skin, soleus and tibia. Even so, we did not adjust for renal function and other potential confounders.

The association between COPD and sodium deposition in the posterior leg skin was an unexpected finding. We postulate four different hypotheses that may explain it. First, since patients with COPD have a significant history of smoking, and fat-soluble toxins can be stored in subcutaneous fat, this pro-inflammatory milieu may be responsible for the increased ^{23}Na skin content. Even so, no correlation was found between current or previous smoking history (latter defined as 20 pack-years or more) and tissue ^{23}Na (data not shown). Second, dialysis and CKD patients may have pulmonary edema which is misdiagnosis as COPD. [62] Increased pulmonary edema would suggest a sodium abundance and an increase use of non-osmotic sodium buffers. Third, sodium deposition may also occur in the airways of the respiratory system – causing inflammation and hyper-reactivity, which in the CKD population may be labelled as COPD. [62] Fourth, this finding could be secondary to chance (given the p -value of 0.05), or other types of bias.

Sodium deposition in the skin, muscle and bone has the potential to provide significant clinical information on different organ systems due to its many associations (be they a result of cause, consequence or confounding). More studies will be needed to unlock the full potential of this novel measurement.

3.4.6 Inter and Intra – Tissue Differences

Another important finding from our pilot study is that the ^{23}Na levels in each tissue appear to be associated with a unique combination of parameters. This finding is outlined in the

different parameters in the regression models in Table 2-3. While alterations in tibia and soleus ^{23}Na can be affected by albumin and PTH levels, these two parameters do not have a statistically significant effect on skin ^{23}Na . Bone and muscle ^{23}Na levels appear to have a stronger link with inflammatory and MBD markers, as evidenced by the higher correlation coefficients and lower p-values. Conversely, diabetes mellitus was found to only play a role in skin ^{23}Na .

Differences between tissues has been previously documented by Schneider *et al.* who found that skin but not muscle ^{23}Na was associated with left ventricular hypertrophy. [14] In addition, Wang *et al.* showed that men have higher accumulation of ^{23}Na in the skin vs muscle, while for women ^{23}Na accumulation has the opposite pattern. [15] In contrast, a number of other studies have demonstrated that skin and muscle ^{23}Na are similarly affected by diabetes mellitus, age, vascular endothelial growth factor levels, end-stage kidney disease, hyperaldosteronism and hypertension. [8,9,12,63] Compared to these latter studies, our study included a more extensive test for correlations between the ^{23}Na levels of the tissues and a variety of parameters. Furthermore, the previous studies' populations varied significantly, from healthy individuals and hypertensive patients, to patients on hemodialysis.

Our study is also unique in highlighting the heterogeneity of ^{23}Na deposition in skin. This heterogeneity can be qualitatively discerned with the naked eye (Fig. 3-4). Accounting for this effect, we intentionally looked at two different skin regions (pretibial skin and posterior leg skin), and the correlations between the ^{23}Na in these two regions with other parameters were quite variable (Table 3-2 and 3-3). In general, post-leg skin associations tended to be more statistically robust; e.g. hypertension was significantly associated with an increase in posterior leg skin ^{23}Na (correlation coefficient 0.547, p -value <0.001), but not with pretibial skin ^{23}Na (correlation coefficient 0.219, p -value 0.548). Such a wide difference in p -values suggest a true divergence, as opposed to a measurement error or a statistical accident.

Skin heterogeneity has not been reported in previous studies, the majority of which do not indicate if the ^{23}Na signal corresponds to a specific skin region. [8-10,14-15,22,64] Similar to this work, in their study, Kopp *et al.*⁶³ used the posterior leg skin in their measurements. Even so, expanded studies are needed to elucidate both the local factors in tissue ^{23}Na storage and the sodium signal (either the average signal or the one from a specific skin region) that best corresponds to clinically significant outcomes.

These inter and intra-tissue divergences alludes to the existence of both macro and micro-environmental factors that likely play a role in the regulation of non-osmotic sodium storage - via alterations in the GAG components of the extracellular matrix. [18-20] Potential factors include local dysregulation, inflammation and ischemia. [7,13,24,27,41-42]

3.4.7 Limitations

Our study did have several limitations. Since it was a cross-sectional observational study, causality effects cannot be determined. Measured and unmeasured confounding factors could be responsible for the differences in sodium levels of tissues, as well as the associations detected. Furthermore, we did not adjust for kidney function in our assessment of these associations.

As a pilot study, the number of subjects in our study groups was relatively modest (ranging from 10 to 23). Thus, generalizability of our data to all severe CKD patients should be done cautiously until larger studies can be conducted. Even so, several statistically-robust associations were discovered, despite the small sample size, adding validity to our results.

3.4.8 Discussion and Conclusions

In our study, we observed that dialysis patients had significantly higher ^{23}Na level in their skin, muscle and bone tissues compared to controls with normal kidney function. Even CKD patients (not on dialysis) had evidence of sodium accumulation - albeit not always statistically significant. No significant difference was found in the tissue ^{23}Na levels of HD, PD and CKD groups. These levels correlated strongly with markers of mineral bone disease and inflammation, while the effect of age and sex seemed relatively limited. Yet, associated factors appeared to be tissue specific. More studies are needed to elucidate the effect and clinical utility of non-osmotic sodium in tissues.

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Chapter 4

4 Conclusions and Future Work

This chapter contains a summary of the achievements in this thesis, as well as the limitations of this research. Furthermore, future directions of research will be explored.

4.1 Discussion

4.1.1 Summary of Thesis Work

Our study investigated non-osmotic tissue sodium levels in patients with different severity of kidney disease (CKD, PD and HD) compared to controls. The results indicated that patients on dialysis have statistically higher sodium levels in all three tissues: skin, bone and muscle. No difference was detected between subjects with CKD (not on dialysis) and subjects on dialysis, and CKD patients also showed trends toward sodium accumulation – albeit not always statistically significant, relative to controls.

Furthermore, we studied the associations between tissue sodium and previously recognized clinical factors in non-osmotic sodium build-up (see Chapter 2), as well as other complications of kidney disease. We did find significant correlations with inflammatory markers and mineral bone disease. The correlations with specific factors were found to be tissue specific: soleus and tibial sodium was strongly correlated with mineral bone disease and inflammatory markers, while skin sodium was better correlated with hypertension.

The main contribution of this study to the existing literature is additional information in the evolving field of non-osmotic sodium storage. The study adds to the current knowledge of NO-Na specifically in renal patients. Previous literature has looked at patients with mild to moderate levels of CKD and acute or chronic patients on hemodialysis. [1-3] Yet, our study is the first to report on patients with CKD 4-5 and on peritoneal dialysis. PD patients are often healthier than HD patients, due to the inherent independence required by this modality. Yet, despite our initial hypothesis that PD patients would have lower levels of tissue sodium because of their preserved residual renal function, no significant difference was observed based on dialysis modality. No statistically significant difference was observed in tissue sodium between

CKD and dialysis patients, either. Even so, there was a trend of lower sodium in CKD patients, which may reach statistical significance with larger sample sizes.

Similar to previous studies, we did find an association with CKD and increased tissue sodium. Yet, due to the observational nature of the study, causes or consequences cannot be ascertained.

Our methodology was similar to those of previous studies – namely utilizing ^{23}Na MRI technology to measure absolute sodium content. [4,5] This study does not help distinguish intra-cellular *vs* extra-cellular sodium, or bound *vs* unbound states. The ^{23}Na MRI pulse sequences detected sodium density levels. Even so, we do report on sodium levels in the tibia (osseous and bone marrow tissue) – as well as the previously reported muscle and skin tissue. Osseous sodium content measured with ^{23}Na MRI technology has not been previously described. Yet, given the physiology and the principles underlying this technology, we have no reason to suspect that sodium measurements in bone would be inaccurate.

This study also highlights the heterogeneity of non-osmotic sodium storage, both inter and intra-tissue. This finding is in keeping with the current view in the literature of an adaptable non-osmotic sodium reservoir based on glycosaminoglycan content. [6,7] Many different factors including the immune system, structural abnormalities, and ischemia (refer to section 2.5) could play a role in the micro-environment and regulate the capacity of this storage system. Furthermore, differences between sodium levels in the skin and muscle tissues has been previously noted. [1,8] The results from our study indicate that biochemical factors affecting sodium storage may be tissue-dependent - a relatively novel hypothesis.

Since this study looked at clinically significant markers – including laboratory markers and baseline characteristics, it highlights some of the possible interactions between non-osmotic sodium and these clinical factors in the study population. These associations are hypothesis-generating and may direct further investigations to shed light into the pathophysiology and consequences of non-osmotic sodium storage. In accordance with the published literature, which show an interdependent relationship between sodium and immune activation, [9-12] a link was demonstrated between inflammatory markers and sodium levels. Similar associations were found with markers of cardiovascular disease and bone mineral disease.

In current literature, osseous storage of sodium is believed to be more static. [13] Yet, due to the chronicity of CKD and dialysis, in our study, sodium stores in the tibia were also

noticed to be higher than controls. This fact could be explained by the increased metabolic activity of bone in CKD, due to mineral bone disease. Even so, our ability to perturb this osseous compartment with current treatments (diuretics or dialysis) is unclear. Similarly, more studies will be needed to assess the effect of treatments in sodium levels in the skin and muscle. Our study highlighted clinical correlations of non-osmotic sodium in patients with kidney disease and adds to the current body of knowledge. Yet, more studies will be needed to understand sodium metabolism and use it for diagnostic and therapeutic purposes in the future.

4.1.2 Study Limitations

This study also had several limitations. First, this was a pilot study, and as such, the study populations (Control, CKD, HD and PD) were relatively small – maximum of 13 subjects for HD. These population sizes make generalization of the results to all patients with CKD a cautious endeavor. Furthermore, the control group did have some comorbidities (for e.g. diabetes mellitus and hypertension), which do affect sodium metabolism and sodium deposition (potentially decreasing the measured effect from kidney function). While it is reassuring that statistically significant trends were detectable even with these sizes and a control group that was not completely healthy, these results need to be demonstrated in larger clinical trials. As a pilot study, the purpose of this research was to roughly test the validity of our hypothesis, before more resource-intensive studies are undertaken. Large studies would be quite financially burdensome – since MRI technology is a relatively expensive imaging modality.

Second, certain aspects of our methodology could be strengthened. The power of the study could have been increased by having completely healthy controls, or sex and age - matching the control group to the patient groups. In addition, our assessment of osseous sodium levels included the bone marrow tissue. As such, the sodium signal detected consists of the average from both these tissues. Ideally these two tissues should be looked at separately, in future studies. Furthermore, when estimating the kidney function of dialysis patients, instead of using creatinine and eGFR, a more appropriate measure, such as the average of urea and creatinine clearances (from 24hour urine collections) could be used. Also, the associations and regression models between tissue sodium and other parameters did not adjust for potential confounding factors. As such, in future studies, more detailed statistical analysis will be necessary to try and assess the main parameters that play a role in tissue sodium.

Thirdly, as an observational study, this research has the inherent limitations of this genre of research. Primarily, causality cannot be established, and only associations (prone to confounding factors) can be generated. Randomized control trials would be the ideal study for showing causality, but unfortunately, these studies would be challenging and unethical to perform in humans in clinical settings. A proper randomized trial would require randomizing individuals to different levels of CKD, and then measuring sodium levels in the tissues – an unethical process given the high burden of morbidity which would be carried by participants.

Fourthly, similar to other clinical studies, sampling error in the population induces further bias. The patient population willing to participate in clinical studies tends to often be comprised of healthier individuals with the capacity to give consent and travel to the research facility. For e.g., since for our specific study participants needed to be able to lie flat for one hour in the MRI scanner, CKD patients with severe pulmonary edema (likely from salt and fluid overload) could not participate. Also, having age and sex matched healthy controls may have increased the power of the study (as mentioned above). The controls were chosen with the aim of expediency given this was a pilot study. Even so, the participant requirements in our study were relatively not cumbersome, and low risk. As such, we do not suspect a significant selection bias.

Lastly, our study methodology did not include measuring sodium content with previously used gold standard techniques, and we did not assess tissue water content. Unfortunately, gold-standard sodium measurements tend to be relatively invasive (requiring tissue biopsies) or exposures to radiation. [4,14-18] Both of these properties often deter subjects from study participation. Furthermore, since multiple previous studies have validated sodium MRI measurements against these gold-standards, [4,19,20] we opted to avoid further undue risks imposed on the study population. On the other hand, we also did not assess tissue water content, since previous studies [21] have demonstrated that sodium accumulation exceeds water increases. Since in this pilot study, our MRI scan time was already 1 hour long – in the setting of multiple measurements averaged to increase SNR for sodium measurements, adding sequences to measure water content would have increased scan time. More robust investigations should be performed in follow up studies.

4.2 Future Work

This section focuses on the future work and other areas that need to be further explored to expand our understanding and knowledge of sodium accumulation in CKD patients.

4.2.1 Cohort Expansion and Clinical Utility

In order to learn more about the effect of kidney function on the sodium deposition in the tissues, larger studies are needed that include patients with all the different stages of CKD (1-5D), and with larger population sizes. As mentioned in Section 1.4.2, the methodology of the study could also be improved by ensuring the control group is completely healthy, or age, sex, BMI-matched controls to the patient groups. Furthermore, more detailed statistical and image analysis could help assess for confounding factors and differentiate between osseous and bone marrow sodium levels. 24hour urine collections can be used to better estimate the residual renal function in dialysis patients. Future studies could also include patients on home hemodialysis or those who have received a renal allograft (both populations have yet to be studied). Since CKD is a big umbrella term for several different pathophysiological mechanisms of injury in the kidney, the effect of the etiology of CKD, and its unique pattern of injury, on sodium accumulation should also be explored. Similarly, acute kidney injury can also result in severe impairment – sometimes requiring renal replacement therapy. This type of injury has also been shown to result in sodium accumulation and needs to be studied further. [3] In addition, the ability of our current treatments (medical or dialysis) to affect the different tissue sodium reservoirs also will need to be studied further. Lastly, the clinical relevance of sodium accumulation will need to be explored in future studies (including causes and consequences). All this information could be utilized clinically to direct CKD patient treatments and provide information on their prognosis. For e.g., dialysis treatments can be individualized to patients' sodium accumulation patterns – by better understanding a patient's intrinsic pattern of sodium accumulation and how dialysis alters this pattern.

4.2.2 Mechanistic Work

As mentioned above, clinical observational studies cannot prove causality, limiting their scientific potential. Thus, further studies are required - using animal models to determine the

direct effect of kidney function (and the different dialysis modalities) on sodium accumulation in the tissues. Furthermore, while some studies have already explored the mechanisms governing this sodium accumulation (Section 1.5), more information is needed to assess if these mechanisms are altered by the uremic toxin-rich environment present in CKD (especially in severe stages). These studies will need to cover a broad spectrum: from cellular-level studies assessing the effect of cellular signaling pathways, to clinical studies looking at the effect of dialysis modalities on tissue sodium fluctuations.

4.2.3 Technological Advancement

In addition, sodium MRI technology is still relatively limited. Due to its low signal-to-noise ratio, large period of scan time is dedicated to obtain limited information on a sample of the anatomy (in this study, a single axial slice of the lower leg). Further advancement in this imaging technology is required to increase SNR and decrease scan time, so that 3-dimensional anatomical images can be obtained. Such advancement could include using a stronger external magnetic field (for e.g. 7T MRI), and improving the sequences to shorten acquisition time.

As mentioned in Section 2.4.6, it is incorrect to assume homogeneity of sodium accumulation in all areas of the skin, likely from the role of the micro-environment in regulating sodium storage. As such, more complete images may help identify specific patterns of sodium storage across different tissues, which may be further associated with both healthy and diseased states. Ideally, ^{23}Na -MRI will become an additional imaging tool in routine clinical use (as opposed to just research settings), to help improve diagnosis and treatment of patients.

4.3 Conclusions

This thesis was a pilot observational study measuring non-osmotic sodium storage in tissues of CKD patients (stages 4-5, HD and PD) compared to controls with normal kidney function, using non-invasive ^{23}Na MRI technology. We observed that dialysis patients had significantly higher ^{23}Na level in all tissues studied: skin, muscle and bone, compared to controls. No statistically significant difference was noted between the three different patient groups (CKD, HD and PD), despite different levels of residual renal function. Even CKD participants had evidence of sodium accumulation - albeit not always statistically significant. Yet, there was a trend of lower sodium levels in CKD participants compared to dialysis ones.

Sodium levels were also correlated with different markers of CKD and other factors previously known to be associated with higher non-osmotic sodium storage. Soleus and tibia sodium levels were strongly associated with markers of mineral bone disease and inflammation, while the effect of age and sex was relatively limited. Even so, significant inter and intra-tissue heterogeneity was observed, and associated factors appeared to be tissue specific. This finding points toward an adaptable non-osmotic sodium compartment that can be influenced by local and global factors.

This study is the first to look at patients on peritoneal dialysis and severe CKD (stages 4-5), in addition to HD patients. Furthermore, it used ^{23}Na MRI to assess multiple tissues, including the osseous compartment, which has not been previously reported. Our findings add to our current understanding of non-osmotic sodium, particularly in the relatively sodium-abundant CKD states, while also generating hypothesis for future studies.

Non-osmotic sodium measured non-invasively with MRI technology has a lot of potential for diagnostic, therapeutic and prognostic applications. Yet, more studies are needed for technological advancement and to better understand sodium metabolism and storage, its causes and effects, before this potential can be harvested.

4.4 References

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Appendices

Appendix A: Institutional Research Ethics Board Approval



Research Ethics

Western University Health Science Research Ethics Board HSREB Amendment Approval Notice

Principal Investigator: Dr. Christopher McIntyre

Department & Institution: Schulich School of Medicine and Dentistry\Medical Biophysics, London Health Sciences Centre

Review Type: Full Board

HSREB File Number: 108765

Study Title: Evaluation of Sodium Deposition in Soft Tissues of Patients with Kidney Disease and its Association with Patient Symptomatology

HSREB Amendment Approval Date: September 22, 2017

HSREB Expiry Date: January 06, 2018

Documents Approved and/or Received for Information:

Document Name	Comments	Version Date
Revised Western University Protocol	Revised Romeo Protocol - Clean	2017/08/30
Revised Letter of Information & Consent	LOI + C - clean	2017/08/30
Sponsor Protocol	Study Protocol - Clean	2017/08/30

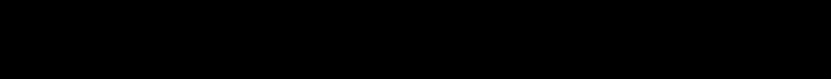
The Western University Health Science Research Ethics Board (HSREB) has reviewed and approved the amendment to the above named study, as of the HSREB Initial Approval Date noted above.

HSREB approval for this study remains valid until the HSREB Expiry Date noted above, conditional to timely submission and acceptance of HSREB Continuing Ethics Review.

The Western University HSREB operates in compliance with the Tri-Council Policy Statement Ethical Conduct for Research Involving Humans (TCPS2), the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use Guideline for Good Clinical Practice Practices (ICH E6 R1), the Ontario Personal Health Information Protection Act (PHIPA, 2004), Part 4 of the Natural Health Product Regulations, Health Canada Medical Device Regulations and Part C, Division 5, of the Food and Drug Regulations of Health Canada.

Members of the HSREB who are named as Investigators in research studies do not participate in discussions related to, nor vote on such studies when they are presented to the REB.

The HSREB is registered with the U.S. Department of Health & Human Services under the IRB



James Street on behalf of Dr. Marcelo Remenchaksky, HSREB Vice Chair

EO: Erika Basile ___ Grace Kelly ___ Katelyn Harris ___ Nicola Morphet ___ Karen Gopaul ___ Patricia Sargeant ✓



Date: 7 December 2017

To: Christopher McIntyre

Project ID: 108765

Study Title: Evaluation of Sodium Deposition in Soft Tissues of Patients with Kidney Disease and its Association with Patient Symptomatology

Application Type: Continuing Ethics Review (CER) Form

Review Type: Delegated

Full Board Reporting Date: December 19, 2017

Date Approval Issued: 07/Dec/2017

REB Approval Expiry Date: 06/Jan/2019

Dear Christopher McIntyre ,

The Western University Research Ethics Board has reviewed the application. This study, including all currently approved documents, has been re-approved until the expiry date noted above.

REB members involved in the research project do not participate in the review, discussion or decision.

Western University REB operates in compliance with, and is constituted in accordance with, the requirements of the TriCouncil Policy Statement: Ethical Conduct for Research Involving Humans (TCPS 2); the International Conference on Harmonisation Good Clinical Practice Consolidated Guideline (ICH GCP); Part C, Division 5 of the Food and Drug Regulations; Part 4 of the Natural Health Products Regulations; Part 3 of the Medical Devices Regulations and the provisions of the Ontario Personal Health Information Protection Act (PHIPA 2004) and its applicable regulations. The REB is registered with the U.S. Department of Health & Human Services under the IRB registration number IRB 00000940.

Please do not hesitate to contact us if you have any questions.

Sincerely,

Kelly Patterson, Ethics Officer, on Behalf of Dr. Joseph Gilbert, HSREB Chair

Note: This correspondence includes an electronic signature (validation and approval via an online system that is compliant with all regulations).

Curriculum Vitae

Name: ELENA QIRJAZI, BAsc.(Hon), MD, FRCPC

EDUCATION

- 2016- current** **Master's Program – Medical Biophysics**
Western University, London, ON
- 2016- 2018** **Home Dialysis Fellowship**
Western University, London, ON
- 2016- 2018** **Clinical Investigator Program – Nephrology Research Fellow**
Western University, London, ON
- 2014- 2016** **Clinical Nephrology Fellowship**
Western University, London, ON
- 2011- 2014** **Internal Medicine**
Western University, London, ON
- 2007-2011** **Doctor of Medicine Program**
University of Toronto, Toronto, ON
- 2003-2007** **Bachelor of Applied Science (Honours) - Engineering Science**
University of Toronto, Toronto, ON

LICENSURE AND CERTIFICATION

- 2016** **Fellow of the Royal College of Physicians of Canada - Nephrology**
- 2015** **Fellow of the Royal College of Physicians of Canada - Internal Medicine**
- 2015** **Fellow of the American Board of Internal Medicine - Internal Medicine**
- 2012** **Licentiate of the Medical Council of Canada**

AWARDS & HONOURS

- May 2017** **CIHR CGS Masters Award (1 year) (*accepted*) – Medical Biophysics Western University**
- April 2016** **Clinical Investigator Program Funding (2 years) (*accepted*) – Medical Biophysics Western University**

PUBLICATIONS & PRESENTATIONS

PUBLICATIONS

- May 2019** *Renal perfusion during hemodialysis: Intradialytic blood flow decline and effects*

- of dialysate cooling*– Raanan Marants, ELENA QIRJAZI, Claire J Grant, Ting Y Lee, Christopher W McIntyre. JASN May 2019
- Aug 2016** *Risk of ventricular arrhythmia with citalopram and escitalopram – A population-based study* – ELENA QIRJAZI, Eric McArthur, Danielle M Nash, Stephanie N Dixon, Matthew A Weir, Akshya Vasudev, Lorne Gula, Matthew J Oliver, Ron Wald, Amit X Garg. PLoS One 2016; 11(8): e0160768.
- Jan 2015** *Cardiac, renal, and central nervous system dysfunction with eosinophilia: eosinophilic granulomatosis with polyangiitis* – Juliya Hemmett, ELENA QIRJAZI, Matthew A Weir, Madeleine Moussa, Yves D Landry, Lakshman Gunaratnam. Lancet 2015; 385-480.
- Mar 2010** *Essentials of Clinical Examinations Handbook (6th Edition). Lincoln M and McSheffrey G (chief editors); Monique Martin, ELENA QIRJAZI and Bertha Wong – co-writers of “Essentials of pain management” chapter, pgs 515-529.*

PRESENTATIONS

POSTER PRESENTATIONS

- Dec 2018** *Improving calcium/phosphate/PTH levels in peritoneal dialysis.* Ryan Marinovich, H. Wang, Robin Wigen, Nancy Woodcock, Arsh Jain, ELENA QIRJAZI; Institute for Healthcare Improvement: National Forum, Orlando, USA.
- Oct 2018** *Hepatic response to cooler hemodialysis.* ELENA QIRJAZI, Ranaan Marants, Megan A. Mio, Brad Urquhart, Ting Lee, Christopher W. McIntyre; American Society of Nephrology – Kidney Week, San Diego, USA.
- Oct 2018** *Viability and fidelity of low-cost gelatin percutaneous renal biopsy phantom in simulation training.* ELENA QIRJAZI, Ian Y. Chan, Juliya Hemmett, Faisal Rehman, Roya Etemad-Rezai; American Society of Nephrology – Kidney Week, San Diego, USA.
- Oct 2018** *Residual renal function loss in hemodialysis patients: Is kidney stunning the culprit and can dialysate cooling help?* Ranaan Marants, ELENA QIRJAZI, Claire Grant, Ting Lee, Christopher W. McIntyre; American Society of Nephrology – Kidney Week, San Diego, USA.
- Oct 2018** *Development of a microparticle-based bio-marker of hemodialysis induced vascular injury.* Janice Gomes, Claire Grant, ELENA QIRJAZI, Hon S. Leong, Christopher W. McIntyre; American Society of Nephrology – Kidney Week, San Diego, USA.
- Oct 2018** *Understanding the effects of haemodialysis on blood flow: An imaging-mathematical modeling study.* Sanjay Kharche, Ranaan Marants, ELENA QIRJAZI, Andrea Kassay, Jermiah Joseph, Kierra D. McDougall, Ting Lee, Christopher W. McIntyre; American Society of Nephrology – Kidney Week, San Diego, USA.
- May 2018** *Improving calcium/phosphate/PTH levels in peritoneal dialysis patients;* Ryan Marinovich, Henry Wang, Robin Wigen, Nancy Woodcock, Arsh Jain, ELENA QIRJAZI; International Society of Peritoneal Dialysis – Vancouver, Canada.
- May 2017** *Successful integration of a simulated goals of care workshop into nephrology curriculum;* Juliya Hemmett, ELENA QIRJAZI (co-first author), Faisal Rehman, Valerie Schultz, Norman Muirhead; Canadian Society of Nephrology – Montreal, Canada.

- Nov 2016** *Improving comfort with comfort care discussions; Juliya Hemmett, ELENA QIRJAZI (co-first author), Faisal Rehman, Valerie Schultz, Norman Muirhead; American Society of Nephrology – Kidney Week, Chicago, USA.*
- May 2016** *Calcineurin-inhibitor related severe adverse drug reaction responsible for complicated course of childhood membranous glomerulonephritis; Amrit Kirpalani, ELENA QIRJAZI, Michael Reider, Kevin Bax, Guido Filler; Pediatrics Research Day, Western University, London, Canada.*
- Mar 2016** *Mind the Gap: A systemic approach to Morbidity and Mortality Rounds in Nephrology; Ghada Ankawi, Juliya Hemmett, Kathleen Quinn, ELENA QIRJAZI (4 co-authors), Louise Moist; London Health Sciences Centre Research Day, London, Canada.*
- May 2015** *Risk of ventricular arrhythmia with citalopram and escitalopram- A Population Based Study; ELENA QIRJAZI, Danielle M Nash, Eric McArthur, Stephanie N Dixon, Matthew A Weir, Akshya Vasudev, Matthew Oliver, Ron Wald, Lorne Gula, Amit X Garg; Medicine Research Day, Department of Medicine, Western University, London, Canada.*

ORAL PRESENTATIONS

- Oct 2018** *Sodium concentration in tissues of dialysis patients. ELENA QIRJAZI, Alireza Akbari, Timothy J. Scholl, Christopher W. McIntyre; American Society of Nephrology – Kidney Week, San Diego, USA.*
- Jun 2018** *Viability and efficacy of low-cost gelatin percutaneous renal biopsy phantom in simulation training. Ian Y. M. Chan, ELENA QIRJAZI, Juliya Hemmett, Faisal Rehman, Roya Etemad-Rezai; London Imaging Discovery Day, London, Canada.*
- Dec 2017** *Managing fluid status and blood pressure in dialysis patients; ELENA QIRJAZI; Nephrology Grand Rounds, Division of Nephrology, University of Calgary, Calgary, Canada. (Invited presentation)*
- May 2017** *Educational workshop on goals of care discussions in nephrology; Juliya Hemmett, ELENA QIRJAZI (co-first author), Faisal Rehman, Valerie Schultz, Norman Muirhead; Medicine Research Day, Department of Medicine, Western University, London, Canada.*
- Aug 2006** *Mass Spectrometry Based Flow Cytometry; ELENA QIRJAZI, Scott Tanner (Assoc.Prof.), Vladimir Baranov (PhD), Olga Ornatsky (PhD), and Robert Kinach; Undergraduate Engineering Research Day, Faculty of Engineering, University of Toronto, Toronto, Canada.*

RESEARCH

July 2016-Current Research Investigator – Master Student

London Health Sciences Centre

Supervisor: Dr. Christopher McIntyre (MD, PhD)

Project: Pathophysiology in chronic kidney disease patients.

- Investigating if liver dysfunction and endotoxin build-up during hemodialysis could be prevented with cooler dialysate.
- Using novel Sodium MRI technology to assess the level of sodium in CKD patients (bone, muscle and skin).

May 2013-June 2015 Research Investigator

London Health Sciences Centre

Supervisor: Dr. Amit Garg (MD, PhD)

Project: SSRI use and incidence of ventricular arrhythmia

- A population based study investigating the incidence of ventricular arrhythmia in patients who initiate treatment with citalopram or escitalopram compared to ones started on referent selective serotonin re-uptake inhibitors (paroxetine or sertraline)

Jun-Aug 2009 Research Investigator

Faculty of Medicine, Discovery Commons, University of Toronto

Supervisor: Dr. Patricia Stewart (PhD)

Co-investigators: Dr. A Hyman (PhD), J Koecher

Project: Understanding the effects of recorded lectures on undergraduate medical student learning and performance

- Developed an online survey to assess the opinions of medical students on lecture capturing and its effect in their education
- Analyzed data over the use of recorded medical school lectures posted on Blackboard

Sept 2008 – May 2009 Research Investigator

Faculty of Medicine & Princess Margaret Hospital, Toronto

Supervisor: Sarah McBain

Co-investigators: Dr. J Trachtenberg, Dr. A Finelli, Dr. A Matthew (PhD)

Project: Erectile dysfunction information-seeking behaviours of prostate cancer patients up to one year post-radical prostatectomy, their partners and patient advocates (Pilot Study)

- Investigated the information-seeking behaviours of prostate cancer patients up to one-year post-radical prostatectomy on the topic of erectile dysfunction
- Compared these behaviours to those of their partners and prostate cancer patient advocates

May 2006 – May 2007 Research Investigator

Institute of Biomaterials and Biomedical Engineering, University of Toronto

Supervisor: Prof. Scott Tanner

Project: Evaluating the novel ICP-MS immunological detection system relative to fluorescent methods by measuring Phospho-STAT3 levels in mouse embryonic stem cells.

- Performed experiments to determine the labeling efficiency of antibodies labeled with elemental metals
- Compared the labeling efficiency of the Phospho-STAT3 molecule found in mouse stem cell, using the novel ICP-MS immunological detection system, with that of the fluorescent standard

EXTRACURRICULAR ACTIVITIES

Leadership Activities

- Sept 2016 – Current Quality Improvement initiative in Peritoneal Dialysis: QI supervisor**
Mentoring medical students through a quality improvement project in Mineral Bone disease management in Peritoneal Dialysis, London
- Sept 2017 – July 2018 Quality Improvement initiative in Hemodialysis: QI supervisor**
Mentoring medical students through a quality improvement project in Mineral Bone disease management in in-centre

hemodialysis, London

Jan 2016 – July 2016 Goals of Care Discussions in Nephrology Workshop: Co-organizer
Organized a workshop teaching nephrology fellow about goals of care discussions with patients and their families, London

Teaching and Educational Activities

Sept 2017 – May 2018 Glomerulonephritis Workshop - Canadian Society of Nephrology 2018
Developing the kidney biopsy simulation section of the Glomerulonephritis Workshop for the Canadian Society of Nephrology pre-course in May 2018

Apr –May 2016 & 2017 Genitourinary System Teaching and Marking for Medical Students –
Faculty of Medicine, Western University