DIETARY POTASSIUM EFFECTS ON BLOOD PRESSURE AND WHOLE-BODY RETENTION OF POTASSIUM, SODIUM, AND CALCIUM FROM A CONTROLLED FEEDING STUDY IN PRE-HYPERTENSIVE-TO-HYPERTENSIVE ADULTS

by

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"Among other things, you'll find that you're not the first person who was ever confused and frightened and even sickened by human behavior. You're by no means alone on that score, you'll be excited and stimulated to know. Many, many men have been just as troubled morally and spiritually as you are right now. Happily, some of them kept records of their troubles. You'll learn from them if you want to. Just as someday, if you have something to offer, someone will learn something from you. It's a beautiful reciprocal arrangement. And it isn't education. It's history. It's poetry."

-J.D. Salinger.

For Tony D. DeGeorge, for instilling in me the value of education and the pursuit of knowledge.

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To my committee, especially my current advisor, for all of their guidance and support. To my family, my parents and sister, for their unconditional love. And to VK, for being the hot fudge to my halibut.

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ABSTRACT

Potassium is an essential nutrient, that has been labeled a shortfall nutrient by recent Dietary Guidelines for Americans Advisory Committees. Increases in potassium intake have been linked to improvements in cardiovascular and other metabolic health outcomes. Blood pressure (BP) has often been cited as the primary criterion for determining potassium requirements. Hypertension (HTN), or high BP, is a primary risk factor for cardiovascular disease and other circulatory diseases. Cardiovascular disease (CVD) is responsible for the 31% of deaths worldwide. Findings from the Agency for Healthcare Research and Quality report (which informed the recently released Dietary Reference Intakes for sodium (Na) and potassium) on potassium intake and chronic disease concluded, with a moderate strength of evidence, that increasing potassium intake decreases BP, particularly among those with HTN. Although, of the 18 randomized controlled trials assessed by the AHRQ, only 4 were dietary interventions, the rest involved potassium supplementation. Observational studies also show a consistent bone benefit with increased potassium rich fruit and vegetable intakes in cohorts spanning adolescents to the elderly. In clinical trials, higher potassium intakes through supplementation have been associated with reduced urinary calcium (Ca) excretion and improvement in Ca balance. Although, similar to BP, intervention trials assessing the impact of dietary potassium on bone are lacking. Controlled feeding studies looking specifically at increases in potassium from food are sparse, leaving a large knowledge gap in the field for a nutrient with an important potential health impact. In general, little is known about whole-body potassium retention, with the few studies conducted lacking consistency and rigor in methods and design. What potassium retention means in terms of adequacy, or how higher or lower retention may influence specific health outcomes is understudied and not well understood.

Utilizing a randomized, cross-over, controlled feeding clinical study with complete metabolic balance measures, our research aims to begin filling these gaps, looking specifically at the effects of potassium intake via potato sources and a potassium supplement on BP and vascular outcomes, as well as how the source of potassium may influence potassium, Na and Ca whole-body balance.

This dissertation will discuss the physiology of potassium intake, how this may affect potassium, Na, and Ca retention, and in turn what influence this has on vascular and bone related

health outcomes. Overall the goal of this research is to address the question: What is the importance of dietary potassium, and how can it benefit cardiovascular and skeletal health?

CHAPTER 1: INTRODUCTION

Portions of this review have been published elsewhere:
Stone, M.S., L. Martyn, and C.M. Weaver, *Potassium Intake, Bioavailability, Hypertension, and Glucose Control.* Nutrients, 2016. **8**(7).
Weaver, C.M., Stone, M. S., et al., *What Is the Evidence Base for a Potassium Requirement?* Nutr Today, 2018. **53**(5): p. 184-195.
Stone, MS, Weaver, CM. Potassium Intake, Metabolism, and Hypertension. *SCAN's Pulse* 2018. 37: 5-10.

Potassium is an essential nutrient, that has been labeled a shortfall nutrient by recent Dietary Guidelines for Americans Advisory Committees. [1, 2]. Physiologically, potassium is the most abundant cation in intracellular fluid where it plays a key role in cell function, maintaining intracellular fluid (ICF) volume and transmembrane electrochemical gradients [3]. Because potassium is a major intracellular ion, it is widely distributed in foods once derived from living tissues. Potassium concentration is highest in fruits and vegetables, but can also be quite high in cereals, grains, dairy and meat [3, 4]. The evolution of dietary practices in the U.S. over the last several decades, and more recently worldwide, have seen a higher intake of low nutrient density convenience foods, coupled with decreased consumption of fruits and vegetables, leading to a diet lower in potassium and higher in sodium [5]. The average intake of potassium of U.S. adults participating in the National Health and Nutrition Examination Survey (NHANES) 2013-2014 was 2668 mg/d, below the adequate intake (AI) of 3000mg/d set forth by the 2019 DRI committee, and well below the previous AI of 4700mg/d [6-8]. This chapter will give a comprehensive overview of potassium as a nutrient, the physiology of how it moves through the body including potassium storage and excretion, and how this may affect vascular pressure, the movement of sodium and calcium throughout the body, and the health consequences of these relationships.

Internal Balance of Potassium

About 90% of dietary potassium (K+) is passively absorbed in the small intestine. In the proximal small intestine (duodenum, jejunum) K+ absorption primarily follows water

absorption, while distal (ileum) movement is more influenced by changes in trans-epithelial electrical potential difference [9]. In the colon, K+ is both endogenously secreted, in exchange for sodium (Na+), as well as absorbed via H+/K+ ATPases. Total body K+ is estimated to be approximately 43 mEq/kg in adults, with only 2% of this found in the extracellular fluid (ECF). Most of the body K+ content is found in the intracellular space of skeletal muscle. Potassium is the primary intercellular cation and plays a key role in maintaining cell function, influencing the transmembrane electro-chemical gradients of each cell [10]. The gradient of K+ across the cell membrane determines cellular membrane potential, which, based on the normal ratio of intracellular to extracellular K+, is −90 mV. This potential difference is maintained in large part by the ubiquitous ion channel, the sodium-potassium (Na+/K+) ATPase pump. Transmembrane electro-chemical gradients cause the diffusion of Na+ out of the cell and K+ into the cell. This process is reversed, and cellular potential difference is held constant, via the Na+/K+ ATPase pumps. When activated, the Na+/K+ ATPase pump exchanges two extracellular K+ ions for three intracellular Na+ ions, influencing membrane potential based on physiological excitation or inhibition. These channels are partially responsible, along with the Na+/K+ chloride (Cl-) symporter, and Na+-calcium (Ca2+) exchanger (NCC), for maintaining the potential difference across the resting cell membrane as well. Both resting membrane potential and the electro-chemical difference across the cell membrane are crucial for normal cell biology, especially in muscle and nervous tissue [3, 10, 11].

Distribution of K+ under normal physiological conditions is referred to as internal balance, which is different from whole body balance or retention that will be discussed later in detail. In healthy individuals, blood K+ concentration ranges between 3.5-5.5 mmol/L, with numerous homeostatic mechanisms in place for maintenance within this narrow margin. Changes in plasma concentrations of K+ alter the electro-chemical gradient and can lead to physiological dysfunction. In hyperkalemia, when K+ concentration exceeds 5.5 mmol/L, membrane depolarization can lead to muscle weakness, paralysis, cardiac dysrhythmias (e.g. sinus bradycardia, ventricular tachycardia, ventricular fibrillation), and death in severe cases. Conversely hypokalemia, when K+ plasma concentration is below 3.5 mmol/L, can cause membrane hyperpolarization, interfering with normal nerve and muscle function leading to muscle weakness and decreases in smooth muscle contraction [12]. Hypokalemia can also cause both atrial and ventricular cardiac dysrhythmias, as well as lead to paralysis, and if left untreated,

death. As mentioned previously, approximately 98% of systemic K+ is found intercellularly, stored primarily in muscle (70%) and to lesser extent bone, liver, skin, and red blood cells. Insulin, catecholamines, acidemia, and osmolarity all effect the transcellular distribution of K+ between plasma and cells [3, 11].

In response to dietary consumption of a high K+ meal, pancreatic beta cells are activated to increase production and release of insulin. Insulin enhances the uptake of K+ via stimulation of Na+/K+ ATPase activity, independent of effects on glucose uptake, in skeletal and cardiac muscle, adipocytes, liver, bone and red blood cells, attenuating postprandial rise in plasma K+ [13]. Na+/K+ ATPase pump activity and expression is also influenced by stimulation of both α and β2 adrenergic receptors by circulating catecholamines (epinephrine, norepinephrine) [10, 11]. Subsequently, alterations in these hormone levels can affect cellular ion movement and serum K+. Stimulation of both β2 adrenergic and insulin receptors may lead to a synergistic cellular influx of K+ in part because insulin release from beta cells is increased via β2 adrenergic stimulation, as well as catecholamine related increases in glycolysis leading to increases in blood glucose. The insulin mediated regulatory pathway leads to Na+/K+ ATPase activation via stimulation of cell surface tyrosine kinase receptors (insulin substrate receptor-1; IRS1), which also stimulates the translocation of intracellular glucose transport proteins (GLUT4 in muscle) facilitating the influx of glucose into the cell. Downstream activation of signaling cascades involving IRS1-phosphatidylinositide-3-kinase (PI3-K) and protein kinase A (Akt) facilitate both K+ and glucose uptake [11]. Catecholamine binding to β2 adrenergic receptors activates pathways mediated by cyclic adenosine-mono-phosphate (cAMP) and protein kinase A (PKA) to increase Na+/K+ ATPase activity and cellular K+ uptake. In contrast, stimulation of α1 and α2 adrenergic receptors, primarily through increased circulating levels of the stress hormone norephinephrine, lead to activation of hepatic Ca2+ dependent K+ channels and increased plasma K+ concentration via K+ release from the liver. Aldosterone, a hormone primarily involved in the renal handling of K+, may also influence transmembrane distribution of K+ via stimulation of cellular Na+ uptake through activation of Na+/H+ or Na+/K+/Cl- transporters and subsequently Na+/K+ ATPases [11, 12]. While hormones play an important role in the movement of K+ within the body, the concentration of other ions (inorganic and organic) are also influential in maintaining proper internal balance [3].

Metabolic acidosis caused by inorganic anions (mineral acidosis) can also stimulate K+ movement. The effect of acidemia on enhancing cellular K+ loss is not related to direct K+-H+ ion exchange, but rather via action on transporters which normally regulate skeletal muscle pH [14]. The decrease in extracellular pH reduces the rate of Na+/H+ exchange and inhibits Na+/bicarbonate (HCO3-) cotransport. The fall in intracellular Na+ reduces Na+/K+ ATPase activity, leading to decreased K+ influx, cellular K+ loses, and possible hyperkalemia [10]. Additionally, a fall in extracellular HCO3- increases inward flux of Cl- via upregulation of Cl-/HCO3- exchange, increasing K+/Cl- cotransport and subsequent K+ efflux. In metabolic acidosis via organic anion (e.g. lactic acid) accumulation, loss of K+ from the cell is much smaller. Accumulation here, through movement of both anions and H+ through monocarboxylate transporters (MCT; MCT1, MCT4), leads to a lower intracellular pH, stimulating the movement of Na+ via Na+/H+ and Na+/HCO3- transporters. An increase of intracellular Na+ maintains Na+/K+ ATPase activity, limiting the efflux of K+. Generally, metabolic acidosis (inorganic or organic) causes greater K+ efflux than respiratory acidosis, HCO3- being the primary anion accumulating in the cell to balance the influx of hydrogen ions [15]. Movement of cellular K+ varies similarly in response to different types of physiological alkalosis as well. In respiratory alkalosis, K+ influx is reduced compared to metabolic alkalosis, due to the efflux of cellular HCO3- [3].

Renal Potassium Handling (External Balance)

The majority of K+ consumed is excreted in the urine, with the remaining excreted in the stool, and, under homeostatic conditions, a variable amount in sweat [16]. Potassium has a higher ratio of dietary intake to extracellular pool size; recall only 2% of the total body K+ is distributed in ECF with the remaining distributed in the ICF of various tissues. To meet the challenge of a high K+ meal, the K+ homeostatic system is very efficient at clearing plasma K+ via an increase in renal excretion. When dietary K+ intake increases or decreases the kidneys modulate excretion accordingly, ensuring the maintenance of plasma K+ concentration. Potassium homeostasis is also thought to be regulated by an enteric K+ sensor. This sensor is proposed to be located in the splanchnic region of the gastrointestinal tract, and utilizes a feedforward mechanism to increase K+ renal excretion after a dietary K+ dose, independent of plasma K+ concentration or hormonal

control [10, 17]. In addition, with the administration of acute K+ loads, only approximately half of the dose appears in the urine after 4–6 h, suggesting that extrarenal tissues (e.g., muscle, bone, liver, adipose) play an important role in K+ homeostasis as well via insulin and catecholamine uptake [18, 19]. Excessive extra-renal K+ losses are usually small, but can occur in individuals with diarrhea, severe burns, or excessive and prolonged sweating [3].

Potassium is freely filtered by the glomerulus of the kidney, with most potassium being reabsorbed (70%–80%) in the proximal convoluted tubule (PCT) and loop of Henle. Under physiological homeostasis, delivery of K+ to the nephron remains constant. Conversely, secretion of K+ in the distal nephron is variable and depends on intracellular K+ concentration, luminal K+ concentration, and cellular permeability [10]. Two major factors of K+ secretion involve the renal handling of Na+ and mineralocorticoid activity. Reabsorption in the proximal tubule is primarily passive and proportional to reabsorption of solute and water, accounting for ~60% of filtered K+ **(Figure 1.1)** [20, 21]. Within the descending limb of Henle's loop, a small amount of K+ is secreted into the luminal fluid, while in the thick ascending limb (TAL), reabsorption occurs together with Na+ and Cl-, both trans- and paracellularly. This leads to the K+ concentration of the fluid entering the distal convoluted tubule to be lower than plasma levels (~2 mEq/L), facilitating eventual K+ secretion into the lumen [21]. Similar to reabsorption in the proximal tubule, paracellular diffusion in Henle's loop is mediated via solvent drag, while transcellular movement occurs primarily through the apical sodium-potassium-chloride (Na+/K+/2Cl-) cotransporter [21]. The renal outer medullary K+ channel (ROMK), also located on the apical membrane, mediates recycling of K+ from the cell to the lumen, sustaining the activation of the Na+/K+-2Cl cotransporter and K+ reabsorption in the ascending limb. The movement of K+ through ROMK induces a positive lumen voltage potential, increasing the driving force of paracellular cation (e.g. Ca2+, Mg2+, K+) reabsorption as well. Na+/K+ ATPase pumps located basolaterally throughout the loop, maintain low levels of intracellular Na+ and further provide a favorable gradient for K+ reabsorption. Potassium can exit the cell through a conductive K+ channel across the basolateral membrane, or in cotransport with Cl− (ClC-Kb). **(Figure 1.1)** [3, 10, 11].

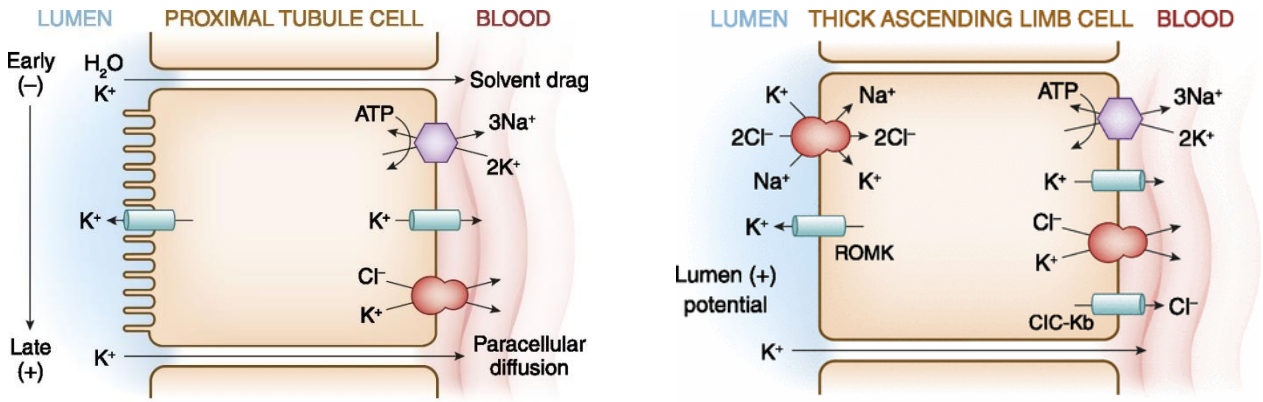

Figure 1.1. A cell model for K+ transport in the proximal tubule (left) the thick ascending limb of Henle (right). *Reprinted with permission from the American Society of Nephrology [10].*

Major regulation of K+ excretion begins in the late distal convoluted tubule (DCT) and progressively increases through the connecting tubule and cortical collecting duct. In the early DCT luminal, Na+ influx is mediated by the apical sodium-chloride co-transporter (NCC), and continues into the late DCT via the epithelial Na+ channel (ENaC) [22]. Both are expressed apically and are the primary means of Na+ reabsorption from the luminal fluid. Na+ reabsorption leads to an electro-chemical potential that is more negative than peritubular capillary fluid. This charge imbalance is matched by an increase in the aforementioned paracellular reabsorption of Cl- from the lumen, as well as increases in Na+/K+ ATPase and ROMK activity. Increased distal delivery of Na+ increases Na+ reabsorption, leading to a more negative luminal/plasma potential gradient and an increase in K+ secretion [10]. Most K+ excretion is mediated by principal cells in the collecting duct. Principal cells possess basolateral Na+/K+ ATPases, which facilitate the movement of K+ from the blood and into the cell. The high cellular concentration of K+ provides a favorable gradient not only for the movement of K+ into the tubular lumen, but for the reabsorption of Na+ as well. Movements of K+ and Na+ occur through the ROMK and ENaC channels, respectively. In conditions of K+ depletion, reabsorption of K+ occurs through H+/K+ ATPases, located on the apical membrane of α-intercalated cells in the collecting duct, thus, providing a mechanism in which K+ depletion increases K+ reabsorption [3, 10, 22].

Two primary types of K+ channels have been identified in the cortical collecting duct, the aforementioned ROMK, as well as the maxi-K channel (also known as the BK large conductance K channel). The ROMK is known to be the major K+ secretory pathway, characterized by

activity during the low conductance of normal physiologic renal fluid excretion. Conversely, the maxi-K channel is quiescent in basal conditions and becomes activated during periods of increased tubular flow, increasing K+ secretion in a flow dependent manner (e.g. hypervolemia, high arterial pressure) **(Figure 1.3)** [10, 17].

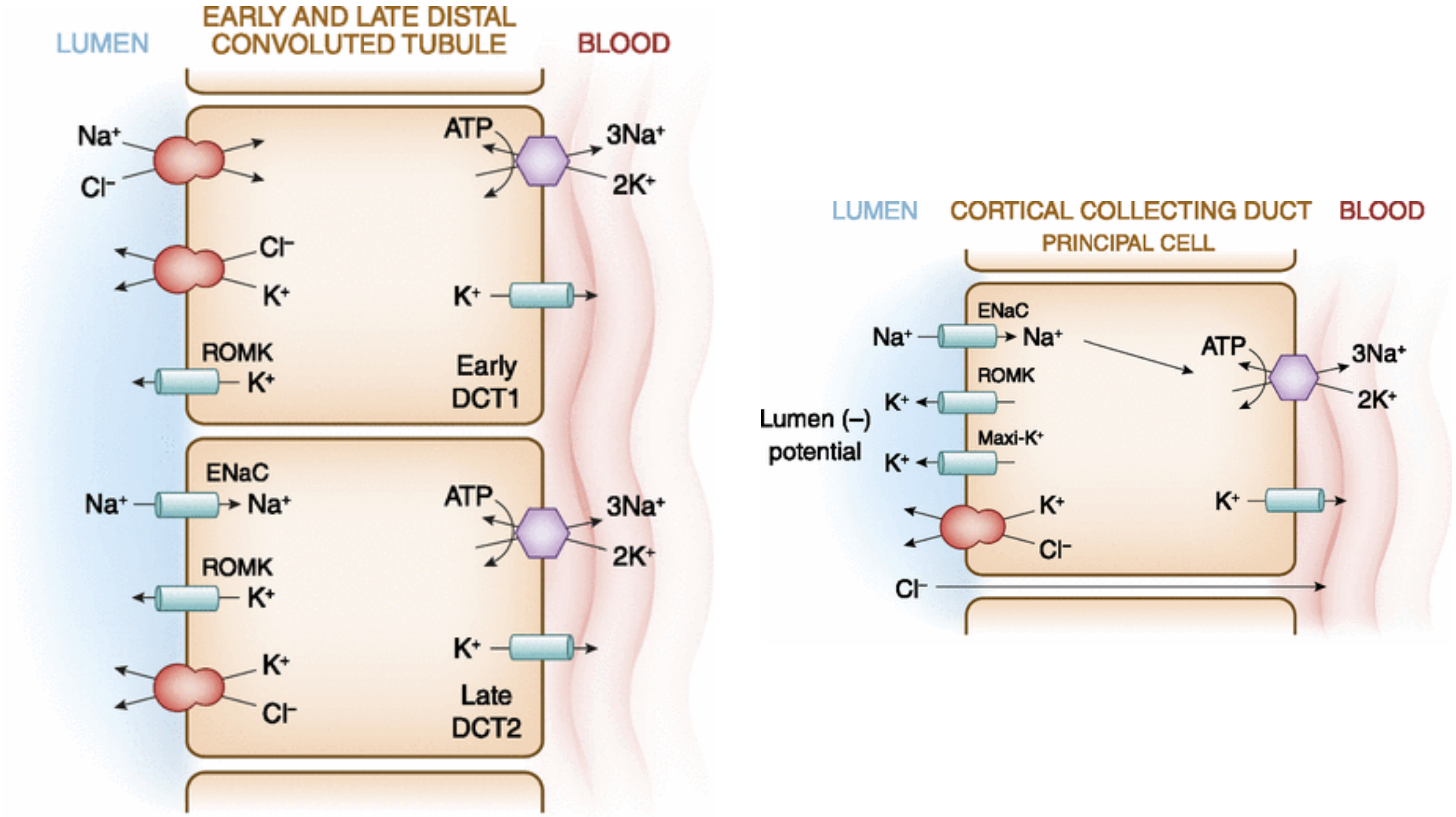

Figure 1.2. A cell model for K+ transport in the distal convoluted tubule (DCT; left) and cortical collecting duct (right). *Reprinted with permission from the American Society of Nephrology [10].*

Interactions with Sodium Balance

Sodium and K+ are the primary electrolytes found in body fluids and work in concert to maintain normal fluid balance. There are no known receptors capable of detecting fluctuations of Na+ within the body, however physiological mechanisms that control extracellular fluid volume effectively control Na+ balance, influencing K+ movement as well. Perturbations in extracellular fluid volume lead to the recruitment of mechanisms that influence both the volume and pressure in the vasculature (cardiac and arterial pressure). Vascular pressure receptors (baroreceptors) sense changes in stretch or tension in vascular beds. Receptors that respond to low-pressure

found in the central venous portion of the vascular tree respond to changes in blood volume, while high-pressure receptors located in the arterial circulation respond to changes in blood pressure [12]. With hypovolemia (low fluid volume) baroreceptors are activated in the vasculature of the pulmonary vein and/or walls of the cardiac atria and send efferent signals to the central nervous system (CNS) to induce both a sympathetic and hormonal response. Hormonally this causes increased release of arginine vasopressin (AVP; antidiuretic hormone) from the posterior pituitary gland, which increases the permeability of the collecting ducts of the kidneys to water, facilitating water reabsorption and increased fluid volume. AVP also increases the reabsorption of Na+ and Cl- in the TAL and collecting duct, overall decreasing Na+ and water loss. As part of a reflex response to a fall in systemic pressure, sympathetic neurons that innervate the afferent/efferent arterioles of the glomerulus release the neurotransmitter norepinephrine, causing an increase in renal vasculature resistance and a decrease in fluid filtration. The decrease in renal blood flow leads to an overall reduction in filtration and reduced Na+ loss. Stimulated α1 and α2 adrenergic receptors in the proximal tubular cells of the kidney also increase the activity of basolaterally located Na+/K+ ATPase and apical Na+/H+ exchanger, respectively, increasing reabsorption of Na+ from the PCT luminal fluid.

In addition to affecting renal hemodynamics, stimulation of α1 adrenergic receptors induce release of renin from the juxoglomerular cells of the kidney afferent and efferent arterioles. Renin, as part of the renin-angiotensin-aldosterone hormonal axis, is a proteolytic enzyme that when released into circulation is responsible for cleaving the hepatically produced protein angiotensinogen into angiotensin 1 [20]. Angiotensin 1 undergoes further cleavage into angiotensin 2 (ANG2), catalyzed by angiotensin converting enzyme (ACE) which is produced primarily by the epithelial cells of the lungs. Angiotensin 2 is a vasoactive hormone, increasing total peripheral vascular resistance in response to low blood volume thus normalizing total pressure. In the CNS ANG2 stimulates the release of AVP from the posterior pituitary, and increases thirst and salt appetite. Angiotensin 2 also has direct and indirect effects on renal Na+ loss. Directly ANG2 increases vascular resistance of the efferent arterioles, decreasing renal plasma flow. Angiotensin 2 also has direct effects on the tubular transport system, increasing expression of the Na+/K+ ATPase and Na+/HCO3-- exchanger in the basolateral and apical membrane of the proximal kidney, respectively, and the Na+/H+ exchanger and ENaC in the distal tubules [9, 10]. Overall decreasing loss and increasing Na+ reabsorption. Indirectly ANG2

in circulation stimulates the release of the mineralocorticoid aldosterone from the adrenal cortex. Aldosterone is secreted in response to low plasma Na+ (hypovolemia), high plasma K+, and increases in ANG-2. Aldosterone increases K+ secretion by stimulating an increase in luminal Na+ reabsorption, increasing renal cellular uptake of Na+ via apical stimulation of ENaC and ROMK expression, and increased activity of basolateral Na+-K+ ATPases [16, 20]. Increased reabsorption of Na also increases the potential difference across the tubular cell, enhancing the secretion of K+ from the cell into the more electro negative lumen [3].

Low K+ intake may lead to excessive Na+ retention independent of fluid dynamics. In animal models, inadequate K+ upregulates the Na+/H+ exchanger in the PCT, leading to increased Na+ reabsorption and fluid expansion [23]. Potassium depletion may also lead to increased activity of the NCC, increasing Na+ and fluid reabsorption in the distal nephron, and promoting arterial pressure dysregulation. Conversely, increased K+ intake may have an acute natriuretic response, higher levels of filtered K+ facilitating a rapid and complete dephosphorylation/deactivation of the NCC [24, 25]. This may also be tied to the proposed enteric K+ sensor, in which gastric delivery of a large K+ dose leads to NCC deactivation, increasing both Na+ and K+ excretion until a new steady state is achieved [17].

Intakes and Dietary Recommendations for Potassium

Dietary Reference Intakes

The reference values for the intake of any nutrient are referred to as the Dietary Reference Intakes (DRIs) and include: the Estimated Average Requirement (EAR), or intake level at which 50% of the population have adequate intakes; the Recommended Dietary Allowance (RDA), based on the EAR, is sufficient to meet the requirements of nearly the entire population (98%); Adequate Intake (AI), used in lieu of an RDA when there insufficient evidence to set an EAR and thus an RDA; and the Tolerable Upper Intake Level (UL), the estimated maximum intake that poses no health risk, developed from a “NOAEL” with a safety factor applied [7, 26-28]. Dietary reference intakes are quantitative values established by review committees commissioned by the National Academy of Sciences, Engineering, and Medicine (NASEM), Health and Medicine Division (formerly the Institute of Medicine), after a review of the appropriate research surrounding any nutrient’s role in eliminating nutritional deficiencies,

and reducing risk of chronic disease. Basic concepts of establishing the proper level of intake for each nutrient are that the needs of healthy (non-diseased) individuals are met, nutrients are grouped by physiological functionality, and age groupings are revised to reflect changes of biological patterns (e.g., gender, growth, pregnancy, etc.) [7, 27, 28]. Chronic disease endpoints are only considered when a sufficient body of knowledge has been established. To this point, the recent Dietary Reference Intake report for sodium (Na) and potassium was the first to establish a chronic disease risk reduction (CDRR) level for Na, a new DRI intended to help differentiate between nutrient intakes necessary for adequacy vs. those which may improve health [29].

Intake Recommendations

Recommended potassium intakes in various countries worldwide often utilize the guidelines set by the North American DRIs or World Health Organization (WHO) [30, 31]. Despite this, large global variation in potassium consumption exists **(Figure 1.3).**

The most recent WHO recommendations for potassium intake come from guidelines published in 2012, examining key chronic disease endpoints related to blood pressure (BP), stroke, CVD, coronary heart disease (CHD), blood lipids, and catecholamines [31]. Based primarily off one large systematic review with meta-analysis [32], the WHO set recommendations to consume at least 90 mmol (~3500 mg) of potassium per day to reduce BP, cardiovascular disease (CVD), stroke, and coronary heart disease [31, 33].

Current recommendations for the United States and Canada were recently revised by the National Academy of Sciences, Health and Medicine Division. According to the 2019 DRI guidelines for potassium, lack of a sensitive biomarker and limitations across potassium retention studies offer insufficient evidence to establish EAR and RDA levels for adequacy or deficiency [8]. Because of this, the committee set AIs using intake data from two nationally representative surveys, NHANES and Canadian Community Health Survey (CCHS). The highest median potassium intake across the two surveys was selected for each DRI group and set as the AI. For adults, the data that informed the potassium AIs were from healthy, normotensive individuals without a self-reported history of CVD. In contrast to the 2005 DRI report, adult AIs were separated by sex, with a potassium intake of 3400mg/d for men and 2600mg/d for women [8, 29]. This is remarkably lower than the AIs established in 2005, set at 4700mg/d for adults 18 and older [7]. Because observational data looking at increased potassium intakes and CVD (and

associated disease) risk are mixed [8, 34], CDRR intake level for potassium could not be established. Blood pressure was considered for a surrogate marker for CVD risk reduction, based on findings that show a reduction in BP with increased supplemental potassium intake [34], but given the lack of clear evidence supporting potassium intake alone in the reduction of CVD and related mortality, the committee decided against this.

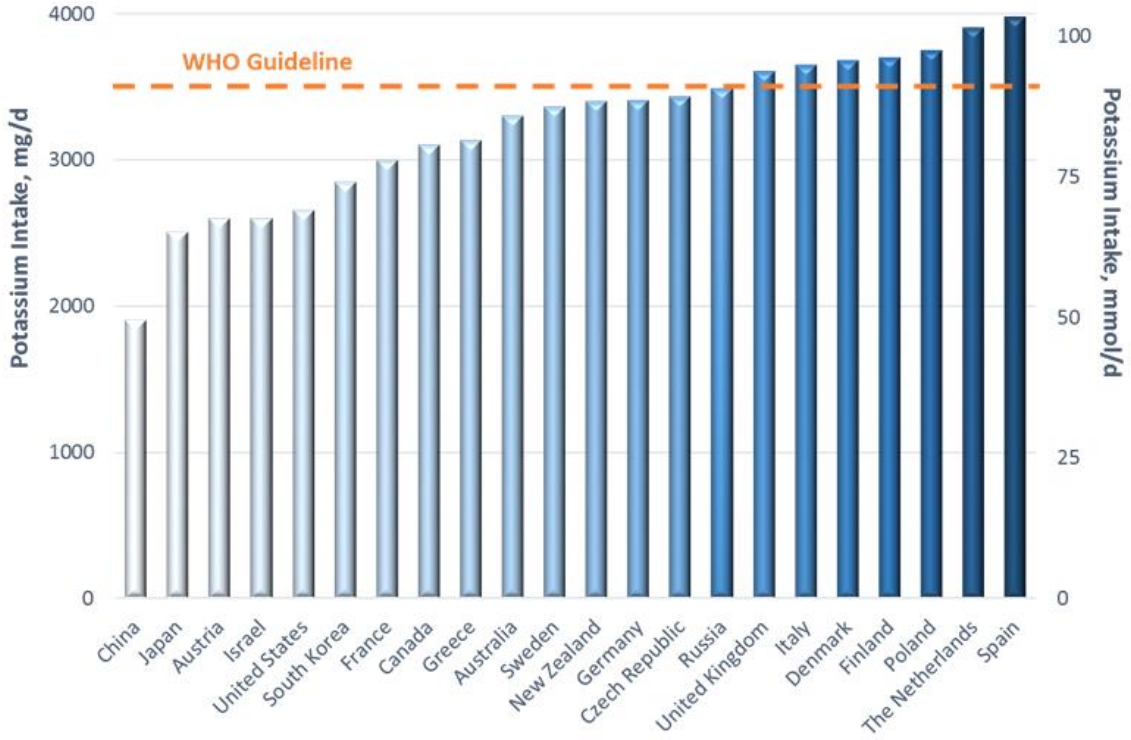

Figure 1.3. Potassium intakes of various countries around the world.

Potassium Retention for Adequacy

The 2005 DRI committee set the adequate intake for potassium at 4700mg/d based on evidence primarily from one study looking at potassium intakes that blunted increases in blood pressure in salt-sensitive African-American men [7]. A specific biomarker of potassium status has yet to be identified. Clinical outcomes such as blood pressure (BP), kidney stone formation, or bone mineral density may describe levels of potassium that establish the newly defined CDRRs, but fail to encompass a level of adequacy for the general population. Dysregulation in either internal or external balance of potassium may lead to low blood concentration and/or

hypokalemia, both serious markers of physiological distress, but unreliable as status indicators in apparently healthy individuals. Because of this, findings from potassium balance studies are utilized in which an intake that results in a positive or neutral overall potassium retention can be considered adequate. In general, potassium retention studies show an increase in urinary potassium with increasing intake [35], fecal losses averaging approximately 420mg/d (10.7mmol/d) at intakes ranging from 2300-3900mg/d [36-38], as well as variable losses due to sweat [39-43].

Sweat potassium losses may be the most unpredictable and difficult to account for. Physical activity and heat exposure show increased potassium loss through sweat, which under homeostatic conditions can be as low as 90mg/d (2.3mmol/L) and as high as 626mg/d (16mmol/L) [39]. Studies show greater potassium loss in sweat during environmental stress (heat) [43, 44] compared to physical stress (exercise) [42], as well as generally unknown/understudied effects due to diet [39, 40]. Researchers accessed differences in sweat K concentrations in seven healthy, young men (18-23y) during both a thermal chamber heat test (40°C, 60% humidity) and 12min running test, finding a higher sweat potassium concentration during heat stress compared to exercise (555 vs. 442 ± 121mg/L (14 ± 5 vs. 11 ± 3mmol/L); $P < 0.05$) [41]. In an older study, conducted in only three healthy male participants exposed to temperatures of 38°C at 70% humidity for 16 days (7.5hr/d), sweat potassium concentrations were reduced comparing the first two days, from 2924 mg/d (75mmol/d) to 810mg/d (21mmol/d) for days 15-16 [39]. Participants were on controlled diets consisting of 2490mg/d of potassium throughout the 16 day study period, possibly pointing to a relationship between dietary intake and sweat potassium accumulation. Similarly, investigators examining potassium losses using two, 4-day exercise interventions at a low vs. high potassium intake (980 vs. 3100mg/d (25 vs 80mmol/d)) found lower sweat potassium concentrations during the low intake compared to high (418.4 vs. 465.3mg/d (10.7 vs. 11.9mmol/d)), significantly lower when comparing days 1-3 of each intervention ($P<0.05$) [40]. While research looking at the effect of dietary intake on sweat potassium accumulation is limited, it seems diet may mediate potassium concentrations in sweat, regardless of stressor, to some degree.

Potassium balance studies are limited in number due to the necessary labor and time associated with such trials for both participants and researchers. Studies that have been conducted often suffer from small sample size, as well as inconsistency in rigor of measurement

and design. Even with the work that has been done, little insight has been given to the physiological significance of potassium retention, and how it relates to health. In an observational study, Holbrook and colleagues assessed normal potassium intake and retention in a cohort of 12 men and 16 women (N=28, 20-53y), during four, 1-week periods over the course of one year. On average men consumed 3300mg/d (84.4mmol/d) of potassium, while women consumed 2400mg/d (61.4mmol/d). Both men and women averaged an apparent absorption of 84.5%, with urinary and fecal losses of approximately 77% (2100mg/d) and 15.5% (395mg/d) of intake [36]. This study revealed that differences in absolute potassium retention do not differ between sexes, but little else can be derived from these findings. In a more recent study, investigators examined differences in fecal and potassium urinary excretion, comparing differences between black (N=11) and white (N=12) healthy men and women (18-45y). Subjects followed a controlled diet of 3910mg/d (100mmol/d) of potassium and 4140mg/d (180mmol/d) of Na for nine days, and primary findings show that urinary potassium was significantly lower in the blacks (2639mg/d (67.5mmol/d)) compared to the whites (3089mg/d (79mmol/d); P=0.015). There were no differences in fecal potassium excretion, however retention was higher in the black cohort (806mg/d (20.6mmol/d)) compared to the whites (400mg/d (10.2mmol/d)) [37]. While sweat losses were not assessed, these should have been relatively minimal given the study setting was a temperature controlled metabolic ward. Overall these findings may show possible differences in potassium retention based on race and/or ethnicity. A balance study conducted on adolescents found similar racial differences in potassium retention. Black (N=30) and white (20) girls consumed a fix potassium intake (2186mg/d (56mmol/d)) at a high (4000mg/d (172mmol/d)) and low (1300mg/d (57mmol/d)) Na intake in cross over design, for two 20-day interventions. Blacks had a significantly higher, positive potassium retention during the low Na diet compared to the whites ($P<0.05$), due primarily to a reduction in urinary excretion, while whites had a small negative cumulative potassium balance [38]. No differences were reported for the high Na diet. Sweat losses were also assessed and found to be ~200mg/d (5mmol/L), with no differences between groups. While these findings may be similar to those of the previously discussed study, it is difficult to compare results because average daily potassium excretions were not reported.

The application of the findings from potassium retention studies is limited, due to both the small number of these interventions, as well as the design limitations and heterogeneity. A

better understanding of how environment (heat), activity, as well as other demographics such as race/ethnicity effect potassium adequacy is also necessary.

Potassium and Hypertension

Potassium and Cardiovascular Disease Risk

Both meta-analyses by the Agency for Healthcare Research and Quality (AHRQ) and the 2019 DRI report for Na and potassium evaluated the evidence from potassium intervention studies on the association between potassium intake with intermediate outcomes, including blood pressure, or final outcomes, such as cardiovascular disease, coronary heart disease (CHD), stroke, and mortality [8, 34]. The two reviews examined 37 observational studies assessing total, cardiovascular disease (CVD), CHD, stroke, and myocardial infarction (MI) mortality. Twenty-five studies investigated dietary intake (e.g. 24hr recall, food frequency questionnaire) while others used the surrogate measure of either 24hr urine or spot urine. Of the 25 dietary intake associations, 14 found an inverse relationship between potassium intake and CVD and related mortality, while only 4 of the 12 studies using urinary excretion as an assessment reported a benefit to mortality risk [8, 34]. This difference may be due to the lack of reliability associated with urinary excretion measures and their ability to infer intake accurately. Like Na, potassium excretion may have an infradian (lasting longer than one day) or possibly an ultradian (less than one day) rhythm [45], making a single 24hr collection an inaccurate assessment of actual intake. Overall both reports concluded there was insufficient evidence to identify a relationship between potassium intake and CVD mortality or related mortality risks, the DRI committee concluding the need for randomized clinical trials. While ideal, a potassium intervention that follows participants for years, and utilizes necessary measures (e.g. controlled diets, multiple 24hr urine collections) to accurately examine potassium intake and CVD risk is impractical. Instead we must rely on clinical trials that have measured reliable indicators for the development of CVD, blood pressure (BP) and its dysregulation, hypertension (HTN), being the most well-established of these markers.

Hypertension, or high blood pressure, is the leading cause of cardiovascular disease and a major contributing risk factor for the development of stroke, CHD, MI, heart failure, and end-stage renal disease, amounting to a US public health financial burden of $50.6 billion [46].

Nearly one in three American adults (~72 million) are estimated to have HTN, while nearly 70 million are at risk for developing HTN (BP between 120/80 mmHg and 140/90 mmHg). Approximately 90% of US adults older than 50 y are at risk for the development HTN, with systolic rises being the most prevalent [47]. Hypertension is a leading cause of morbidity and mortality worldwide and second only to smoking as a preventable cause of death in the US [3, 48].

Numerous epidemiological studies suggest diet as a key component in BP control, with some studies showing lower BP in populations consuming higher amounts of fruits and vegetables [49-51]. Dietary patterns shown to lower BP include increased potassium and reduced Na intake, increases in fruit and vegetable consumption, as well as other foods rich in antioxidants [52, 53]. A population study conducted by Khaw et al. in St. Lucia, West Indies suggested an increase in potassium by ~700-1200 mg/d (20-30mmol/d) resulted in a 2 to 3 mmHg reduction in systolic blood pressure (SBP) [54]. In adults a 2-mmHg reduction in BP can reduce CHD and stroke mortality rates by 4 and 6%, respectively [55]. The INTERSALT study, a worldwide epidemiologic study (n = 10079 men and women aged 20-59 y from 32 countries) that looked at the relationship between 24hr Na excretion and BP, provided evidence of potassium intake as an important factor effecting population BP, independent of Na, among diverse population groups [55]. The American Heart Association has estimated that increasing potassium intake may decrease HTN incidence in Americans by 17% and lengthen life span by 5.1 years [46].

Mechanisms of Arterial Pressure Control

Regulation of systemic BP, or arterial pressure, is the most important role of the cardiovascular system, maintaining proper blood flow and the delivery of oxygen and nutrients to all tissues throughout the body. Arterial pressure is a result of cardiac output (CO; heart rate x stroke volume), or blood being pumped from the heart into systemic circulation, and total peripheral vascular resistance (TPR), or the degree to which the systemic vasculature is in a state of constriction or dilation [56]. Blood pressure is regulated by both short-term and long-term mechanisms. In the short-term arterial baroreceptors, located predominately in the walls of the aorta and the carotid arteries, respond to sensory inputs of increased stretch in the vasculature sending afferent signals to the medullary cardiovascular center in the CNS. Subsequently, the

CNS integration process is such that increased input from the arterial baroreceptor reflex, caused by increases in arterial pressure, will elicit a decrease in the tonic activity of cardiovascular sympathetic nerves and an increase in cardiac parasympathetic nerve activity. The result from this negative feedback system is a decrease in BP, via a decrease in CO and TPR. Conversely, a decrease in mean arterial pressure would increase sympathetic and decrease parasympathetic neural activity [56, 57]. If arterial pressure remains elevated for several days the baroreceptor reflex will gradually adjust to this new pressure set point and cease firing. Because of this, it is not considered a good mechanism for long-term control. Long-term pressure regulation is closely tied to the prevalence and potential causes of HTN. Long-term regulation is theorized to be primarily dependent on the way the kidneys handle Na (e.g. extracellular osmolarity) and regulate blood volume. Arterial pressure has a marked effect on urinary output rate and total body fluid volume. A disturbance that leads to an increase in arterial pressure will in turn cause an increase in urinary output, decreasing total fluid volume, bringing arterial pressure back to a homeostatic level. Again conversely, a decrease in arterial pressure would lead to fluid volume expansion. Similar to short-term regulation, long-term regulation works as a negative feedback loop, utilizing modulation of fluid volume as a means for pressure regulation. As discussed previously, the kidneys play a major role in regulating electrolyte balance and the osmolarity of blood plasma. Plasma is filtered within the glomerular capillaries before entering the renal tubules of the nephron. The rate at which this process occurs is referred to as the glomerular filtration rate (GFR) and is influenced by both the hydrostatic and oncotic aspects of arterial pressure. Increased blood volume and pressure will increase GFR, and when the body is at physiological steady-state, arterial pressure must remain at a level that ensures urinary output equals fluid intake [56]. Filtered fluid enters the renal tubules where it is either reabsorbed and reenters the cardiovascular system, or is excreted as urine. The kidneys regulate blood osmolarity primarily via modulation of total body water rather than total solutes, although some fluid reabsorption occurs because Na is actively pumped out of the renal tubules. The previously discussed hormonal influences of arginine vasopressin (anti-diuretic hormone; AVP), and the renin-angiotensin-aldosterone axis stimulate both water and Na reabsorption in response to low fluid volume/low blood pressure. The resulting increase in BP, and overall dysregulation of this long-term control mechanism, may explain the incidence of HTN to some degree, although the majority of primary HTN remains idiopathic **(Figure 1.4)** [56, 57].

Systemic hypertension is defined as an elevation of systolic blood pressure (vascular pressure during cardiac muscle contraction) above 130 mmHg and diastolic blood pressure (vascular pressure during cardiac muscle relaxation) above 80 mmHg [58]. Secondary HTN can be traced to a pre-existing comorbidity such as kidney disease, obesity and/or diabetes, and various forms of cancer. Primary or essential HTN ("essential" to drive blood through the vasculature) often has no diagnosable cause, leaving only the symptom of high BP to be treated either pharmacologically, or through lifestyle modification (e.g. exercise and diet) [56].

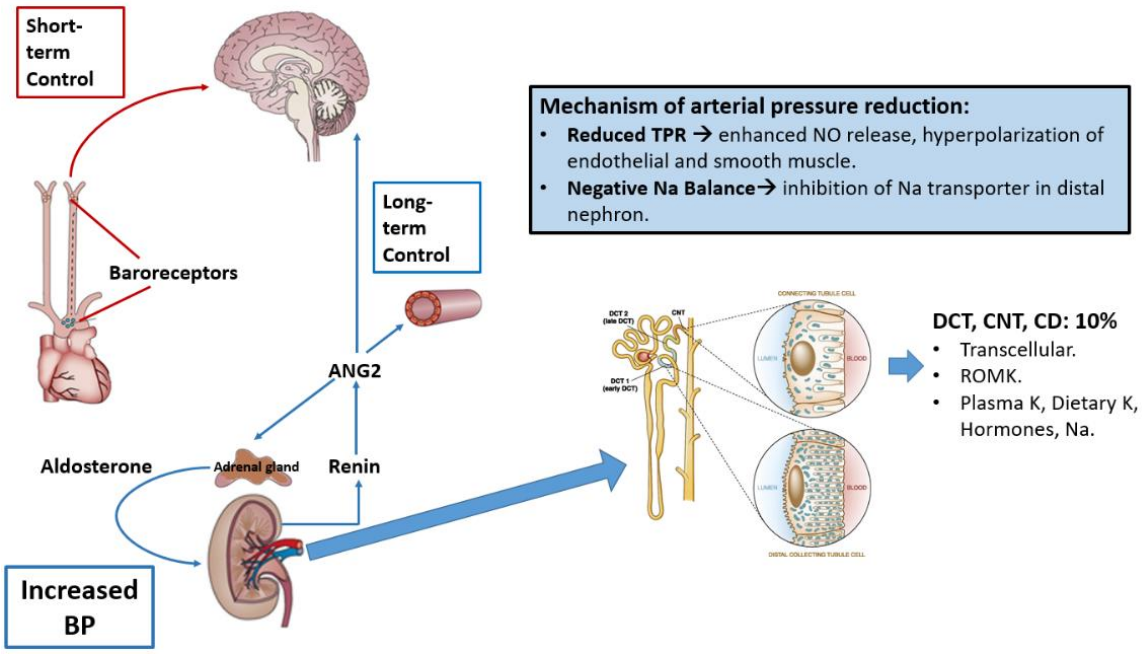

Figure 1.4. Arterial Pressure Control and Renal K Handling. Short-term arterial pressure control involves sensory input from baroreceptors and rapid response from CNS/autonomic neural integration. Long-term control relies on the kidneys ability to regulate blood volume and solute concentration. The role of K may involve enhanced nitric oxide release and vasodilation in the vasculature (short-term), as well as increased Na secretion and excretion via renal handling. Angiotensin-II (ANG2), total peripheral resistance (TPR), thick ascending limb (TAL), distal convoluted tubule (DCT), connecting tubule (CNT), collecting duct (CD), renal outer medullary potassium channel (ROMK), sodium (Na), potassium (K). *Portions reprinted and modified with permission from Springer Nature [59], and the American Society of Nephrology [60].*

Potassium and Arterial Pressure

The antihypertensive effects of increased potassium intake may be related to numerous processes. Theorized mechanisms include influences of the renin-angiotensin-aldosterone system, reduction in adrenergic tone, increased Na excretion (natriuresis), and increases in

vasodilation. Acutely, increased plasma potassium is associated with endothelial dependent vasodilation. Endothelial cells are a monolayer of cells that control the tone of the underlying smooth muscle throughout the vasculature. Potassium can induce endothelial hyperpolarization via a stimulation of Na+/K+ ATPase pumps and the activation of plasma membrane potassium channels. The response is then transmitted to vascular smooth muscle cells by the accumulation of potassium in the myoendothelial space or direct electrical coupling through myoendothelial gap junctions [61]. Hyperpolarization of endothelial cells may also lead to an efflux of Ca from vascular smooth muscle, resulting in smooth muscle relaxation and increased blood flow [57]. An increase in potassium intake may also improve overall endothelial function, promote vascular smooth muscle relaxation through inhibition of vascular sympathetic neural transmissions, and increase the release of nitric oxide from endothelial cells [61, 62]. In addition to enhanced vasodilation, potassium is proposed to effect adrenergic tone through modulation of baroreceptor sensitivity, reduced sensitivity to catecholamine related vasoconstriction, improved insulin sensitivity, and decreases in oxidative stress and inflammation [58].

As discussed before briefly, increases in potassium can lead to increased Na excretion, or naturesis, and in turn decreased fluid volume and BP. Recall that active Na reabsorption and potassium secretion take place in the DCT and collecting duct of the kidneys. The primary transporters involved in this process include ENaC (Na reabsorption) and ROMK (potassium excretion). In addition, Na is also actively reabsorbed in DCT by the NCC, which is activated upon phosphorylation. The activity of the NCC determines the delivery of Na to the downstream ENaC and ROMK, and the extent to which Na is reabsorbed and potassium is excreted. Shown primarily in animal models, increases potassium load (via feeding) cause a subsequent increase in extracellular potassium concentration which may lead to singling that results in the dephosphorylation and decrease in NCC activity, reducing Na reabsorption in the DCT and increasing Na urinary excretion [24]. In humans, prospective population studies point to increased dietary potassium, via higher fruit and vegetable intake, influencing Na excretion, and possibly leading to a benefit for overall fluid balance and arterial pressure [63]. It is clear that physiological relationship between potassium and Na influence arterial pressure, although the complete understanding of the mechanisms related to this interaction have yet to be fully elucidated.

Also discussed previously in relation to renal potassium excretion, the renin-angiotensin-aldosterone system (RAAS) is important in the regulation of arterial pressure. Falls in BP or blood volume depletion cause the release of renin from the kidneys, activating this counter-regulatory hormonal axis, which increases pressure via Na and fluid reabsorption, and vasoconstriction. BP lowering interventions (e.g. reduced Na) and therapies (e.g. diuretics) activate RAAS, increasing plasma renin activity (PRA). In an ancillary study to the initial Dietary Approaches to Stop Hypertension (DASH) trial (N=381), PRA change was inversely associated with systolic BP (SBP) change for the control diet (slope= -0.35, P=0.001), while it did not differ by BP change on interventions of a high fruit and vegetable diet or DASH diet [64]. These results suggest a DASH type diet (higher potassium and moderate Na intake) that leads to BP reduction may blunt the counter-regulatory actions of RAAS.

Very few dietary trials have attempted to evaluate the possible mechanisms driving the proposed beneficial effect of potassium on BP, with the majority of the findings coming from the afore mentioned DASH collaborative research group. From this group, Lin and colleagues looked to elucidate the specific mechanisms in which the DASH dietary pattern lowers BP in hypertensive individuals. Subjects (N=20, 44.3 ± 78y) included non-medicated hypertensive (144.2/88.5 mmHg) adults randomized to consume a control diet (3400mg Na/d, 1700mg K/d) or DASH diet (3400mg Na/d, 4700mg K/d) for two weeks. With the DASH diet, SBP fell by 10.65 (P = 0.023) and 9.60mmHg (P = 0.039) and DBP by 5.95 (P = 0.069) and 8.60mmHg (P = 0.011) at the end of week one and two, respectively. Plasma nitrite (NO_2^-) levels, a marker of vasodilation and microvascular health, following hyperemia showed a significant treatment effect (P=0.014), however, researchers found no evidence of BP reduction due to suppression of RAAS, or blunting of androgenic tone. There was also no difference in urinary Na excretion between the DASH group and control, contrary to previous findings by this group in which the DASH diet in conjunction with Na intake reduction had an overall natriuretic effect [65]. While the latter conclusion may be attributed to a much larger sample size (N=375 vs. 20), this shows the importance of manipulating Na in eliciting long lasting effects of BP reduction. It may also highlight the need to specifically look at the manipulations of Na and potassium in the diet to fully understand these mechanisms.

Findings from the Agency for Healthcare Research and Quality Report

As mentioned previously, the systematic review undertaken by the AHRQ evaluated the evidence from potassium interventions on the association between potassium intake with blood pressure [34]. The report concluded there was moderate strength evidence supporting the benefit of increased potassium intake from supplements on both systolic blood pressure (SBP) and diastolic blood pressure (DBP) in adults, based on 11 parallel randomized control trials (RCTs) and 8 cross-over RCTs. The report also concluded that a moderate strength of evidence exists to support the effect of increased potassium from either supplements or foods on BP (both SBP and DBP) in individuals with pre-hypertension or HTN based on 17 RCTs (11 parallel, 6 cross-over). In contrast, a low strength of evidence, based only on 3 RCTs (2 parallel, 1 crossover), suggests that increased potassium does not affect BP in normotensive individuals. The report cited insufficient evidence for the moderating effects of age, sex, race/ethnicity, comorbidities (diabetes, kidney disease, obesity), intake of other minerals (calcium, magnesium, Na), and potassium form (potassium chloride vs. others) in relation to potassium and BP. There is also insufficient evidence to suggest an effect of increased potassium intake on BP in children and adolescents. Perhaps of most interest to the field of nutrition science, the AHRQ report found insufficient evidence on the effect of increasing potassium intake via dietary changes alone on BP in adults. Only 4 out of the 26 trials reviewed for this topic consisted of any type of dietary potassium manipulation, highlighting the need for more dietary interventions to properly answer this important question [33, 34]. Findings from the 2019 DRI committee's review were similar, although they completely excluded any dietary potassium interventions due to concerns of collinearity with intake of other nutrients [8].

Dietary Potassium Interventions

Numerous meta-analyses conducted over the past 30 years support the findings of the AHRQ, generally concluding a positive relationship between increased potassium supplementation and a reduction in blood pressure in adults [32, 66-70]. In contrast, overall findings on the effect of increased dietary potassium intake and BP have been conflicting. The majority of these systematic reviews and meta-analyses included dietary interventions with supplement trials, despite the fact that only a few dietary interventions exist. Since RCTs looking

specifically at dietary potassium intake represent a large gap in the literature, it is important to evaluate these trials separately for their scientific quality and effect size [33].

In an early dietary intervention trial, Chalmers et al. assessed the effects of both an increase in dietary potassium and a reduction in dietary sodium on blood pressure in hypertensive individuals (DBP between 90 and 100mmHg) from an Australian population [71]. Two hundred twelve subjects (age 52.3±0.8 years; 181 males and 31 females) were placed in one of the 4 following diet groups: normal diet (control), high potassium diet (>100mmol/3900mg potassium/d), reduced Na diet (50-75mmol/1150-1725mg Na/d), or high potassium/low Na diet. During the 12-week intervention, subjects were regularly counseled on how to adequately modify their food choices based on their group (e.g. avoiding salt/high Na foods or increasing fruit and vegetable intake). Both SBP and DBP were significantly reduced in each dietary intervention compared to controls, but no significant differences were observed between intervention groups. Reductions in the high potassium group were 7.7±1.1 and 4.7±0.7 mmHg for SBP and DBP, respectively. The significant decrease in overall BP within each intervention group may point to the importance of the interaction between Na and potassium in regulating BP [33].

A more recent study based in the United Kingdom, assessed the effects of increased potassium intake from both dietary sources and supplements on BP in untreated pre-hypertensive individuals (DBP: 80-100 mmHg) [72]. In a cross-over design, subjects (n=48, 22-65 years) completed four, 6 week dietary interventions including a control diet, an additional 20 or 40mmol potassium/d (780 or 1560mg/day) from fruit and vegetables and 40mmol potassium-citrate/d. Similar to the Chalmers study, nutrition coaching was used to modulate participant food choice during each dietary intervention, focused on increasing fruit and vegetable intake. However, this study found no significant changes in BP (ambulatory and supine BP) between the control group and any of the dietary or supplement interventions. A smaller increase in dietary potassium (780-1560mg/d increase compared to 3900mg/d) as well as lower baseline blood pressure (SBP: 137.7 vs >150mmHg, DBP: 88.6 vs. >95mmHg) may explain some of the contrasting findings between these two trials. The focus on only increasing dietary potassium from fruit and vegetable sources in the Berry et al. study [72] compared to controlling both potassium and Na intake in the Chalmers study [71] could also be the reason for the disparity in

their findings. However, similar to the Berry study, Miller and colleagues found null results from an RCT designed to assess the effect of a potassium focused Dietary Approaches to Stop Hypertension (DASH) diet on BP in an urban African American population [73]. Subjects (N=123) were primarily female (71%) with controlled hypertension (SBP: 120-140mmhg, DBP: 80-90mmHg) on stable doses of antihypertensive medication. In a parallel design, the intervention group (DASH-plus) received extensive nutrition coaching geared toward increasing the purchase and consumption of high potassium foods (e.g. fruits, vegetables, beans, nuts), while the control group received initial instruction on how to adopt the DASH diet to improve health, but minimal dietary advice thereafter. Despite an estimated intake of 3700mg/d of potassium (2300mg/d over baseline) there was no significant difference in SBP (1.5mmHg (95% CI: −2.6, 5.6, p=0.48) or DBP (1.3 (95% CI: −1.3, 3.9, p=0.33) in the DASH-plus intervention group compared to the control. Researchers point out that the lack of effect may have been due to medication changes, which occurred in a large percentage of participants despite a design aimed for a population on stable doses. The primary concern with studies designed around nutrition coaching is the ability of the participants to follow the diet correctly, and report their dietary choices accurately. While compliance can be measured somewhat accurately in these trials (via spot urine collections in the Miller study [73], and 24hr urine collection in the previously mentioned studies [71, 72]) one of the primary limiting factors in dietary nutrition advice interventions is the lack of a controlled diet [33].

While evidence on the effect of increased dietary potassium from clinical trials is extremely limited, controlled feeding interventions are almost nonexistent, with the majority of findings being extrapolated from The Dietary Approaches to Stop Hypertension (DASH) studies. [47, 52, 74]. The DASH intervention revealed that a dietary pattern rich in fruit and vegetables, fiber, and low fat dairy products, with reductions in saturated and total fat and Na could reduce BP compared to the average American diet [52]. Although the initial DASH trial diet led to a dramatic increase in potassium consumption (DASH diet = 4100mg/d, DASH combination diet (Dash diet + low-fat dairy, low saturated and total fat) = 4400 mg/d) and reduction in BP, due to other dietary modifications these beneficial effects cannot be attributed to potassium alone. In a subsequent study conducted by the DASH collaborative research group assessing the effects of the DASH diet, plus reduced dietary Na on BP. Investigators randomly assigned participants (BP >120/80mmHg) to a control diet (N=204; 49±10y), similar to typical intake in the US, or the

DASH diet (N=208; 47±10y), which as in the previous study aimed for a high potassium intake level (≈4700mg/d) [74]. Within each diet participants consumed foods at levels of high (150mmol/d; 3450mg/d), intermediate (100mmol/d; 2300mg/d), and low (50mmol/d; 1150mg/d) Na for 30 consecutive days, in a random order cross-over design. Researchers found that the DASH diet resulted in a significantly lower SBP at every Na level and a significantly lower DBP at the high and intermediate Na levels. Further, the combination of the DASH diet and low Na lowered SBP by 11.5 and 7.1mmHg in participants with, and without HTN, respectively. Findings were similar in an Australian study utilizing dietary advice and supplements in a cross-over design to assess the effects of high vs. low Na on BP in the context of a high potassium diet (≈3400mg/d) [75]. Despite Na supplementation up to 120 mmol/d (2760mg/d), 24h ambulatory BP, and resting auscultatory SBP were reduced by 2.9 mm Hg, 1.4mmHg, and 5.5mmHg compared with baseline measurements, respectively. Low Na reduced BP more than high Na diets, similar to the DASH study. The study also lacked a low potassium control arm making it difficult to conclude if the effects were related to potassium intake [33].

Potassium and Bone

Osteoporosis, or a severe reduction in bone mass leading to decreased bone health and increased fracture risk, is a global health problem with great financial impact. Over 200 million people worldwide suffer from osteoporosis, including 30% of postmenopausal women in both the US and Europe [76]. Peak bone mass is achieved by the third decade of life, after which bone loss begins, accelerating with aging in both men and women [77]. The bone mass present at any given point during life is determined by factors that influence the acquisition, maintenance, or loss of bone throughout the lifespan, many of which are modifiable lifestyle factors [33].

Adequate potassium intake may benefit overall bone health, and has been proposed to do so through its effect on acid-base balance **(Figure 1.5)** [78, 79]. Support for the acid-base bone theory stems from the idea that the Western diet is high in meats and cereal grains and low in fruits and vegetables, creating an environment of low-grade metabolic acidosis (net acid excretion (NAE) = 75 to 100mEq acid/d) [78]. Buffering of this increased acid load via bone tissue derived calcium (Ca) salts, is proposed to lead to bone loss. Alkaline potassium salts

produced from metabolizing fruits and vegetables or potassium supplements (potassium bicarbonate or citrate) are thought to provide bicarbonate precursors and help to maintain pH homeostasis (~7.35-7.45). The impact of excess systemic acid on bone is suggested to be mediated by two mechanisms: pH buffered through the dissolution of the bone matrix, and cell-based mechanisms (e.g. up-regulation of bone resorbing cell (osteoclast) activity) [78, 79]. However, opposition to the acid-base balance theory exists. In a rat model, looking at the relationship between the inhibitory effect of vegetables on bone resorption and base excess, addition of potassium citrate at levels that neutralized urinary acid excretion from an acidogenic diet had no effect on bone turnover [80]. Researchers measured bone turnover via a urinary excretion tracer from prelabeled bone, and concluded reductions in bone resorption via increased vegetable intake and subsequent base excess were not causally related. The authors suggested that bioactive compounds (e.g. flavonoids) in fruits and vegetables may be responsible for benefits to bone. Despite this, there is some consistency in the literature that increased potassium intake benefits bone, though the mechanisms behind this remain unclear [3].

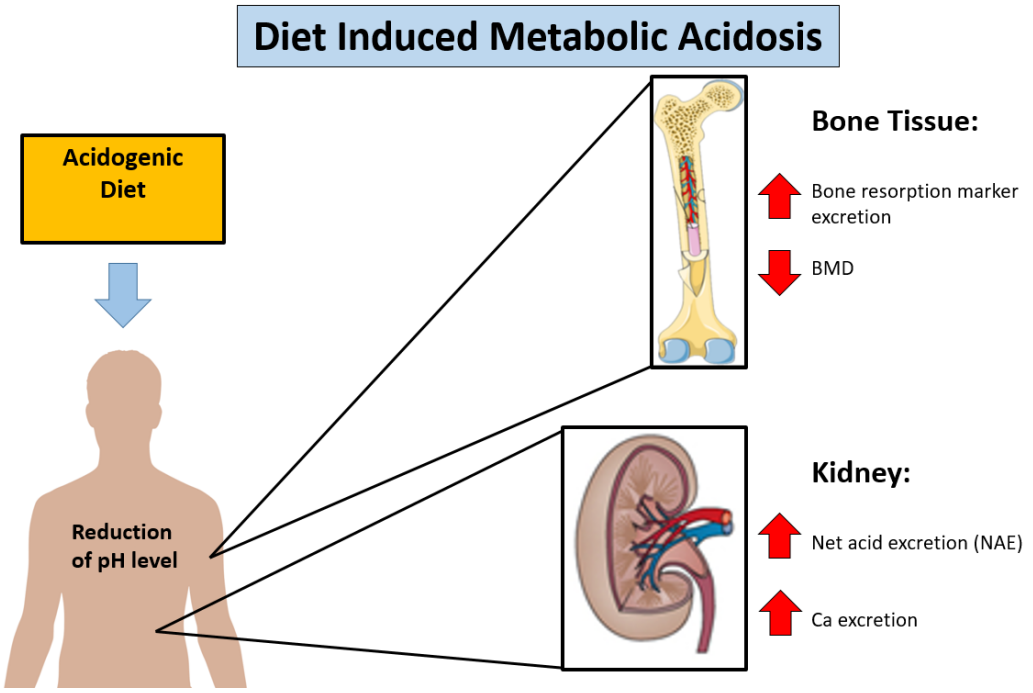

Figure 1.5. The Acid-Ash Hypothesis and Potassium. The acid-ash hypothesis proposes that a diet higher in meats and cereal grains, precursors to acid metabolites, causes chronic acidemia leading to the dissolution of bone tissue. This leads to hypercalciuria, and a decrease in Ca retention. In contrast, the consumption of fruits and vegetables, and other food sources or supplements that are more alkaline (e.g. K, Ca) would decrease urinary Ca excretion and have a protective effect on bone. Bone mineral density (BMD). *Portions reprinted and modified with permission from Springer Nature [59]*.

Cross-sectional, observational studies show a consistent bone benefit with increased dietary potassium associated with higher fruit and vegetable intakes in adolescent boys and girls [81-84], pre- and postmenopausal women [83, 85-87] and elderly men and women [81, 88]. Higher potassium intakes have been associated with reduced urinary Ca excretion, and improvement in overall Ca balance [89]. Current and past diet history was investigated in the Aberdeen Prospective Osteoporosis Screening study (APOSS), looking at specific associations of mineral intake and bone outcomes in premenopausal women (45-49y, n=994). Women who reported lower intake of potassium via fruits and vegetables during early adulthood, compared to those who reported medium or high intake (median Ca intake ~1000 mg/d for all participants), had significantly lower BMD at the lumbar spine (LS) and femoral trochanter (FT) ($P < 0.01$). Although significant differences in LS, femoral neck (FN), and FT BMD between the lowest and highest quartiles of potassium intake were lost after adjustment for cofounding variables ($P < 0.06$) [85]. However, in a second study by the same group in a similar population, women (45-55y) with lower intakes of fruits and vegetables had lower forearm BMD and higher bone resorption, with potassium intake accounting for 7.4% of the variation in forearm BMD [90]. Observational studies have also examined the association between potassium intake specifically and bone health. In a cohort of 266 elderly women, subjects in the highest quartile of potassium intake (measured via 24hr urinary excretion) had significantly higher total body BMD and hip BMD compared to the lowest quartile [33, 91].

Several clinical supplementation trials have accessed the influence of potassium on biochemical markers of bone turnover. Studies have shown decreases in the bone resorption markers (C- and N-telopeptide), and increases in bone formation markers (procollagen type I N-terminal propeptide), with potassium supplementation, as well as decreases in urinary Ca excretion [92-94]. In postmenopausal women (N=18) potassium bicarbonate (60-120 mmol/d) taken for 18 days decreased urinary hydroxyproline excretion by 10%, and increased serum osteocalcin, a marker of bone formation [94]. Findings from potassium supplementation trials typically show persistent increased calciuria with treatment in both men and women [89, 95]. In a randomized double-blind placebo-controlled study, men and women (n= 52) randomly assigned to six months of 60 or 90 mmol/d of potassium citrate supplementation had decreases in urinary Ca and net acid excretion resulting in positive Ca balance in the group on the highest dose (90 mmol/d) compared to control (0 mmol/d) [96]. Serum C-telopeptide, a marker of bone

turnover, also decreased significantly in both potassium groups. Similar studies have also found decreases in both net acid and Ca excretion, as well as a positive influence on bone biomarkers with increases in potassium supplementation [3, 33, 92, 93].

Clinical dietary intervention trials accessing the impact of potassium on bone are lacking. In an ancillary study to the main DASH trial, Lin and colleagues examined the effects of two dietary patterns (DASH vs. control) and three sodium levels (1150, 2300, 3450mg/d) on bone metabolism in a subset of 186 individuals (23-76y). The investigators found that the DASH diet significantly reduced bone turnover from baseline (osetocalcin by 8-11% and CTX by 16-18%) as well as compared to control, while a reduction in Na intake decreased Ca excretion for both diet groups. Although the Ca intake for the control was low compared to the DASH intervention (450 mg/d vs. 1250 mg/d), which may have influenced the results [33, 97].

There have been few studies examining the relationship between potassium supplementation and BMD, and/or bone microarchitecture. In a randomized, prospective controlled trial Jehle and colleagues investigated the effect of 30mmol/d of potassium chloride (KCl) vs. potassium citrate (K-citrate) on spine (L2-L4) and hip BMD in postmenopausal women (N=161, 58.6 ± 4.8y) for 12 months. Women taking K-citrate showed significant increases in spine BMD from baseline at 3, 9, and 12 months, reaching an increase of 0.89% ($P < 0.05$) at month 12, whereas women in the KCl group showed a decreased spine BMD of -0.98% ($P < 0.05$) [98]. In second study, the same group examined the effect of 60 mEq of K-citrate/d for 24 months on bone outcomes in 201 elderly (>65 y old) men and women. A significant 1.7% difference in spine BMD and significant improvements in trabecular thickness, trabecular number, and FRAX index score in the K-citrate supplementation group compared to the control group showed the potential for potassium to improve bone health in the elderly [33, 99].

Potassium and Calcium Balance

Potassium intake has been associated with reduced urinary Ca excretion. Clinical trials show persistent increased calciuria in both men and women given potassium supplements (bicarbonate or citrate) vs. similar Na supplements, suggesting potassium may have a role in bone benefit beyond acid balance [89, 100]. In the kidney Ca is reabsorbed via solvent drag in the PCT (60-70%) and the TAL (20%). Active reabsorption of Ca takes place in the DCT via

specific transport proteins. Calcium is reabsorbed via the Ca channel TRPV5 from the tubular fluid into the cell where it binds to the transfer protein calbindin 28K and is shuttled across and out of the cell via the plasma membrane Ca ATPase (PMCA) and Na/Ca exchanger (NCX). High Na intakes have been shown to increase urinary Ca losses, with a loss of approximately 24-40mg of Ca for a Na intake of ≈2.3g [16]. The mechanism for this is not well defined, but most likely involves Ca following Na excretion via solvent drag. Increased intracellular Na within the kidney tubular cells may also affect the dynamics of the NCX (which exchanges 3 Na for 1 Ca), leading to its dysregulation and possible reversal. Increased intakes of potassium may have the opposite effect on Ca, in which paracellular reabsorption in the TAL is facilitated by movement of Na, potassium, and Cl across the Na+/K+/2Cl- cotransporter (NKCC) on the apical membrane. Potassium shuttled into the cell via NKCC is subsequently re-secreted into the lumen via ROMK, maintaining an electropositive lumen, facilitating the passive reabsorption of Ca, decreasing urinary loss and improving Ca balance.

The Role of Dietary Potassium in Health

There is still much to learn about the role of potassium in overall health. The importance of potassium in normal physiology is clear, but how adequate to greater than adequate intakes can help facilitate benefit to these systems is not well understood. The application of potassium retention studies is limited, due both to the small number of these interventions, as well as the lack of rigor and heterogeneity of their designs. The data necessary for the prescription of an intake that meets the physiological requirements of the healthy, general population is currently not available. Despite this, it appears increased potassium intake improves the function of the cardio-vasculature and may benefit bone, though the form (supplement vs. food) and dose of potassium in these interventions have been inconsistent, and the mechanisms behind these effects need further study. The research that follows will attempt to help fill these gaps in the potassium literature, through a carefully controlled dietary potassium intervention study with BP outcomes and whole-body potassium, Na, and Ca retention measures in prehypertensive adults.

References

1. *Dietary Guidelines Advisory Committee. Report of the Dietary Guidelines Advisory Committee on the Dietary Guidelines for Americans, 2010.*, in *Washington, DC: U.S. Department of Agriculture, Agricultural Research Service.*2011.

2. *U.S. Department of Health and Human Services and U.S. Department of Agriculture. 2015-2020 Dietary Guidelines for Americans. 8th Edition.* 2015.

3. Stone, M.S., L. Martyn, and C.M. Weaver, *Potassium Intake, Bioavailability, Hypertension, and Glucose Control.* Nutrients, 2016. **8**(7).

4. DeSalvo, K.B., R. Olson, and K.O. Casavale, *DIetary guidelines for americans.* JAMA, 2016. **315**(5): p. 457-458.

5. Weaver, C.M., *Potassium and health.* Adv Nutr, 2013. **4**(3): p. 368S-77S.

6. U.S. Department of Agriculture, A.R.S., *What We Eat in American, 2013-2014.* 2017.

7. Institute of, M., *Dietary Reference Intakes for Water, Potassium, Sodium, Chloride, and Sulfate*. 2005, Washington, DC: The National Academies Press.

8. National Academies of Sciences, E., et al., *The National Academies Collection: Reports funded by National Institutes of Health*, in *Dietary Reference Intakes for Sodium and Potassium*, M. Oria, M. Harrison, and V.A. Stallings, Editors. 2019, National Academies Press (US) Copyright 2019 by the National Academy of Sciences. All rights reserved.: Washington (DC).

9. Gumz, M.L., L. Rabinowitz, and C.S. Wingo, *An Integrated View of Potassium Homeostasis.* N Engl J Med, 2015. **373**(1): p. 60-72.

10. Palmer, B.F., *Regulation of Potassium Homeostasis.* Clin J Am Soc Nephrol, 2015. **10**(6): p. 1050-60.

11. Unwin, R.J., F.C. Luft, and D.G. Shirley, *Pathophysiology and management of hypokalemia: a clinical perspective.* Nat Rev Nephrol, 2011. **7**(2): p. 75-84.

12. Stipanuk, M.H., *Biochemical, physiological, & molecular aspects of human nutrition*. 2nd ed.. ed. Biochemical, physiological, and molecular aspects of human nutrition. 2006, St. Louis: St. Louis : Saunders Elsevier.

13. Greenlee, M., et al., *Narrative review: evolving concepts in potassium homeostasis and hypokalemia.* Ann Intern Med, 2009. **150**(9): p. 619-25.

14. Aronson, P.S. and G. Giebisch, *Effects of pH on Potassium: New Explanations for Old Observations.* Journal of the American Society of Nephrology : JASN, 2011. **22**(11): p. 1981-1989.

15. Perez, G.O., J.R. Oster, and C.A. Vaamonde, *Serum potassium concentration in acidemic states.* Nephron, 1981. **27**(4-5): p. 233-43.

16. Shils, M.E. and M. Shike, *Modern nutrition in health and disease*. 2006: Lippincott Williams & Wilkins.

17. Palmer, B.F. and D.J. Clegg, *Physiology and pathophysiology of potassium homeostasis.* Advances in Physiology Education, 2016. **40**(4): p. 480-490.

18. Youn, J.H., *Gut sensing of potassium intake and its role in potassium homeostasis.* Semin Nephrol, 2013. **33**(3): p. 248-56.

19. Bia, M.J. and R.A. DeFronzo, *Extrarenal potassium homeostasis.* Am J Physiol, 1981. **240**(4): p. F257-68.

20. Penton, D., J. Czogalla, and J. Loffing, *Dietary potassium and the renal control of salt balance and blood pressure.* Pflugers Arch, 2015. **467**(3): p. 513-30.

21. Ludlow, M., *Renal handling of potassium.* ANNA journal/American Nephrology Nurses' Association, 1993. **20**(1): p. 52-58.

22. Meneton, P., J. Loffing, and D.G. Warnock, *Sodium and potassium handling by the aldosterone-sensitive distal nephron: the pivotal role of the distal and connecting tubule.* Am J Physiol Renal Physiol, 2004. **287**(4): p. F593-601.

23. Soleimani, M., et al., *Potassium depletion increases luminal Na+/H+ exchange and basolateral Na+:CO3=:HCO3- cotransport in rat renal cortex.* J Clin Invest, 1990. **86**(4): p. 1076-83.

24. Veiras, L.C., et al., *Potassium Supplementation Prevents Sodium Chloride Cotransporter Stimulation During Angiotensin II Hypertension.* Hypertension, 2016. **68**(4): p. 904-12.

25. Sorensen, M.V., et al., *Rapid dephosphorylation of the renal sodium chloride cotransporter in response to oral potassium intake in mice.* Kidney Int, 2013. **83**(5): p. 811-24.

26. Fulgoni, V.L., 3rd, *Limitations of data on fluid intake.* J Am Coll Nutr, 2007. **26**(5 Suppl): p. 588S-591S.

27. Lupton, J.R., et al., *Nutrient reference value: non-communicable disease endpoints--a conference report.* Eur J Nutr, 2016. **55 Suppl 1**: p. S1-10.

28. Millen, B.E., et al., *The 2015 Dietary Guidelines Advisory Committee Scientific Report: Development and Major Conclusions.* Adv Nutr, 2016. **7**(3): p. 438-44.

29. *Updated dietary reference intakes for sodium and potassium.(Report).* Pediatrics Week, 2019: p. 284.

30. Strohm, D., et al., *Revised Reference Values for Potassium Intake.* Annals of Nutrition and Metabolism, 2017. **71**(1-2): p. 118-124.

31. *Guideline: Potassium Intake for Adults and Children*. 2012, Geneva: World Health Organization.

32. Aburto, N.J., et al., *Effect of increased potassium intake on cardiovascular risk factors and disease: systematic review and meta-analyses.* BMJ, 2013. **346**: p. f1378.

33. Weaver, C.M., et al., *What Is the Evidence Base for a Potassium Requirement?* Nutr Today, 2018. **53**(5): p. 184-195.

34. Newberry, S.J., et al., *Sodium and Potassium Intake: Effects on Chronic Disease Outcomes and Risks*. 2018, Rockville MD.

35. Hene, R.J., et al., *Adaptation to chronic potassium loading in normal man.* Miner Electrolyte Metab, 1986. **12**(3): p. 165-72.

36. Holbrook, J.T., et al., *Sodium and potassium intake and balance in adults consuming self-selected diets.* Am J Clin Nutr, 1984. **40**(4): p. 786-93.

37. Rosa, R.M., et al., *Gastrointestinal and renal excretion of potassium in African-Americans and White Americans.* J Hypertens, 2012. **30**(12): p. 2373-7.

38. Palacios, C., et al., *Racial differences in potassium homeostasis in response to differences in dietary sodium in girls.* Am J Clin Nutr, 2010. **91**(3): p. 597-603.

39. Consolazio, C.F., et al., *Excretion of sodium, potassium, magnesium and iron in human sweat and the relation of each to balance and requirements.* J Nutr, 1963. **79**: p. 407-15.

40. Costill, D.L., R. Cote, and W.J. Fink, *Dietary potassium and heavy exercise: effects on muscle water and electrolytes.* Am J Clin Nutr, 1982. **36**(2): p. 266-75.

41. Fukumoto, T., et al., *Differences in composition of sweat induced by thermal exposure and by running exercise.* Clin Cardiol, 1988. **11**(10): p. 707-9.

42. Armstrong, L.E., et al., *Voluntary dehydration and electrolyte losses during prolonged exercise in the heat.* Aviat Space Environ Med, 1985. **56**(8): p. 765-70.

43. Malhotra, M.S., et al., *Effect of restricted potassium intake on its excretion and on physiological responses during heat stress.* Eur J Appl Physiol Occup Physiol, 1981. **47**(2): p. 169-79.

44. Malhotra, M.S., K. Sridharan, and Y. Venkataswamy, *Potassium losses in sweat under heat stress.* Aviat Space Environ Med, 1976. **47**(5): p. 503-4.

45. Rakova, N., et al., *Long-term space flight simulation reveals infradian rhythmicity in human Na(+) balance.* Cell Metab, 2013. **17**(1): p. 125-31.

46. Roger, V.L., et al., *Executive summary: heart disease and stroke statistics--2012 update: a report from the American Heart Association.* Circulation, 2012. **125**(1): p. 188-97.

47. Svetkey, L.P., et al., *Effect of the dietary approaches to stop hypertension diet and reduced sodium intake on blood pressure control.* J Clin Hypertens (Greenwich), 2004. **6**(7): p. 373-81.

48. Lopez, A.D. and C.D. Mathers, *Measuring the global burden of disease and epidemiological transitions: 2002-2030.* Ann Trop Med Parasitol, 2006. **100**(5-6): p. 481-99.

49. Young, D.B., H. Lin, and R.D. McCabe, *Potassium's cardiovascular protective mechanisms.* Am J Physiol, 1995. **268**(4 Pt 2): p. R825-37.

50. *Intersalt: an international study of electrolyte excretion and blood pressure. Results for 24 hour urinary sodium and potassium excretion. Intersalt Cooperative Research Group.* BMJ, 1988. **297**(6644): p. 319-28.

51. Elford, J., et al., *Migration and geographic variations in blood pressure in Britain.* BMJ, 1990. **300**(6720): p. 291-5.

52. Appel, L.J., et al., *A clinical trial of the effects of dietary patterns on blood pressure. DASH Collaborative Research Group.* N Engl J Med, 1997. **336**(16): p. 1117-24.

53. Svetkey, L.P., et al., *Effects of dietary patterns on blood pressure: subgroup analysis of the Dietary Approaches to Stop Hypertension (DASH) randomized clinical trial.* Arch Intern Med, 1999. **159**(3): p. 285-93.

54. Khaw, K.T. and G. Rose, *Population study of blood pressure and associated factors in St Lucia, West Indies.* Int J Epidemiol, 1982. **11**(4): p. 372-7.

55. Stamler, R., *Implications of the INTERSALT study.* Hypertension, 1991. **17**(1 Suppl): p. I16-20.

56. Mohrman, D.E. and L.J. Heller, *Cardiovascular Physiology, Seventh Edition*. 2010: McGraw-Hill Education.

57. Ekmekcioglu, C., et al., *The role of dietary potassium in hypertension and diabetes.* Journal of Physiology and Biochemistry, 2016. **72**(1): p. 93-106.

58. Chopra, H.K. and C.V.S. Ram, *Recent Guidelines for Hypertension.* Circulation Research, 2019. **124**(7): p. 984-986.

59. Victor, R.G., *Carotid baroreflex activation therapy for resistant hypertension.* Nature Reviews Cardiology, 2015. **12**: p. 451.

60. Subramanya, A.R. and D.H. Ellison, *Distal convoluted tubule.* Clin J Am Soc Nephrol, 2014. **9**(12): p. 2147-63.

61. Haddy, F.J., P.M. Vanhoutte, and M. Feletou, *Role of potassium in regulating blood flow and blood pressure.* Am J Physiol Regul Integr Comp Physiol, 2006. **290**(3): p. R546-52.

62. He, F.J., et al., *Effects of potassium chloride and potassium bicarbonate on endothelial function, cardiovascular risk factors, and bone turnover in mild hypertensives.* Hypertension, 2010. **55**(3): p. 681-8.

63. Cogswell, M.E., et al., *Dietary Sodium and Cardiovascular Disease Risk--Measurement Matters.* N Engl J Med, 2016. **375**(6): p. 580-6.

64. Chen, Q., et al., *The effects of dietary patterns on plasma renin activity: results from the Dietary Approaches to Stop Hypertension trial.* J Hum Hypertens, 2012. **26**(11): p. 664-9.

65. Akita, S., et al., *Effects of the Dietary Approaches to Stop Hypertension (DASH) diet on the pressure-natriuresis relationship.* Hypertension, 2003. **42**(1): p. 8-13.

66. Whelton, P.K., et al., *Effects of oral potassium on blood pressure. Meta-analysis of randomized controlled clinical trials.* JAMA, 1997. **277**(20): p. 1624-32.

67. Cappuccio, F.P. and G.A. MacGregor, *Does potassium supplementation lower blood pressure? A meta-analysis of published trials.* J Hypertens, 1991. **9**(5): p. 465-73.

68. Geleijnse, J.M., F.J. Kok, and D.E. Grobbee, *Blood pressure response to changes in sodium and potassium intake: a metaregression analysis of randomised trials.* J Hum Hypertens, 2003. **17**(7): p. 471-80.

69. Binia, A., et al., *Daily potassium intake and sodium-to-potassium ratio in the reduction of blood pressure: a meta-analysis of randomized controlled trials.* J Hypertens, 2015. **33**(8): p. 1509-20.

70. Dickinson, H.O., et al., *Potassium supplementation for the management of primary hypertension in adults.* Cochrane Database Syst Rev, 2006(3): p. CD004641.

71. Chalmers, J., et al., *Australian National Health and Medical Research Council dietary salt study in mild hypertension.* J Hypertens Suppl, 1986. **4**(6): p. S629-37.

72. Berry, S.E., et al., *Increased potassium intake from fruit and vegetables or supplements does not lower blood pressure or improve vascular function in UK men and women with early hypertension: a randomised controlled trial.* Br J Nutr, 2010. **104**(12): p. 1839-47.

73. Miller, E.R., 3rd, et al., *A Dietary Intervention in Urban African Americans: Results of the "Five Plus Nuts and Beans" Randomized Trial.* Am J Prev Med, 2016. **50**(1): p. 87-95.

74. Sacks, F.M., et al., *Effects on blood pressure of reduced dietary sodium and the Dietary Approaches to Stop Hypertension (DASH) diet. DASH-Sodium Collaborative Research Group.* N Engl J Med, 2001. **344**(1): p. 3-10.

75. Nowson, C.A., T.O. Morgan, and C. Gibbons, *Decreasing dietary sodium while following a self-selected potassium-rich diet reduces blood pressure.* J Nutr, 2003. **133**(12): p. 4118-23.

76. Sözen, T., L. Özışık, and N.Ç. Başaran, *An overview and management of osteoporosis.* European Journal of Rheumatology, 2017. **4**(1): p. 46-56.

77. Weaver, C.M. and R.K. Fuchs, *Chapter 12 - Skeletal Growth and Development*, in *Basic and Applied Bone Biology*, D.B.B.R. Allen, Editor. 2014, Academic Press: San Diego. p. 245-260.

78. Barzel, U.S., *The skeleton as an ion exchange system: implications for the role of acid-base imbalance in the genesis of osteoporosis.* J Bone Miner Res, 1995. **10**(10): p. 1431-6.

79. Brandao-Burch, A., et al., *Acidosis inhibits bone formation by osteoblasts in vitro by preventing mineralization.* Calcif Tissue Int, 2005. **77**(3): p. 167-74.

80. Muhlbauer, R.C., A. Lozano, and A. Reinli, *Onion and a mixture of vegetables, salads, and herbs affect bone resorption in the rat by a mechanism independent of their base excess.* J Bone Miner Res, 2002. **17**(7): p. 1230-6.

81. Prynne, C.J., et al., *Fruit and vegetable intakes and bone mineral status: a cross sectional study in 5 age and sex cohorts.* Am J Clin Nutr, 2006. **83**(6): p. 1420-8.

82. Tylavsky, F.A., et al., *Fruit and vegetable intakes are an independent predictor of bone size in early pubertal children.* Am J Clin Nutr, 2004. **79**(2): p. 311-7.

83. Li, J.J., et al., *Fruit and vegetable intake and bone mass in Chinese adolescents, young and postmenopausal women.* Public Health Nutr, 2013. **16**(1): p. 78-86.

84. Vatanparast, H., et al., *Positive effects of vegetable and fruit consumption and calcium intake on bone mineral accrual in boys during growth from childhood to adolescence: the University of Saskatchewan Pediatric Bone Mineral Accrual Study.* Am J Clin Nutr, 2005. **82**(3): p. 700-6.

85. New, S.A., et al., *Nutritional influences on bone mineral density: a cross-sectional study in premenopausal women.* Am J Clin Nutr, 1997. **65**(6): p. 1831-9.

86. Nieves, J.W., et al., *Nutritional factors that influence change in bone density and stress fracture risk among young female cross-country runners.* Pm r, 2010. **2**(8): p. 740-50; quiz 794.

87. Macdonald, H.M., et al., *Effect of potassium citrate supplementation or increased fruit and vegetable intake on bone metabolism in healthy postmenopausal women: a randomized controlled trial.* Am J Clin Nutr, 2008. **88**(2): p. 465-74.

88. Tucker, K.L., et al., *Potassium, magnesium, and fruit and vegetable intakes are associated with greater bone mineral density in elderly men and women.* Am J Clin Nutr, 1999. **69**(4): p. 727-36.

89. Frassetto, L., R.C. Morris, Jr., and A. Sebastian, *Long-term persistence of the urine calcium-lowering effect of potassium bicarbonate in postmenopausal women.* J Clin Endocrinol Metab, 2005. **90**(2): p. 831-4.

90. New, S.A., et al., *Dietary influences on bone mass and bone metabolism: further evidence of a positive link between fruit and vegetable consumption and bone health?* Am J Clin Nutr, 2000. **71**(1): p. 142-51.

91. Zhu, K., A. Devine, and R.L. Prince, *The effects of high potassium consumption on bone mineral density in a prospective cohort study of elderly postmenopausal women.* Osteoporos Int, 2009. **20**(2): p. 335-40.

92. Dawson-Hughes, B., et al., *Treatment with potassium bicarbonate lowers calcium excretion and bone resorption in older men and women.* J Clin Endocrinol Metab, 2009. **94**(1): p. 96-102.

93. Marangella, M., et al., *Effects of potassium citrate supplementation on bone metabolism.* Calcif Tissue Int, 2004. **74**(4): p. 330-5.

94. Sebastian, A., et al., *Improved mineral balance and skeletal metabolism in postmenopausal women treated with potassium bicarbonate.* N Engl J Med, 1994. **330**(25): p. 1776-81.

95. Lemann, J., Jr., R.W. Gray, and J.A. Pleuss, *Potassium bicarbonate, but not sodium bicarbonate, reduces urinary calcium excretion and improves calcium balance in healthy men.* Kidney Int, 1989. **35**(2): p. 688-95.

96. Moseley, K.F., et al., *Potassium citrate supplementation results in sustained improvement in calcium balance in older men and women.* J Bone Miner Res, 2013. **28**(3): p. 497-504.

97. Lin, P.H., et al., *The DASH diet and sodium reduction improve markers of bone turnover and calcium metabolism in adults.* J Nutr, 2003. **133**(10): p. 3130-6.

98. Jehle, S., et al., *Partial neutralization of the acidogenic Western diet with potassium citrate increases bone mass in postmenopausal women with osteopenia.* J Am Soc Nephrol, 2006. **17**(11): p. 3213-22.

99. Jehle, S., H.N. Hulter, and R. Krapf, *Effect of potassium citrate on bone density, microarchitecture, and fracture risk in healthy older adults without osteoporosis: a randomized controlled trial.* J Clin Endocrinol Metab, 2013. **98**(1): p. 207-17.

100. Lemann, J., Jr., et al., *Oxalate is overestimated in alkaline urines collected during administration of bicarbonate with no specimen pH adjustment.* Clin Chem, 1989. **35**(10): p. 2107-10.

CHAPTER 2: SHORT-TERM INCREASED DIETARY POTASSIUM FROM POTATO AND POTASSIUM GLUCONATE: EFFECT ON BLOOD PRESSURE AND MICROCIRCULATION IN PRE-HYPERTENSIVE-TO-HYPERTENSIVE ADULTS

Abstract

Potassium is an essential nutrient, that has been labeled a shortfall nutrient by recent Dietary Guidelines for Americans Advisory Committees. Increases in potassium intake have been linked to improvements in cardiovascular and other metabolic health outcomes. Blood pressure (BP) is currently the primary criterion for determining potassium requirements. In this clinical trial, we assessed the effects of increasing potassium intake through food or supplements on BP and microcirculation (endothelial function). Thirty pre-hypertensive-to-hypertensive (Systolic BP ≥ 120mmHg) men (N=15) and women (N=15) with a mean ± SD age, BMI, and BP (systolic(SBP)/ diastolic(DBP)) of 48.2 ± 15 y, 31.4 ± 6.1kg/m^2, and 136.3 ± 11.9/ 86.1 ± 7.1mmHg, respectively, were enrolled in a cross-over, randomized control feeding trial. Participants were assigned to a random order of four 16-day dietary potassium interventions including a basal diet (control) of 2300mg/d (~60mmol/d), and three phases of an additional 1000mg/d(3300mg/d (~85mmol/d) total) of potassium in the form of potatoes (baked, boiled, or pan-heated with no additional fat), French fries, or a potassium (K)-gluconate supplement. Each intervention was separated by two or more weeks of wash out. Blood pressure was measured in triplicate using manual auscultation on days 1, 4, 6, 8, 11, 13, 15, 16, and 17 of each intervention. Cutaneous microvascular and endothelial function were assessed via thermal hyperemia, utilizing laser Doppler flowmetry (LDF), at baseline and at the end of each intervention. Differences in both SBP and DBP ± SE were assessed at the end treatment (average of day 15, 16, and 17 measurements) using a mixed model ANOVA with adjustment for baseline BP as a covariate. There were no significant differences among treatments (all values mmHg: control: 129.3 ± 0.9/ 83.5 ± 1.3, potatoes: 126.2 ± 0.93/ 83.6 ± 1.3, French fries: 127.8 ± 0.94/ 83.6 ± 1.3, supplement: 128.5 ± 0.9/ 83.8 ± 1.6; SBP:P=0.07, DBP: P=0.97). However, differences between SBP for control and potato treatments approached significance (P=0.059), and secondary BP analysis of change in SBP over time was significantly reduced by feeding

baked/boiled potatoes compared to control (-6.01mmHg vs. -2.6mmHg; P = 0.017). Utilizing the same statistical analysis, there were also no significant differences in endothelial function (measured as percent of cutaneous vascular conductance max (%CVCmax) ± SE) among treatments (control: 87.4 ± 1.5%, French Fries: 87.7 ± 1.4%, potatoes: 88.3 ± 1.1%, supplement: 88.9 ± 1.0%). In this controlled feeding study, increased potassium from potatoes had a slight benefit to SBP at end treatment, and may improve SBP over time. However, there was no significant benefit to DBP or microvascular function in men and women with higher cardiometabolic risk. This trial was registered at ClinicalTrials.gov as NCT02697708.

Introduction

Potassium is an essential nutrient, that has been labeled a shortfall nutrient by recent Dietary Guidelines for Americans Advisory Committees [1, 2]. Increases in potassium intake have been linked to improvements in cardiovascular and other metabolic health outcomes. Blood pressure (BP) is currently the primary criterion for determining potassium requirements. Cardiovascular disease (CVD) is responsible for the 31% of deaths worldwide [3]. Hypertension (HTN), or high blood pressure (BP), the primary risk factor for cardiovascular disease (CVD) and other circulatory diseases, is a leading cause of mortality worldwide and second only to smoking as a preventable cause of death in the United States [4]. Findings from the recent Agency for Healthcare Research and Quality (AHRQ) report on potassium intake and chronic disease concluded, with a moderate strength of evidence, that increasing potassium intake decreases BP, particularly in those with HTN [5]. Although, of the 18 randomized controlled trials assessed by the AHRQ, only 4 were dietary interventions, the rest involved potassium supplementation. Numerous meta-analyses conducted over the past 30 years support the findings of the AHRQ, generally concluding a beneficial relationship between increased potassium supplementation and a reduction in BP in adults [6-11]. In contrast, overall findings on the effect of increased dietary potassium intake and BP have been conflicting, with some studies confirming benefit [12] and others finding null results [13, 14]. The majority of these trials have utilized dietary advice or coaching to increase participant potassium consumption. This lack of dietary intervention control is a major limiting factor and may explain differing results.

Evidence on the effect of increased dietary potassium on BP from clinical trials is extremely limited, and there are currently no known controlled feeding interventions investigating specifically potassium as the primary variable of interest. Because of this, controlled feeding trials utilizing a dietary pattern that increases potassium consumption can be examined. These findings have primarily been extrapolated from The Dietary Approaches to Stop Hypertension (DASH) studies [15-17]. The DASH intervention revealed that a dietary pattern rich in fruit and vegetables, fiber, and low-fat dairy products, with reductions in saturated and total fat, and moderate sodium (Na) could reduce BP compared to the average American diet [15]. Although the initial DASH trial diet did lead to a dramatic increase in dietary potassium consumption (DASH diet = 4100md/d, DASH combination diet (Dash diet + low-fat dairy, low saturated and total fat) = 4400 mg/d) and reduction in BP, these beneficial effects cannot be attributed to potassium alone due to the other concurrent dietary modifications.

Because of this large gap in the potassium literature, the goal of this research was to utilize a controlled feeding study to examine the effect of increased dietary potassium from potato sources and a potassium supplement on blood pressure and microvascular outcomes, compared to a control diet. We hypothesized that the interventions with an increased potassium intake would reduce BP, and improve microvascular function compared to the control diet.

Methods

Subjects

Subjects (N=30, age 21y and older) were recruited from a Midwest community. Subjects were pre-hypertensive-to-hypertensive (SBP >120mmHg) men and women who were otherwise apparently healthy. Exclusion criteria included: medications to treat hyper or hypotension (hypertensive monotherapy was allowed), or those known to affect electrolyte metabolism or contain high levels of potassium or Na; history of myocardial infarction, diabetes mellitus, renal disease, GI disease, pancreatitis, cholestatic liver disease, cancer, smoking, use of illegal drugs, or excessive alcohol intake; subjects who were pregnant or lactating, allergy or intolerance to study foods, unwillingness to refrain from dietary supplements; and weight loss > 3kg in the past 2 months. Screening consisted of two visits at least one week apart. Participants came to the Indiana Clinical and Translational Science Institute (CTSI) clinical research center at Purdue

University where they consented, had their BP measured, a fasting blood draw, and filled out screening questionnaires. They were also given 1-week diet and activity logs to complete at home before their second screening visit, which consisted of another BP measurement to ensure eligibility, and return of the logs. If eligible, subjects completed a 4-day run-in period (described below), subjects who chose to continue following the 4-day run-in were randomized to one of 24 possible sequences of the 4 phases including control, potato, French fry, and K-gluconate interventions. Subjects were enrolled in May 2016 to September 2017. The study protocol was approved by the Purdue University Institutional Review Board, and all subjects provided written, informed consent.

Dietary Intervention

Study design was a randomized, cross-over, controlled feeding trial with the primary outcome of end of treatment SBP and DBP, and secondary outcomes of change in SBP and DBP over each intervention, as well as changes in microvascular function (Figure 2.1). After initial screening eligible subjects entered the run-in period. Each day of the 4-day run-in diet represented one of the four diet phases: control, potato, French fries, and potassium supplement. During the run-in, subjects came to the clinical facility each morning, had their BP taken, either stayed to eat breakfast or had all their meals and snacks for the day packed out in a cooler to take with them. After the 4-day run-in period, participants were assigned to a random order of four 16-day dietary potassium interventions including a basal diet (control) of 2300mg potassium/d (~60mmol/d), and three phases of an additional 1000mg potassium/d (3300mg/d(~85mmol/d) total) of potassium in the form of potatoes (baked, boiled, or pan-heated with no additional fat), French fries (FF), or a K-gluconate supplement. Each intervention was separated by two or more weeks of wash out. A 4-day menu cycle at three calorie levels (1800, 2200, and 2600 kcal/d) was designed to manipulate potassium levels while keeping other micro- (e.g. Na, Ca), macro- (e.g. fat, protein, CHO), and other (e.g. fiber) nutrients constant. Subjects picked up study foods approximately every other day (on days 1, 4, 6, 8, 11, 13, 15, 16). For each day of controlled feeding, the subjects recorded their intake by checking off each item listed on daily menu sheets provided. They were also instructed to indicate whether they ate any non-study foods, and/or whether they did not eat all the study foods they were provided. The food and beverages were prepared with deionized water and weighed to the nearest one tenth gram on digital scales.

Deionized bottle water was also given to control water intake. Meal composites of each menu cycle day were homogenized and chemically analyzed. Potassium gluconate supplements were sent out to a food chemistry lab for analysis (Eurofins Food Chemistry Testing US, Inc.). Researchers adjusted energy intakes with snack items that contained mostly sugar. Participants were instructed to return any uneaten items, which were analyzed for mineral content.

Blood Pressure and Cutaneous Microvascular Measures

A slightly modified version of the DASH BP assessment protocol was utilized to measure BP [15, 18]. Blood pressure was measured by trained and certified study staff using manual auscultation with a McKesson Deluxe Aneroid Sphygmomanometer (Richmond, Va), after the participant sat for at least 5 minutes at rest in a quiet, temperature-controlled room. Three readings (first and fifth Korotkoffs sounds) were taken from the left arm at each visit and averaged. Measurements were taken in the morning after overnight fast, at approximately the same time of day for each timepoint. We assessed BP throughout the study approximately every other day (on days 1, 4, 6, 8, 11, 13, 15, 16, 17) during each phase. Treatment differences in both systolic blood pressure (SBP) and diastolic blood pressure (DBP) were assessed at the end of treatment (average of day 15, 16, and 17 measurements). Secondary BP analysis assessed change in BP over time (end of treatment – baseline), with baseline BP defined at an average of measures from Days 1 and 4 of each intervention.

Cutaneous microvascular and endothelial function were assessed via thermal hyperemia, utilizing laser Doppler flowmetry (LDF), at baseline (Day 1 or 2 of first intervention) and at the end of each intervention (Day 15 or 16). Direct local heating of the skin, a physiological endothelial nitric oxide (NO) synthase (eNOS)-dependent dilator stimulus, is a standardized and reliable method to measure NO-dependent dilation and has been used to examine microvascular function in a variety of pathologies [19]. Cutaneous red blood cell flux was continually measured with an integrated LDF probe placed in a local heating unit (Skin Heater/Temperature Monitor SHO2, Moor Instruments, Devon, UK) positioned medially on the left forearm. Baseline measurements were collected for 15 min at a local skin temperature of 33° C. After a stable baseline period, the local skin temperature was increased to 42°C at a rate of 0.5°C every 5s for 45min to achieve a 10min plateau, after which maximal vasodilation was induced by increasing skin temperature to 43°C (20 min) [19]. Mean arterial pressure (MAP) was measured at the

brachial artery throughout the protocol using an automated blood pressure monitor (Omron BP791IT; Omron Global). Cutaneous vascular conductance (CVC) was calculated as red blood cell flux divided by MAP and expressed as a percent of site-specific maximal vasodilation (%CVCmax).

Statistical Methods

Mean differences in both SBP, DBP, and endothelial function (measured as percent of cutaneous vascular conductance max (%CVCmax) among treatments were analyzed using a mixed model ANOVA with Tukey post hoc analyses ($P < 0.05$). The primary BP outcome was mean differences among treatments in end of treatment BP (average of day 15, 16, 17), with adjustment for baseline BP (average of day 1 and 4) as a covariate ($P<0.0001$). Secondary BP outcomes were change in BP over time (end of treatment – baseline) using a mixed model ANOVA and contrast analysis. A sample size of 30 cross-over participants with two sided alpha=0.05, provided 80% power to detect differences in the primary outcome of differences in SBP of 3.7 mmHg at end of treatment. All computations were performed using JMP (SAS) statistical software. Alpha was set at 0.05.

Results

Baseline Characteristics of Study Subjects

Data from 30 subjects were included in the final analyses (Table 2.1). On average, subjects were 48.2 ± 15.0y, had a BMI bordering overweight to obese (31.4 ± 6.1kg/m2), and SBP and DBP of 136.3 ± 11.9mmHg and 86.1 ± 7.1mmHg, respectively (all data Mean ± SD).

Subject Adherence

A total of 25 subjects completed all four interventions, one subject completed three, and four subjects completed two (Figure 2.2). Three subjects were on some form of hypertensive monotherapy, and were monitored to ensure timing and dose of medication did not change during the study duration. The mean body weight change for the control, potato, FF, and supplement interventions was -1.1kg, -0.9kg, -0.9kg, -1.0kg, respectively. Supplement intake compliance by pill count was 92%.

Chemical Analysis of Controlled Diets

There was some unexpected variation in both potassium and sodium (Na) content of the diets. Potassium content for the intervention phases of control, potato, French Fry (FF), and supplement were 2238 ± 45, 3008 ± 4, 2977 ± 31, and 3299 ± 43mg/d, respectively. The potassium levels for the control and supplement phases were close to the target of 2300 and 3300mg/d, respectively, while the potato and FF were slightly lower at approximately 3000mg/d compared to the 3300mg/d target. For Na, the target for all four interventions was set at 3300 mg/d, however analysis showed Na content to be slightly higher for the potato (3417 ± 83mg/d) and FF (3466 ± 117mg/d), and slightly lower for the control and supplement phases (2974 ± 61mg/d). All results reported as means ±SE.

Blood Pressure

Primary BP analysis was a comparison of end of treatment SBP and DBP for all interventions. There were no significant differences among interventions (all values mmHg; control: 129.3 ± 0.9/ 83.5 ± 1.3, potatoes: 126.2 ± 0.93/ 83.6 ± 1.3, French fries: 127.8 ± 0.94/ 83.6 ± 1.3, supplement: 128.5 ± 0.9/ 83.8 ± 1.6; SBP:P=0.07, DBP: P=0.87. Mean ± SE). However, differences for SBP between control and potato phases approached significance (P=0.059; Figure 2.4). Secondary BP analysis assessed change in BP over time. Looking at change in BP over the course of each intervention there were also no significant differences among interventions (all values mmHg control: -2.6 ± 0.9/ -1.2 ± 0.6, potatoes: -6.0 ± 1.1/ -1.3 ± 0.7, French fries: -3.6 ± 1.3/ -1.5 ± 0.9, supplement: -3.4 ± 0.9/ -0.8 ± 0.8; SBP: P=0.09, DBP: P=0.9. Mean ± SE). However, the potato intervention resulted in a SBP change of -6.01mmHg which, after post hoc contrast analysis, was significantly lower (P=0.017) when compared to the SBP change in the control diet (-2.6mmHg; Figure 2.5).

Cutaneous Microvascular Function

There were no significant differences in endothelial function (measured as percent of cutaneous vascular conductance max (%CVCmax ± SE) among diets (control: 87.8 ± 1.5%, French Fries: 87.7 ± 1.4%, potatoes: 88.5 ± 1.1%, supplement: 88.9 ± 1.0%; P > 0.05. Mean ±SE) (Figure 2.6).

Discussion

Research focused on dietary potassium as a specific nutrient of interest, in the context of effects on BP, is extremely limited. Currently, evidence only exists from interventions utilizing dietary advice with mixed results [12-14]. In an earlier study, Chalmers et al. assessed the effects of both increasing dietary potassium and dietary sodium (Na), via nutrition coaching, on BP in hypertensive individuals from an Australian cohort [12]. After 12 weeks subjects (age 52.3±0.8 years; 181 males and 31 females) placed on the high potassium intake diet (>100mmol/3900mg potassium/d) had significant decreases in both SBP and DBP of 7.7±1.1 and 4.7±0.7 mmHg, respectively. However, the other invention arms (low Na arm, low Na/high potassium arm) had similar reductions in BP, highlighting the possibility that the effect was due to overall diet change, and not a specific nutrient (e.g. potassium). Findings were similar in another Australian study using dietary advice and supplements in a cross-over design to assess the effects of high vs. low Na on BP in the context of a high potassium diet (≈3400mg/d (~87mmol/d)) [20]. Despite Na supplementation up to 2760mg/d (120/mmol/d), SBP was reduced by 5.5mmHg, however the study lacked a control arm, making it difficult to conclude if the effects were due specifically to potassium intake. More recent studies utilizing dietary advice to manipulate dietary potassium have returned null results [13, 14]. In a study based in the United Kingdom, investigators assessed the effects of increased potassium intake from both dietary sources and supplements on BP in pre-hypertensive individuals (N=48, 22-65 years) [13]. In a cross-over design, subjects completed four, 6-week dietary interventions including a control diet, an additional 20 or 40mmol potassium/d (780 or 1560mg/day) from fruit and vegetables, and 40mmol potassium citrate/d, with no significant differences in BP between interventions. Compared to the Chalmers study [12], less of an increase in dietary potassium (780-1560mg/d increase compared to 3900mg/d) as well as lower baseline BP (SBP: 137.7 vs >150mmHg, DBP: 88.6 vs. >95mmHg) may explain the difference in results to some degree. The focus on only instructing participants to increase dietary potassium from fruit and vegetable sources, compared to coaching focused on both potassium and Na intake could also be the reason for the disparity in findings. However, Miller et al. found similar results from an RCT designed to assess the effect of a potassium focused DASH diet on BP in an urban African American population [14]. While compliance can be measured somewhat accurately in these trials (via spot in Miller et al.[14],

and 24hr urine collection in Chalmers et al.[12]) one of the primary limiting factors in dietary advice interventions is the lack of a controlled diet. Although there are controlled feeding interventions that increase potassium due to a change in overall dietary pattern (e.g. DASH) resulting in BP benefit, because of the manipulation of many nutrients to achieve the desired dietary pattern, these findings cannot be attributed to potassium alone [15, 16]. The lack of controlled feeding trials looking specifically at potassium as the nutrient of interest for BP, and other health outcomes, represents a large gap in the literature.

We assessed the effect of dietary potassium from potato sources (bake or boiled (potato) and French fry (FF)) or a potassium supplement (K-gluconate) on BP outcomes in pre-hypertensive to hypertensive adults via a cross-over, highly controlled feeding trial. Potatoes comprise ~20% of the vegetable intake in the American diet, with white potatoes and French fries representing ~7 and 3% of overall potassium intake [2]. This is the first controlled feeding trial, to our knowledge, looking specifically at dietary potassium as the primary nutrient of interest on effects on BP. While there were no significant differences between interventions for either SBP or DBP outcomes, increased potassium from baked or boiled potatoes did appear to benefit SBP compared to the control group (126.2 vs. 129.3mmHg), a difference of 3.1 mmHg that approached significance (P = 0.059). Similarly, there was a significant change from baseline in SBP between the potato (-6.01mmHg) compared to the control diet (-2.6mmHg) (P=0.029). We assessed BP both ways to ensure we captured complete treatment effects. While a large change in BP over time may be considered clinically relevant in a healthcare setting, and many other interventions assessing BP outcomes have reported change in BP over intervention time course as a primary outcome, comparing differences between interventions at the end of each treatment is often considered more relevant. Measures of screening and baseline BP are often found to be much higher, attributed to white coat syndrome, or similar participant unsettling due to unfamiliar circumstances, or just simply having their BP measured [21]. In research looking at BP changes over time may inflate differences between groups. Because of this possible inflation, we considered end of treatment SBP and DBP as the primary BP outcomes to better understand the true effects of the intervention.

There were also no significant differences in endothelial function/cutaneous microcirculation, assessed utilizing thermal hyperemia, measured via laser Doppler flowmetry. Local cutaneous thermal hyperemia is the measurement of skin blood flow in response to a heat

stimulus, and an indirect measure of endothelial function via NO release. It has been consistently demonstrated that approximately 60-70% of the thermal hyperemic vasodilatory response is mediated by the production and release of NO [22, 23]. It is also thought that initial changes in the microvasculature, as measured in the capillaries and arterioles of the skin, may be directly related to endothelial dysfunction, which is often considered the initiating event in the development of CVD and related mortalities. The lack of significant differences in endothelial function between interventions may have been due to measurement methodological issues [24]. The temperature used in our protocol to elicit a maximal vasodilatory response was only one degree Celsius above the plateau phase (indirect measure of NO release and endothelial function). This may have produced a ceiling effect in which the plateau phase was already causing maximal vasodilation, making it difficult to determine any treatment effects. The decrease in perfusion pressure for each intervention (especially during the first week) also may have blunted any possible vasodilatory benefit the increase in potassium may have had.

Despite these null findings, it is interesting to note that the FF intervention of the study had no negative impact on BP or cutaneous microvascular outcomes overall. While this may seem counter intuitive to common nutrition advice, it is important to point out that this was a highly controlled dietary intervention where all nutrients, aside from potassium, were designed to be the same within each diet. The added fat and Na from the French fries was adjusted for within the FF arm, however the French fries that we used were comparable in energy, fat and Na (~500 kcals, 20g of fat, 500 mg of Na) to a large serving from a fast food restaurant.

Our dietary potassium intake intervention level was set at 3300mg/d, or 70% of the potassium AI at the time of the trial, based on guidelines from the 2005 Dietary Reference Intake (DRI) report [25]. Chemical analysis of the diets showed a potassium content of closer to 3000mg/d for both potato groups (baked/boiled potatoes and FF), and approximately 3300mg/d for the supplement group. The difference in target potassium intake vs. actual intake based on chemical analysis is similar to findings of other controlled feeding trials [15, 16], and could have been due to variations in potassium content of fruits and vegetables, which may differ based on season, location, and crop. Despite this, the potassium content of the diets was still higher than the average American intake for healthy normotensive individuals (≈2668mg/d), and of those with hypertension (≈2646mg/d) [26].

According to the recently published DRI guidelines for potassium, lack of a sensitive biomarker offer insufficient evidence to establish Estimated Average Requirement and Recommended Daily Allowance levels for potassium adequacy or deficiency [27]. Because of this, the DRI committee again set an adequate intake (AI) level for potassium, although at a much lower level than the 2005 DRI committee (4700mg/d). The highest median potassium intakes in healthy, normotensive individuals across the National Health and Nutrition Examination Survey (NHANES) and Canadian Community Health Survey (CCHS) were selected for each DRI group and set as the AIs. In contrast to the 2005 DRI report adult intake levels were separated by sex, with a potassium intake of 3400mg/d for males and 2600mg/d for females [27]. With this recent review the DRI committee also established criteria for a new intake value for Chronic Disease Risk Reduction (CDRR), a DRI intended to help differentiate between nutrient intakes necessary for adequacy vs. those which may improve health. Because observational data looking at increased potassium intakes and CVD (and associated disease) mortality risk are mixed, CDRR intake level for potassium could not be established.

The two recent, large systematic reviews by the AHRQ and the 2019 DRI committee examined 37 observational studies assessing total, cardiovascular disease (CVD), coronary heart disease (CHD), stroke, and myocardial infarction (MI) mortality. Twenty-five studies assessed dietary intake (e.g. 24hr recall, food frequency questionnaire) while others used the surrogate measure of either 24hr urine or spot urine. Of the 37 associations, 18 found an inverse relationship between potassium intake and CVD and related mortality. Observational and prospective cohort studies to date have primarily used 24hr urine, spot urine, and dietary recalls to assess potassium intake. In addition to the inherent error associated with these assessment methods, there is also a large inter-individual variability with excretion measures, especially when only represented by one time point. Like Na, potassium excretion may have an infradian (lasting longer than one day) or possibly an ultradian (less than one day) rhythm [28], making a single 24hr collection an inaccurate assessment of actual intake. In addition, the aforementioned potassium content of potassium rich fruit and vegetables, which may vary greatly based on crop and season, can add more error to dietary intake assessment methods. From these findings the DRI committee concluded there is a need for more randomized clinical trials assessing CVD outcomes. While ideal, a potassium intervention that follows participants for years, and utilizes necessary measures (e.g. controlled diets, multiple 24hr urine collections) to accurately examine

potassium intake and mortality risk is impractical. Both groups also assessed evidence related to the associated between potassium intake and BP, each concluding increased potassium had a moderate benefit to BP outcomes, although out of the 26 trials reviewed only 4 included manipulation of potassium via dietary intake, pointing the need for more clinical trials looking at potassium from food as the primary nutrient of concern.

It is clear that more dietary interventions looking specifically at the effects of increasing potassium from food sources are needed to elucidate the true effect of dietary potassium on BP, as well as other CVD outcomes. Our intervention may be an important first step in this direction. While there were no statistically significant differences among treatments for primary outcomes, both potato groups (baked/boiled and FF) had a greater drop in SBP compared to control, with the baked/boiled potato group approaching significance in the primary end of treatment SBP outcome. Lack of significance may have been due to a smaller sample size compared to other BP trials, or the relatively short duration. Intervention duration may be a major limiting factor in the clinical research setting, as the potassium supplementation trials reviewed by the AHRQ ranged from 4 weeks to 36 months, and the longest dietary interventions 8-12 weeks. A study by Siani et al. saw a reduction in hypertensive drug therapy in a group of patients given dietary instruction on how to consume a high potassium diet compared to a control group. Reductions in medication usage occurred in both groups for the first six months of intervention, but only continued for the potassium group thereafter [29]. In contrast to this, a recent analysis by the DASH investigators showed diet-induced changes in BP were achieved within 2 weeks on a controlled DASH dietary pattern [30]. Overall, dietary potassium interventions to date, not utilizing a controlled diet or only manipulating a single nutrient, may not be long enough to see the effect of the intervention. In additional to studying potassium form (diet vs. supplement), future research will need to assess dose-response evidence for potassium to assess at what of level of potassium intake maximum benefit can be obtained. Overall little is known about how increasing dietary potassium alone may affect BP, and understanding this specific relationship may pave the way for understanding the relationship between potassium and other health outcomes.

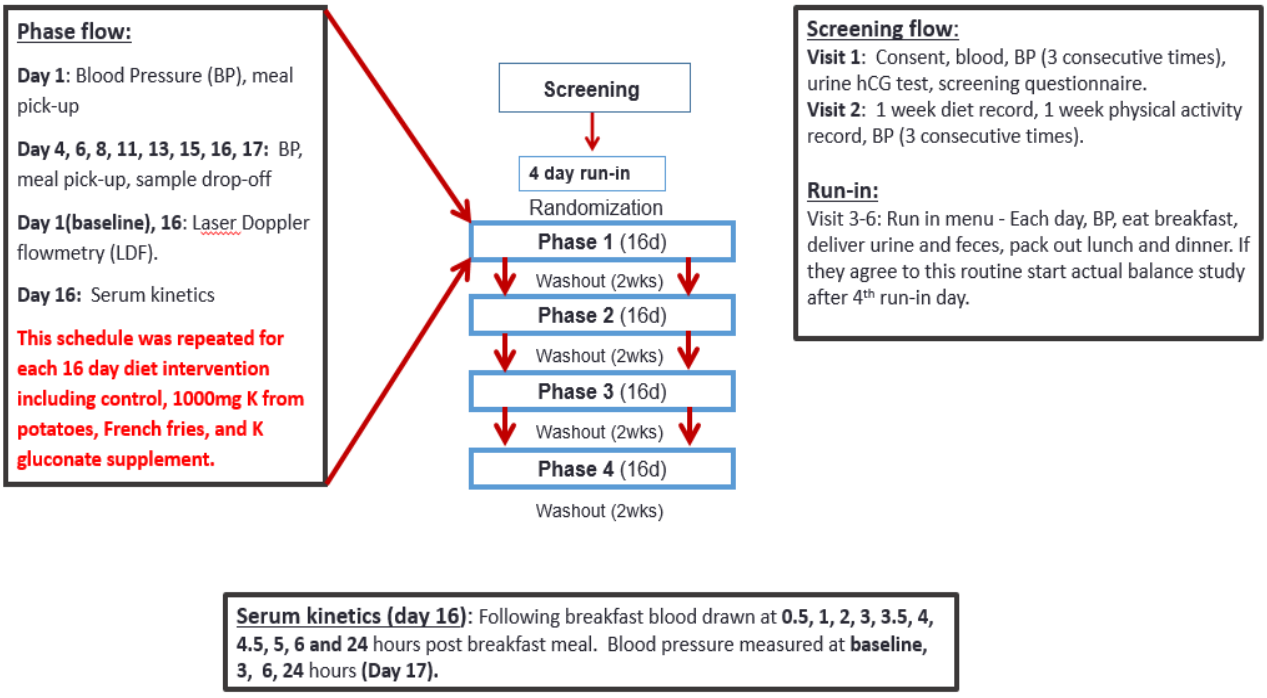

Figure 2.1. Overall study design and measures. Study design was a randomized, cross-over, controlled feeding study with primary repeated measures of blood pressure.

Table 2.1 Baseline characteristics of all subjects who completed at least two interventions.

	All Mean (SD)	Male Mean (SD)	Female Mean (SD)
n	30	15	15
Age (y)	48.2 (15.0)	43.8 (13.7)	52.7 (15.4)
Height (cm)	172.2 (10.2)	179.4 (7.1)	165.0 (7.2)
Weight (kg)	93.86 (22.9)	99.1 (20.7)	88.6 (24.4)
BMI (kg/m^2)	31.4 (6.1)	30.5 (4.8)	32.3 (7.2)
Systolic Blood Pressure (mmHg)	136.3(11.9)	134.6(12.7)	138.0(11.3)
Diastolic Blood Pressure (mmHg)	86.12(7.1)	85.9(7.0)	86.4(7.4)

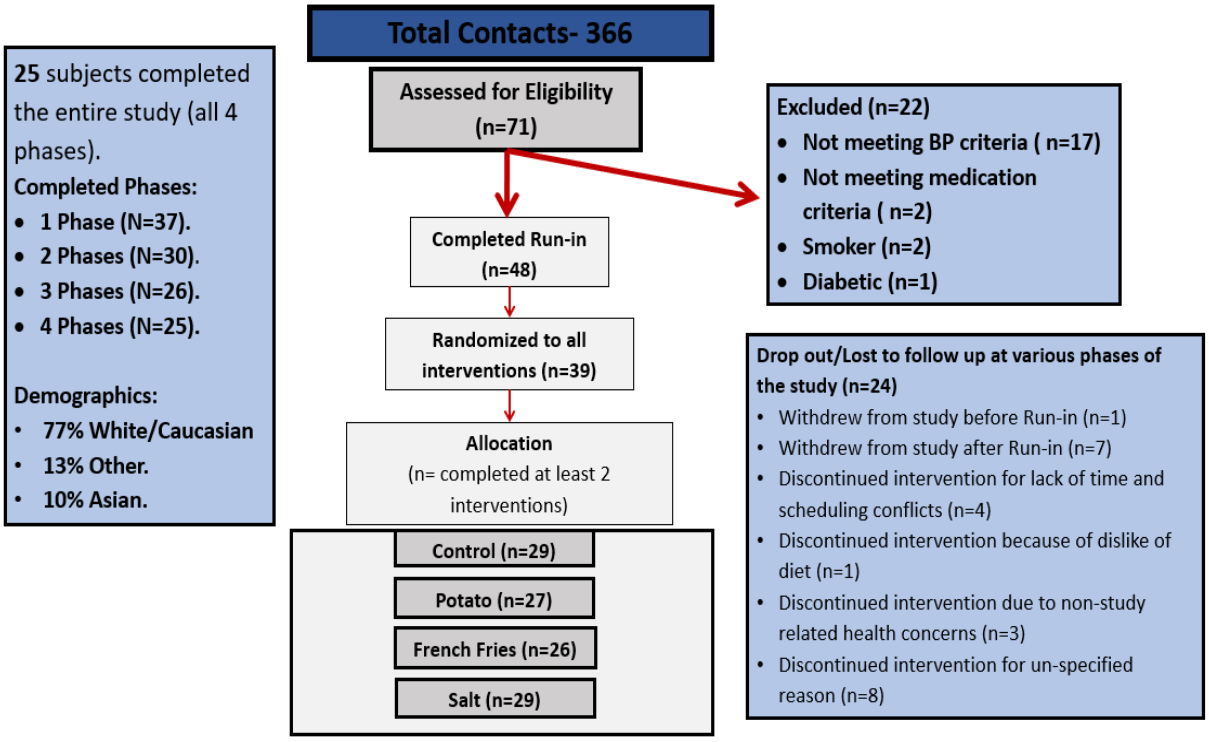

Figure 2.2. Study recruitment and flow diagram.

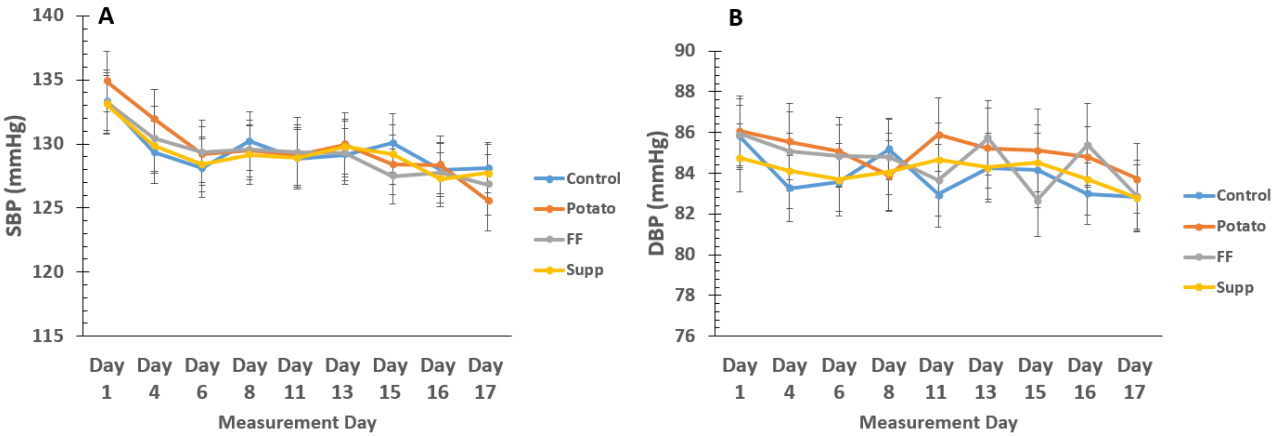

Figure 2.3. Blood pressure throughout each intervention. (A) Changes in systolic blood pressure (SBP) and (B) diastolic blood pressure (DBP) for all intervention phases (N=30). No significant differences among groups at end of treatment ($P > 0.05$).

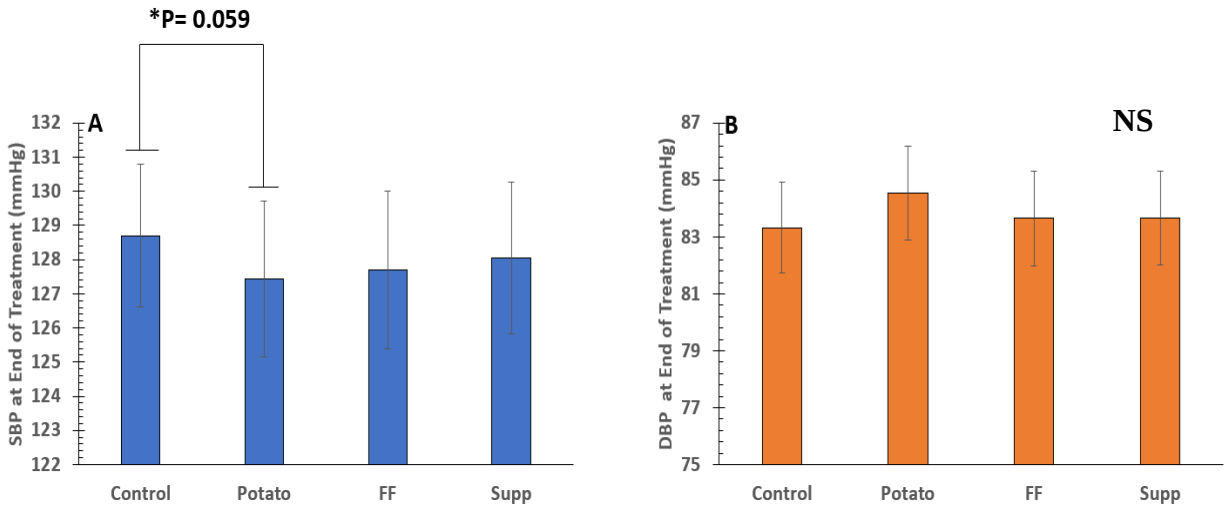

Figure 2.4. Primary BP analysis: Comparison of end of treatment systolic (SBP) and diastolic (DBP) blood pressure for all intervention phases (N=30). Changes in systolic blood pressure (A; SBP) and diastolic blood pressure (B; DBP) for all intervention phases (N=30). Results for differences in systolic blood pressure were assessed at the end of treatment (average of day 15, 16, and 17 measurements) using a mixed model with repeated measures with adjustment for baseline BP as a covariate, with no significant differences (NS) among phases (control: 129.3 ± 0.9/ 83.5 ± 1.3, potatoes: 126.2 ± 0.93/ 83.6 ± 1.3, French fries: 127.8 ± 0.94/ 83.6 ± 1.3, supplement: 128.5 ± 0.9/ 83.8 ± 1.6; SBP:P=0.07, DBP: P=0.97). However, differences between SBP for control and potato phases approached significance (P=0.059). Means ±SE.

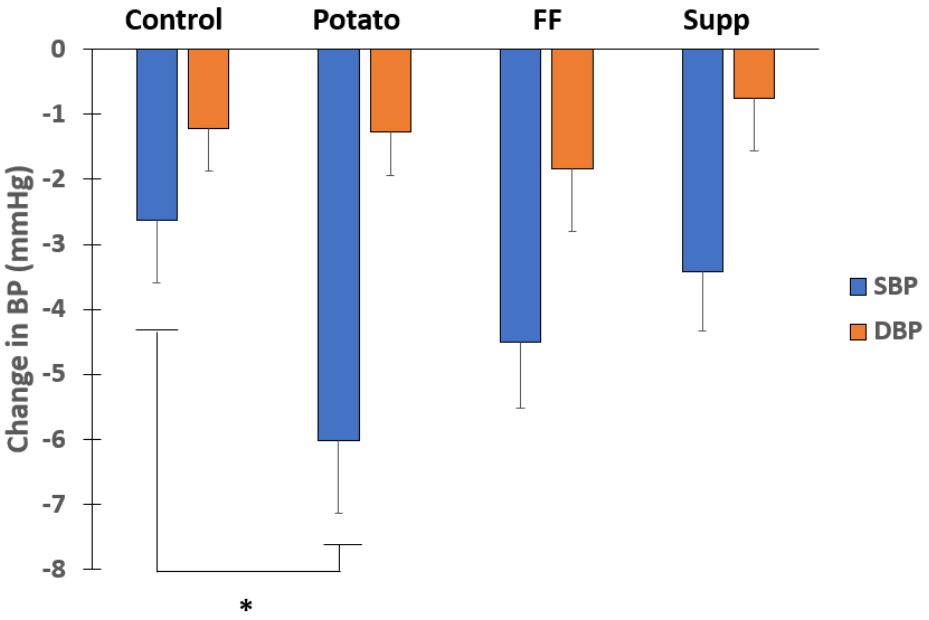

Figure 2.5. Change in BP over time. BP over the course of each intervention, the potato arm resulted in a SBP change of -6.01 mmHg which, after contrast analysis, was significantly lower (P=0.02) when compared to the SBP change in the control group (-2.6 mmHg). Means ±SE.

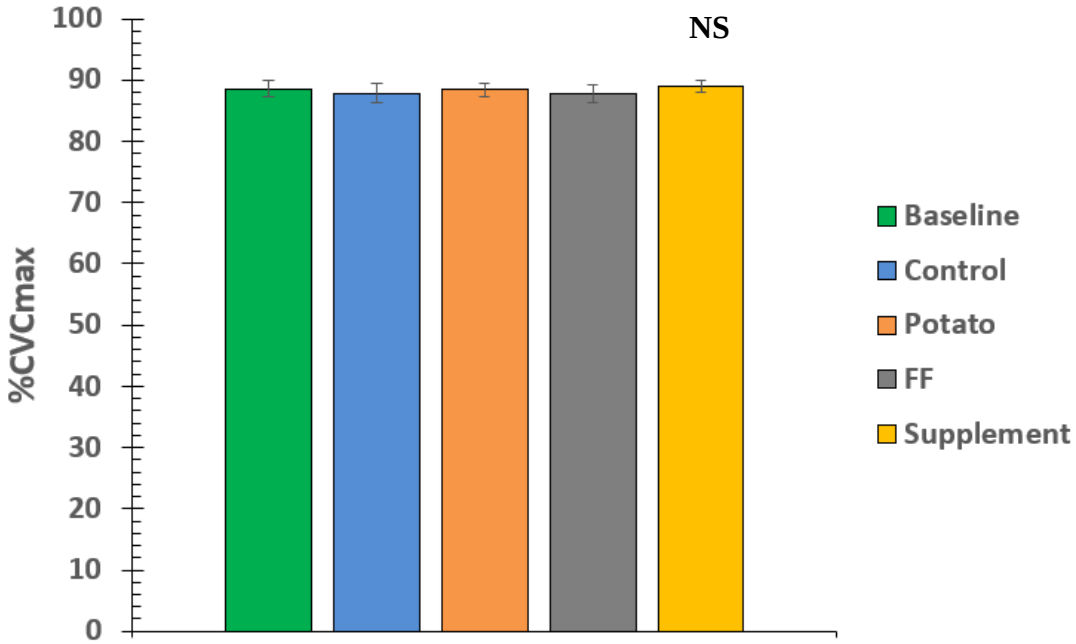

Figure 2.6. Cutaneous Microvascular function among groups. Changes in microvascular function as measured via laser Doppler flowmetry (LDF) for all intervention phases (N=30). No significant differences (NS) among groups at end of treatment ($P > 0.05$). Means ±SE.

References

1. *Dietary Guidelines Advisory Committee. Report of the Dietary Guidelines Advisory Committee on the Dietary Guidelines for Americans, 2010.*, in *Washington, DC: U.S. Department of Agriculture, Agricultural Research Service*. 2011.

2. *U.S. Department of Health and Human Services and U.S. Department of Agriculture. 2015-2020 Dietary Guidelines for Americans. 8th Edition.* 2015.

3. Roger, V.L., et al., *Executive summary: heart disease and stroke statistics--2012 update: a report from the American Heart Association.* Circulation, 2012. **125**(1): p. 188-97.

4. Lopez, A.D. and C.D. Mathers, *Measuring the global burden of disease and epidemiological transitions: 2002-2030.* Ann Trop Med Parasitol, 2006. **100**(5-6): p. 481-99.

5. Newberry, S.J., et al., *Sodium and Potassium Intake: Effects on Chronic Disease Outcomes and Risks*. 2018, Rockville MD.

6. Aburto, N.J., et al., *Effect of increased potassium intake on cardiovascular risk factors and disease: systematic review and meta-analyses.* BMJ, 2013. **346**: p. f1378.

7. Whelton, P.K., et al., *Effects of oral potassium on blood pressure. Meta-analysis of randomized controlled clinical trials.* JAMA, 1997. **277**(20): p. 1624-32.

8. Cappuccio, F.P. and G.A. MacGregor, *Does potassium supplementation lower blood pressure? A meta-analysis of published trials.* J Hypertens, 1991. **9**(5): p. 465-73.

9. Geleijnse, J.M., F.J. Kok, and D.E. Grobbee, *Blood pressure response to changes in sodium and potassium intake: a metaregression analysis of randomised trials.* J Hum Hypertens, 2003. **17**(7): p. 471-80.

10. Binia, A., et al., *Daily potassium intake and sodium-to-potassium ratio in the reduction of blood pressure: a meta-analysis of randomized controlled trials.* J Hypertens, 2015. **33**(8): p. 1509-20.

11. Dickinson, H.O., et al., *Potassium supplementation for the management of primary hypertension in adults.* Cochrane Database Syst Rev, 2006(3): p. CD004641.

12. Chalmers, J., et al., *Australian National Health and Medical Research Council dietary salt study in mild hypertension.* J Hypertens Suppl, 1986. **4**(6): p. S629-37.

13. Berry, S.E., et al., *Increased potassium intake from fruit and vegetables or supplements does not lower blood pressure or improve vascular function in UK men and women with early hypertension: a randomised controlled trial.* Br J Nutr, 2010. **104**(12): p. 1839-47.

14. Miller, E.R., 3rd, et al., *A Dietary Intervention in Urban African Americans: Results of the "Five Plus Nuts and Beans" Randomized Trial.* Am J Prev Med, 2016. **50**(1): p. 87-95.

15. Appel, L.J., et al., *A clinical trial of the effects of dietary patterns on blood pressure. DASH Collaborative Research Group.* N Engl J Med, 1997. **336**(16): p. 1117-24.

16. Sacks, F.M., et al., *Effects on blood pressure of reduced dietary sodium and the Dietary Approaches to Stop Hypertension (DASH) diet. DASH-Sodium Collaborative Research Group.* N Engl J Med, 2001. **344**(1): p. 3-10.

17. Svetkey, L.P., et al., *Effect of the dietary approaches to stop hypertension diet and reduced sodium intake on blood pressure control.* J Clin Hypertens (Greenwich), 2004. **6**(7): p. 373-81.

18. *The effects of nonpharmacologic interventions on blood pressure of persons with high normal levels. Results of the Trials of Hypertension Prevention, Phase I.* Jama, 1992. **267**(9): p. 1213-20.

19. Alba, B.K., et al., *Acute dairy milk ingestion does not improve nitric oxide-dependent vasodilation in the cutaneous microcirculation.* Br J Nutr, 2016. **116**(2): p. 204-10.

20. Nowson, C.A., T.O. Morgan, and C. Gibbons, *Decreasing dietary sodium while following a self-selected potassium-rich diet reduces blood pressure.* J Nutr, 2003. **133**(12): p. 4118-23.

21. Gorostidi, M., et al., *Prevalence of white-coat and masked hypertension in national and international registries.* Hypertens Res, 2015. **38**(1): p. 1-7.

22. Brunt, V.E. and C.T. Minson, *Cutaneous thermal hyperemia: more than skin deep.* J Appl Physiol (1985), 2011. **111**(1): p. 5-7.

23. Fujii, N., V.E. Brunt, and C.T. Minson, *Tempol improves cutaneous thermal hyperemia through increasing nitric oxide bioavailability in young smokers.* Am J Physiol Heart Circ Physiol, 2014. **306**(11): p. H1507-11.

24. Cracowski, J.L., et al., *Methodological issues in the assessment of skin microvascular endothelial function in humans.* Trends Pharmacol Sci, 2006. **27**(9): p. 503-8.

25. Institute of, M., *Dietary Reference Intakes for Water, Potassium, Sodium, Chloride, and Sulfate*. 2005, Washington, DC: The National Academies Press.

26. *Updated dietary reference intakes for sodium and potassium.(Report).* Pediatrics Week, 2019: p. 284.

27. National Academies of Sciences, E., et al., *The National Academies Collection: Reports funded by National Institutes of Health*, in *Dietary Reference Intakes for Sodium and Potassium*, M. Oria, M. Harrison, and V.A. Stallings, Editors. 2019, National Academies

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28. Rakova, N., et al., *Long-term space flight simulation reveals infradian rhythmicity in human Na(+) balance.* Cell Metab, 2013. **17**(1): p. 125-31.

29. Siani, A., et al., *Increasing the dietary potassium intake reduces the need for antihypertensive medication.* Ann Intern Med, 1991. **115**(10): p. 753-9.

30. Juraschek, S.P., et al., *Time Course of Change in Blood Pressure From Sodium Reduction and the DASH Diet.* Hypertension, 2017. **70**(5): p. 923-929.

CHAPTER 3: SHORT-TERM SUPPLEMENTAL DIETARY POTASSIUM FROM POTATO AND POTASSIUM GLUCONATE: EFFECT ON POTASSIUM AND SODIUM RETENTION IN PRE-HYPERTENSIVE-TO-HYPERTENSIVE ADULTS

Abstract

Potassium is an essential nutrient, that has been labeled a shortfall nutrient by recent Dietary Guidelines for Americans Advisory Committees. Current recommendations established by the National Academy of Sciences 2019 DRI committee are given as an Adequate Intake (AI) of 3400 mg/d for men and 2600 mg/d for women. Little is known about potassium tissue retention, and less is understood on how retention may affect health outcomes. In this clinical trial, we assessed the effects of potassium intake from potato sources and a potassium supplement on overall whole-body potassium and sodium (Na) balance. Twenty-eight pre-hypertensive-to-hypertensive (Systolic BP ≥ 120mmHg) men (N=14) and women (N=14) with a mean ± SD age, BMI, and BP (systolic(SBP)/ diastolic(DBP)) of 47.3 ± 14.8 y, 31.2 ± 6.3kg/m^2, and 136.3 ± 12.0/ 85.6 ± 6.3mmHg, respectively, were enrolled in a cross-over, randomized controlled diet trial. Participants were assigned to a random order of four 16-day dietary potassium interventions including a basal diet (control) of 2300mg/d(~60mmol/d), and three phases of an additional 1000mg/d(3300mg/d(~85mmol/d) total) of potassium in the form of potatoes (baked, boiled, or pan-heated with no additional fat), French fries (FF), or a potassium (K)-gluconate supplement. Each intervention was separated by two or more weeks of wash-out. Twenty-four hour urine and stool samples were collected throughout each intervention phase, and assessed via ICP-OES for potassium and sodium (Na) content. Results for differences in mineral balance were assessed as 13-day averages as follows: retention (mg/d) = dietary intake – (urine + stool losses) using a mixed model ANOVA with Tukey post hoc analyses. Due to slight nutrient differences in the calculated vs. the chemical analysis of the controlled diets, retention was assessed as absolute (mg/d) and as a percent of intake. Potassium mineral retention as percent (%) of intake (± SE) for interventions of control, potato, FF, and K-gluconate was 23.3 ± 5.6%, 26.4 ± 5.3 %, 26.8 ± 4.9 %, and 33.1 ± 5.3 %, respectively. Potassium retention was significantly higher (P = 0.05) during the supplement intervention compared to the control

(P=0.04). Sodium retention in the potato phase (11.8 ± 6.3%) was lower compared to the FF (22.8 ± 4.8) phase, and significantly lower (P = 0.0006) compared to the control (26.1 ± 5.4; P = 0.0004), and supplement (30.6 ± 5.0% P=0.01) interventions. There is little understanding of what constitutes a potassium intake for adequacy. Further assessment of potassium and Na retention across many different groups is needed, especially in vulnerable populations, which may benefit from higher potassium intakes and an understanding of what is necessary for that benefit to be achieved. This trial was registered at ClinicalTrials.gov as NCT02697708.

Introduction

Potassium is an essential nutrient, that has been labeled a shortfall nutrient by recent Dietary Guidelines for Americans Advisory Committees [1, 2]. Increases in potassium intake have been linked to improvements in cardiovascular and other metabolic health outcomes. Recently, the dietary reference intake (DRI) was reassessed for potassium, as well as sodium (Na). The review committee established a new potassium adequate intake (AI) for healthy adults (≥19y) at 3400mg/d for males and 2600mg/d for females [1]. The new AIs are set at the highest median intake for both age and gender, based on two national health surveys, the National Health and Nutrition Examination Survey (NHANES) and Canadian Community Health Survey (CCHS). While a comprehensive review of the potassium literature was undertaken, the 2019 DRI review committee concluded there was insufficient evidence to establish potassium intake levels for adequacy for any population [1].

A specific biomarker of potassium status has yet to be clearly identified. Observational research shows associational benefit from increased potassium intakes related to CVD and related mortality risk [2-5], while randomized clinical trials have primarily used potassium supplementation to examine effects of higher potassium intakes showing benefit to BP outcomes [6-9]. Despite this, findings from two recent large systematic reviews, by the Agency for Healthcare Research and Quality (AHRQ) and the 2019 DRI committee, concluded that there was insufficient data to establish intake values based on disease risk benefit in healthy, or unhealthy individuals [1, 10]. Because of this, the primary evidence the 2019 DRI committee reviewed for the establishment of potassium intakes for adequacy came from potassium retention studies conducted in normotensive, healthy populations. The application of the findings from

potassium retention studies is limited, due to the small number of these interventions, as well as the lack of rigor in measurements and heterogeneity of their designs. Further, potassium balance studies are limited in number due to the necessary labor and time associated with such interventions for both participants and researchers. Only seven studies with assessment of complete intake, urinary, and fecal losses were reviewed by the DRI committee for determination of potassium adequacy. Overall, potassium balance studies show an increase in urinary potassium with increasing intake (primary mode of excretion) [11], fecal losses averaging approximately 420mg/d (10.7mmol/d) at intakes ranging from 2300-3900mg/d [12-14], as well as variable losses due to sweat [15-19]. Adequacy was defined as a potassium intake that resulted in a positive or neutral overall potassium retention. However, given the limited number, and overall heterogeneity in study design and findings, the committee concluded there was insufficient evidence to establish any type of potassium intake recommendation.

Also important in understanding the significance of potassium retention in the context of adequacy, is how potassium intake affects sodium (Na) retention. Potassium and Na have a dynamic physiological relationship, and the way in which these nutrients interact to influence fluid balance has important health implications, primarily related to BP and CVD risk. Studies have shown that consuming potassium-salts in place of Na-salts increases urinary Na excretion [20], as well as decreases CVD related mortality risk [21], trials also show improvements in BP with higher potassium intakes in the context of a high Na diet [22], while others show no improvement over an Na intake that is low [23, 24]. The relationship between potassium retention and Na retention is understudied, and how this interaction potentially effects health outcomes is less well understood.

The gap in the literature related to what level of potassium intake is necessary for adequacy, as well as disease risk reduction, is apparent. Understanding the role of potassium retention and how it relates to Na retention, and ultimately potassium adequacy is especially important in at risk populations such as pre-hypertensives/hypertensives, whose adequacy levels may be different. The goal of this research was to utilize a controlled feeding study to examine the effect of increased dietary potassium from potato sources and a potassium supplement on potassium and Na retention, compared to a control diet. We hypothesized that the interventions with an increased potassium intake would increase potassium retention and decrease Na retention, compared to control.

Methods

Subjects

Subjects (N=28, age 21y and older) were recruited at Purdue University and the surrounding area. Subjects were pre-hypertensive-to-hypertensive (SBP >120mmHg) men and women who were otherwise apparently healthy. Exclusion criteria included: medications to treat hyper or hypotension (hypertensive monotherapy was allowed later in recruitment), known to affect electrolyte balance or contain high levels of potassium or Na; history of myocardial infarction, diabetes mellitus, kidney disease, gastrointestinal disease, pancreatitis, cholestatic liver disease, cancer, smoking; use of illegal drugs or excessive alcohol intake; subjects who were pregnant or lactating, had allergy or intolerance to study foods, were unwillig to refrain from dietary supplements, or weight loss > 3kg in the past 2 months. Screening consisted of two visits at least one week apart. Subjects came to the Indiana Clinical and Translational Science Institute Clinical Reseaerch Center at Purdue University in the Department of Nutrition Science where they were consented, had their BP measured, a screening blood draw, and filled out screening questionnaires. They were also given 1-week diet and activity logs to fill out before their second screening visit, which consisted of another BP measurement to ensure eligibility, and return of the logs. When eligible, subjects completed a 4-day run-in period (described below), continuing subjects were randomized to one of 24 possible sequences of the 4 phases including, control, potato, French fry (FF), and potassium-gluconate interventions. Subjects were enrolled in May 2016 to September 2017. The study protocol was approved by the Purdue University Institutional Review Board, and all subjects provided written, informed consent.

Dietary Intervention

Study design was a randomized, cross-over, controlled feeding trial with primary measures of potassium excretion and balance and blood pressure (not reported here), and secondary measures of Na excretion and balance (Figure 3.1). After initial screening eligible subjects entered the run-in period. Each day of the 4 day run-in diet represented one of the four diet phases: control, potato, French fries, and supplement. During the run-in, subjects came to the clinical facility each morning, had their BP taken, either stayed to eat breakfast or had all their meals and snacks for the day packed out in a cooler to take with them. After the 4 day run-in

period, participants were assigned to a random order of four 16-day dietary potassium interventions including a basal diet (control) of 2300mg/d (~60mmol/d), and three phases of an additional 1000mg/d (3300mg/d (~85mmol/d) total) of potassium in the form of potatoes (baked, boiled, or pan-heated with no additional fat), French fries, or a K-gluconate supplement. Each intervention was separated by two or more weeks of wash out. A 4-day menu cycle at three calorie levels (1800, 2200, and 2600 kcal/d) was designed to manipulate potassium levels while keeping other micro (e.g. Na, Ca) and macro (e.g. fat, protein, CHO) constant. Subjects picked up study foods approximately every other day (on days 1, 4, 6, 8, 11, 13, 15, 16). For each day of controlled feeding, the subjects recorded their intake by checking off each item listed on daily menu sheets provided for each day. They were also instructed to indicate whether they ate any non-study foods and whether they did not eat all the study foods. The food and beverages were prepared with deionized water and weighed to the nearest one tenth gram on digital scales. Deionized bottle water was also given to control water intake Composites of each menu cycle day were homogenized and analyzed. Potassium gluconate supplements were sent to a food chemistry lab for analysis (Eurofins Food Chemistry Testing US, Inc.). Researchers adjusted energy intakes with snack items that contained mostly sugar. Any uneaten items were instructed to be returned and analyzed for mineral content.

Mineral Balance and Net Absorption

Complete daily urine and feces were collected in acid washed containers for each 16-day intervention of the study, on a daily basis. Instructions and all necessary supplies (e.g. containers for urine and stool collections) were provided. Urine was pooled as 24-hr collections, which were analyzed for creatinine to assess urinary collection compliance. Fecal samples were also pooled for each 24-h period, and completeness of collections was determined by measuring fecal polyethylene glycol (PEG) recovery by turbidimetric assay. PEG was administered as two 500 mg capsules, instructed to be taken three times per day with each meal. Urine for mineral analysis was acidified with 1% (by vol) HNO_3 and stored at −20 °C. Fecal samples were homogenized with ultra-pure water and concentrated HCl using a laboratory stomacher (Tekmar Co, Cincinnati, OH); they were then heated in a drying oven at 50 °C for a minimum of 24 h, ashed in a muffle furnace at 600 °C for 96 h, and diluted in 1N HNO_3 for total mineral analysis. Urine was analyzed for creatinine, potassium and Na. Stools were analyzed for PEG and

minerals. Dietary, urinary, and fecal minerals were measured by ICP-OES (5100 PC; Perkins Elmer, Waltham, MA). Unacidified urine was measured for creatinine by a kinetic modification of Jaffe's colorimetric assay (Cobas Mira Plus; Roche Diagnostic Systems, Nutley, NJ). The first 2 days of each phase were excluded from balance calculations as equilibration days, as was the last day (day 16), which was an in-clinic testing day. Thus, a 13-day period was assessed for potassium and Na excretion and retention for each intervention. Balance and percent absorption (%ab) were determined using the following equations averaged over the 13 days:
Daily potassium/sodium balance (mg/d) = daily potassium/sodium intake (mg/d) – daily potassium/sodium excretion (mg/d) (urine and stools).

% Net absorption = daily potassium/sodium intake (mg/d) - daily potassium/sodium fecal excretion (mg/d) x 100.

Statistical Methods

Results for differences between interventions in potassium and Na for urinary loss, fecal loss, and retention (both absolute and as % of intake), were analyzed using a mixed model ANOVA, and Tukey post hoc adjustment for pairwise comparisons between diet types. All computations were performed using JMP (SAS Institute, Cary, NC, USA) statistical software. Alpha was set at 0.05. A sample size of 20 cross-over participants with two-sided alpha=0.05, provided 80% power to detect differences in change in potassium and Na retention of 350 and 450 mg/d or 10 and 14% of daily intake, respectively. All values are reported as mean ± SE unless otherwise stated.

Results

Baseline Characteristics of Study Subjects

Overall data from 28 subjects were included for urinary excretion measures (Table 3.1), and 20 subjects were included in fecal excretion, and mineral retention analysis, for whom these measures were available. On average, subjects (N=20) were middle-aged to older adults (48.2 ± 15.0y), had a BMI bordering overweight to obese (31.4 ± 6.1kg/m^2), and a SBP and DBP of 136.3 ± 11.9mmHg and 86.1 ± 7.1mmHg, respectively (all data mean ± SD).

Subject Adherence

A total of 23 subjects completed all four interventions, one subject completed three, and four subjects completed two. Three subjects were on some form of hypertensive monotherapy, and were monitored to ensure timing and dose of medication did not change during the study duration. The mean weight change for the control, potato, FF, and supplement interventions was -1.1kg, -0.9kg, -0.9kg, -1.0kg, respectively. Supplement intake compliance by pill count was 92%. For potassium and Na fecal excretion, retention, and %ab, all 20 subjects for which these measures are available completed all four phases of the study.

Chemical Analysis of Controlled Diets

There was some unexpected variation in the potassium and sodium content of the diets (Figure 3.2). Potassium content for the interventions of control, potato, French Fry (FF), and supplement were 2238 ± 45, 3008 ± 4, 2977 ± 31, and 3299 ± 43mg/d, respectively. The potassium levels for the control and supplement phases were close to their targets of 2300 and 3300 mg/d, respectively, while the potato and FF were slightly lower at approximately 3000mg/d compared to the 3300mg/d target. Sodium levels were set at 3300mg/d for all diets, however analysis showed Na content to be slightly higher for the potato (3417 ± 83mg/d) and FF (3466 ± 117 mg/d) phases, and slightly lower for the control and supplement phases (2974 ± 61mg/d).

Urinary Potassium and Sodium Excretion

There were significant differences among groups in both urinary potassium ($P < 0.0001$) and Na ($P < 0.0001$) excretion. For potassium, the control phase urinary excretion (1516 ± 101.9mg/d) was significantly lower than the potato (1932.6 ± 153.1mg/d; $P = 0.0002$), FF (1824.4 ± 119.7mg/d; $P = 0.006$), and supplement (1981.7 ± 162.4; $P = 0.006$) phases (Figure 3.3). For Na, the potato phase (2817.0 ± 174.7mg/d) was significantly higher than both the control (2202.3 ± 123.5mg/d; $P < 0.0001$) and supplement (2106.0 ± 129.3mg/d; $P < 0.0001$) interventions, and was also significantly higher with the FF intervention (2530 ± 143.8mg/d) compared to the control ($P = 0.04$) and supplement ($P = 0.002$) phases. (Figure 3.4).

Potassium and Sodium Fecal Excretion, Balance, and % Absorption

There were no significant differences ($P =0.18$) in fecal potassium excretion with losses of 336.5 ± 34.0, 348.5 ± 27.6, 382.7 ± 37.0, 317.6 ± 31.5mg/d, for control, potato, FF, and

supplement interventions, respectively (Figure 3.3). Differences in potassium and Na retention were assessed as absolute and % of intake. Absolute potassium retention was significantly higher during the supplement (1087.0 ± 176.5mg/d) compared to the control (550.3 ± 126.7mg/d; P < 0.0001), potato (797.0 ± 155.8mg/d; P=0.04), and FF (804.0 ± 139.2mg/d; P = 0.05) study phases. However, when assessed as percent of intake (%) the only a significant difference remained between only the supplement (33.1 ± 5.3 %) and the control (23.3 ± 5.6%; P=0.04) (Figure 3.5).

There were also no significant differences (P =0.18) in fecal Na excretion with losses of 123.8 ± 13.5, 141.3 ± 17.7, 122.8 ± 15.2, 111.8 ± 13.7mg/d, for control, potato, FF, and supplement interventions, respectively (Figure 3.4). Absolute Na retention was significantly lower during the potato (458.9 ± 208.0 mg/d) compared to the control (858.5 ± 164.9mg/d; P = 0.002), FF (834.8 ± 162.0mg/d; P=0.047), and supplement (1005.1 ± 158.3mg/d; P = 0.0015) study phases. Sodium retention in the potato group, when assessed as % of intake (11.8 ± 6.3), remained significantly lower than both the control (26.1 ± 5.4; P = 0.01) and supplement (30.6 ± 5.0; P = 0.0004) phases (Figure 3.6).

There were significant differences in percent net absorption (%ab) for both potassium (P = 0.005) and Na (P =0.025). For potassium, the %ab in the supplement phase (91.9 ± 1.0) was significantly higher compared to the control (88.3 ± 1.3; P = 0.003). For Na, %ab was significantly lower in the potato intervention (96.2 ± 0.5) compared to the FF (97.0 ± 0.4; P = 0.047) and supplement (97.1 ± 0.4; P = 0.03) interventions (Figure 3.7).

Discussion

Potassium and sodium retention studies are limited in number due to the necessary labor and time associated with such interventions for both participants and researchers. Studies that have been conducted often suffer from small sample size, as well as design and measurement inconsistencies. Even with the work that has been done, little insight has been given to the physiological significance of potassium retention. Of the seven trials examined by the 2019 DRI committee, potassium intake levels of approximately 2200mg/d or greater resulted in a positive balance [11, 12, 14-16, 25], levels of ~2100mg/d or less resulted in a negative balance [14, 16,

25], and one study reported neutral balance at an intake of 2034mg/d [26]. However, it is difficult draw absolute conclusions from these findings, given the large amount of heterogeneity between these trials. In an observational study, Holbrook and colleagues assessed normal potassium intake and retention in a cohort of 12 men and 16 women (N=28, 20-53y) during four, 1-week periods over the course of one year. On average, men consumed 3300mg/d (84.4mmol/d) of potassium, while women consumed 2400mg/d (61.4mmol/d). Both men and women averaged an apparent absorption of 84.5%, with urinary and fecal losses of approximately 77% (2100mg/d) and 15.5% (395mg/d) of intake, respectively [12]. Sodium intake and balance were also assessed in this study, with men consuming 4200mg/d (181.8mmol/d) and retaining 730mg/d (31.6mmol/d), and women consuming 2700mg/d (116.8mmol/d) and retaining 260mg/d (11.3mmol/d). On average, both men and women had Na apparent absorption of 98.5% (3349 mg/d), with urinary losses of 85.8% (2917mg/d), and fecal losses of ~2% (56mg/d). These findings were important in establishing a basic understanding of how both potassium and Na are retained and excreted in a healthy adult population. It also revealed that at intake levels slightly higher than the median intakes reported by the 2019 DRIs, a positive or adequate potassium balance is achieved, but it is difficult to apply these findings from a small, homogenous cohort to the general population. In a more recent study, investigators examined differences in fecal and urinary potassium excretion between black (N=11) and white (N=12) healthy adults (18-45y) on a 9-day controlled diet of 3910mg/d (100mmol/d) potassium and 4140mg/d of Na (180mmol/d) [13]. Researchers found that urinary potassium was significantly lower in the blacks (2639mg/d (67.5mmol/d)) compared to the whites (3089mg/d (79mmol/d); P=0.015), while potassium fecal losses did not differ. Extrapolating from these results, it appears retention was higher in the black cohort (806mg/d (20.6mmol/d)) compared to the whites (400mg/d (10.2mmol/d)) [13]. Results for differences in Na excretions (urine and fecal) were not statistically significant. However, blacks also retained a higher amount of Na (917.9 mg/d (39.9mmol/d)) compared to whites (521.3 mg/d (22.7mmol/d)). Overall these findings may show possible differences in potassium and Na retention based on race and/or ethnicity. We previously conducted a balance study in adolescents and found similar racial differences in potassium retention. Black (N=30) and white (N=20) girls consumed a fix potassium intake (2186mg/d (56mmol/d)) at a high (4000mg/d (172mmol/d)) and low (1300mg/d (57mmol/d)) Na intake in cross over design, for two 20-day interventions. Blacks had a significantly higher, positive potassium retention during the low Na

diet compared to the whites (P<0.05), due primarily to a reduction in urinary excretion, while whites had a small negative cumulative potassium balance [14]. No race differences were reported for the high Na diet. Sodium retention was significantly higher in blacks (991mg/d (43.1mmol/d)) compared to whites (334mg/d (14.5mmol/d)) on the high Na diet. Sweat losses were also assessed and found to be ~200mg/d (5mmol/L) for potassium, and ~100mg/d (4.3 mmol/d) for Na, with no differences between groups. While these findings may be similar to those of the previously discussed study, it is difficult to compare results because average daily potassium excretions were not reported, however, findings from both of these studies may point to differences in Na retention as a possible contributing factor to the well documented racial differences in hypertension risk [27].

We assessed the effect of dietary potassium from potato sources (bake or boiled and FF) or a potassium supplement (K-gluconate) on mineral retention outcomes in pre-hypertensive to hypertensive adults via a cross-over, highly controlled feeding trial. Potatoes comprise ~20% of the vegetable intake in the American diet, with white potatoes and French fries representing ~7 and 3% of overall potassium intake [1]. Overall our findings are relatively consistent with the current literature, having a positive potassium retention with a potassium intake of ~2200mg/d or greater. Retention as a result of the supplement intervention was significantly higher (1087mg/d; $P < 0.0001$) than the other three study phases in absolute terms (mg/d), but this significance only remained over the control phase after adjusting as percent of intake (31.1 vs. 23.3%; $P = 0.04$). Sodium retention in the potato intervention, in absolute terms, was significantly lower (458.9 mg/d; $P = 0.0006$) compared to the other three interventions, and this significance remained over both the control (26.1; $P = 0.01$) and supplement (30.6; $P = 0.0004$) arms when assessed as % of the diet. This finding was even more interesting considering the higher Na intake in the potato intervention (~3400mg/d) compared to both the control and supplement (~3000mg/d). Urinary losses of both potassium and Na behaved in accordance with the controlled diets, serving as another tool of compliance. Potassium urinary excretion average 63%, and Na excretion averaged 75% of total intake across all interventions, both similar to values that have been reported in other controlled diet studies [28, 29]. There were also no significant differences in fecal losses for either mineral, also something that was expected given the primary site of regulation for both potassium and Na is the kidney. Differences in % absorption for both potassium and Na align well with findings for retention. The potato arm having a lower Na %ab

(96.1%) than the three other interventions, significantly lower than both FF (97.0%) and supplement (97.1%), and the supplement phase having a higher potassium % absorption (91.9%) than the other study arms, significantly higher than the control (88.3%) phase.

Our dietary potassium intake intervention level was set at 3300mg/d, or 70% of the potassium AI at the time of the trial, based on guidelines from the 2005 Dietary Reference Intake (DRI) report (4700mg/d) [30]. Chemical analysis of the diets show a potassium content of closer to 3000mg/d for both potato groups (baked/boiled potatoes and FF), and approximately 3300mg/d for the supplement group. The difference in aimed potassium intake vs. actual intake based on analysis is similar to findings of other controlled feeding trials [28, 31], and could have been due to variations in potassium content of fruits and vegetables, which may differ based on season, location, and crop. There was also some variation in Na content, with potato groups having an intake of ~3400mg/d, and both the control and supplement phases being closer to ~3000mg/d. Sodium intake levels for all diets were designed to be 3300mg/d.

Potassium and Na are absorbed in the small intestine primarily via passive diffusion, this may explain the difference between the supplement and control phase for % potassium absorption. A greater more concentrated amount of potassium (supplement) may have been absorbed more readily than a lower dose dispersed in food, but it is unclear as to why % Na absorption was lower in the potato group, although this does align well with the retention results. It is also unclear as to why Na retention was lower in the potato intervention compared to the others. The FF Na retention level was similar to the control, although overall Na intake was higher (~3400 vs. 3000 mg/d) in the FF diet, perhaps both dose and form are important factors in potassium delivery in the context of influencing Na retention. Sodium and water reabsorption in the kidney are tightly linked, and the role of potassium within this relationship and overall fluid balance is thought to be important, but not well understood. In animal models, both potassium depletion and potassium loading can lead to an increase, via Na/H+ exchanger upregulation in the proximal kidney, and decrease, via Na-chloride cotransporter deactivation in the distal kidney, in Na reabsorption [32-34]. This may in part explain some of our results. Differences in Na retention may have also been the result of the non-mineral component of the potassium supplement, gluconate. Gluconic acid is an oxidized product of glucose, and active Na absorption in the upper small intestine occurs through the Na+/glucose cotransporter, a process largely dependent on the presence of glucose. This may explain the higher % absorption of Na in

the K-gluconate group (97.1%), and the difference in Na retention, although the amount (~460 mg/90mg of potassium (per pill)) of gluconate administered may not have been enough to cause this effect. There may have also been some inflation error related to fecal processing in both potassium % absorption and retention in the supplement phase. All stool samples were collected and homogenized, and a sample of the homogenate was aliquoted for storage before analysis and the rest discarded. Because the potassium in the supplements would have been more concentrated when consumed compared to food, any potassium not absorbed may have been more concentrated in the stool samples as well. Even with homogenization a small concentrated pocket of unabsorbed K-gluconate may have been missed, and could in part explain our results.

To our knowledge this is the first potassium retention study conducted in an unhealthy population (pre-hypertensive-to-hypertensives). Overall the study was tightly controlled but may have had some limitations based on design. The intervention was free-living, meaning subjects visited the clinical testing area frequently for meal pick-up and sample drop-off (~every other day), but this study was not conducted in an in-patient metabolic ward, making it difficult to not only ensure complete subject compliance, but also to control for inadvertent sample (urine or fecal) loss in the process of collection. This could have added error, causing inflation of retention results. Although, this being a hypertensive population with a physiological dysregulation centering around potassium and Na renal handling and fluid balance, it is difficult to discern if our results are atypical or normal for this population. Another limitation to this balance study was a lack of sweat mineral loss assessment. Sweat potassium losses are variable, depending on environment, activity, and the individual. Physical activity and heat exposure show increased potassium loss through sweat, which under homeostatic conditions can be as low as 90mg/d (2.3mmol/L) and as high as 626mg/d (16mmol/L) [15]. Studies show greater potassium loss during environmental stress (heat) [19, 35] compared to physical stress (exercise) [18], as well as generally unknown/understudied effects due to diet [15, 16]. Sweat Na losses can be even more unpredictable, and are equally understudied [1]. While the majority of our subjects were sedentary adults who spent most of their days in a temperature controlled setting (campus office building), not capturing both potassium and Na sweat losses may have led to an overall incomplete assessment of retention.

According to the 2019 DRI guidelines for potassium, lack of a sensitive biomarker and limitations across potassium retention studies offer insufficient evidence to establish EAR and

RDA levels for adequacy or deficiency [1]. But while acknowledging the insufficiencies in evidence related to potassium adequacy, the committee's recommendation to essentially have the population continue their current dietary pattern in terms of potassium intake and potassium rich foods (e.g. fruits and vegetables), seems questionable. With evidence related to potassium retention sparse, it would seem a call for more research in this area accompanied by not establishing any potassium intake level may have been more appropriate. Currently the application of the findings from potassium retention studies is limited, due to both to their small number, and overall heterogeneity of measurement and design. A better understanding of how environment (heat), activity, as well as other demographics such as race/ethnicity effect potassium adequacy is necessary. This study adds to that research, and will hopefully spark future intrigue in further assessment of potassium and Na retention across many different groups, especially unhealthy populations, which may benefit from higher potassium intakes and a better understanding of potassium adequacy.

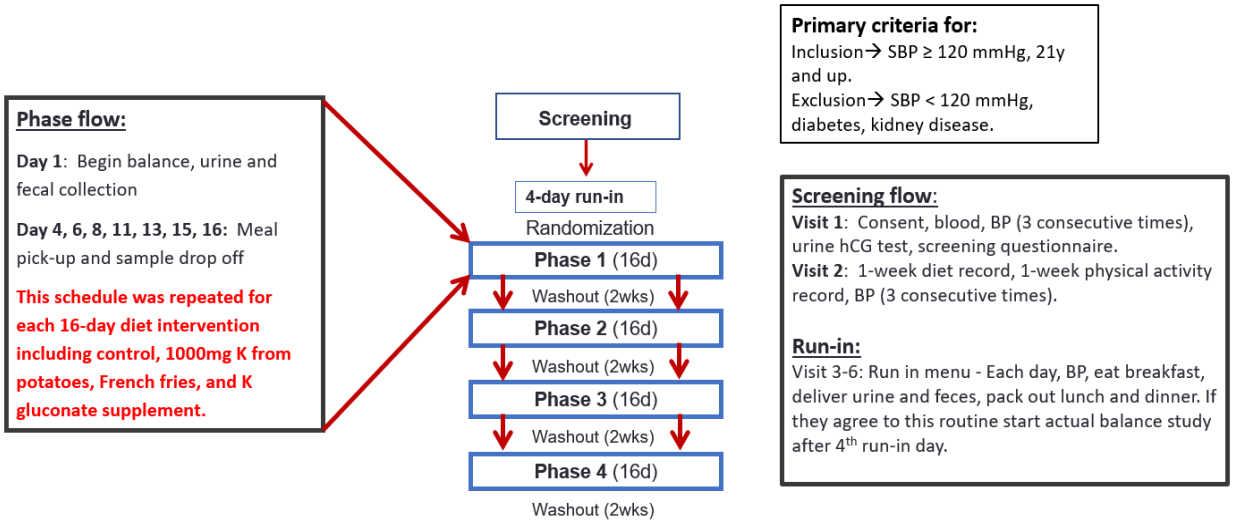

Figure 3.1. Overall study design and measures. Study design was a randomized, cross-over, controlled feeding study with primary measures of potassium balance, and secondary outcomes of sodium balance (24-hr urine and stool collection).

Table 3.1. Baseline characteristics of all subjects who completed at least two interventions.

	All Mean (SD)	Male Mean (SD)	Female Mean (SD)
n	28	14	14
Age (y)	47.3 (14.8)	43.2 (14.0)	51.3 (14.9)
Height (cm)	172.0 (10.2)	179.1 (7.3)	164.9 (7.5)
Weight (kg)	93.0 (23.2)	97.7 (20.8)	88.3 (25.3)
BMI (kg/m^2)	31.2 (6.3)	30.3 (4.8)	32.2 (7.5)
Systolic Blood Pressure (mmHg)	136.3(12.0)	135.2(12.9)	137.4(11.5)
Diastolic Blood Pressure (mmHg)	85.6(6.3)	86.2(7.2)	85.0(5.6)

Table 3.2. Baseline Characteristics of subjects included in potassium and sodium fecal excretion, retention, and percent absorption.

	All Mean (SD)	Male Mean (SD)	Female Mean (SD)
n	20	10	10
Age (y)	49.5 (15.3)	44.4 (15.2)	54.7 (14.2)
Height (cm)	172.0 (9.4)	177.9 (7.3)	166.0 (7.4)
Weight (kg)	93.73 (23.2)	96.7 (20.0)	90.8 (26.8)
BMI (kg/m^2)	31.5 (6.4)	30.4 (4.9)	32.7 (7.8)
Systolic Blood Pressure (mmHg)	137.3(13.3)	137.7(14.5)	137.0(12.7)
Diastolic Blood Pressure (mmHg)	85.9(6.9)	86.6(8.1)	85.3(5.8)

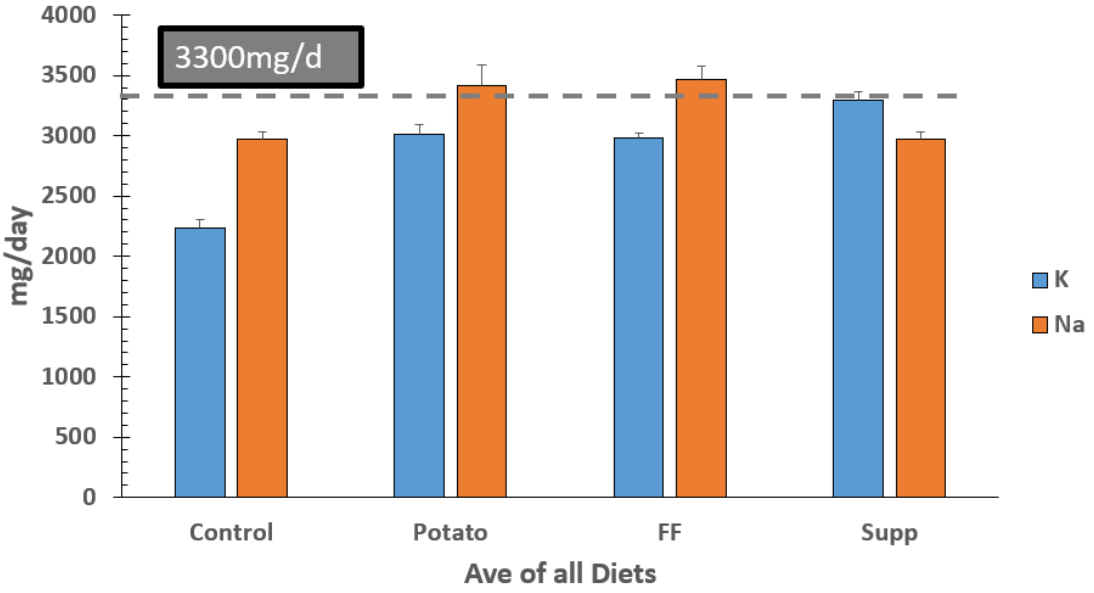

Figure 3.2. Chemical Analysis of Controlled Diets. Potassium (K) content for the interventions of control, potato, French Fry (FF), and Supplement were 2238 ± 43, 3008 ± 29, 2977 ± 4, and 3282 ± 43 mg/d, respectively. The K levels for the control and supplement phases were close to the target of 2300 and 3300 mg/d, respectively, while the potato and FF were slightly lower at approximately 3000 mg/d compared to the 3300 mg/d target. For Na, the target for all four interventions was set at 3300 mg/d, however analysis showed Na content to be slightly higher for the potato (3417 ± 77 mg/d) and FF (3466 ± 112 mg/d), and slightly lower for the control and supplement phases (2974 ± 60 mg/d). Means ±SE.

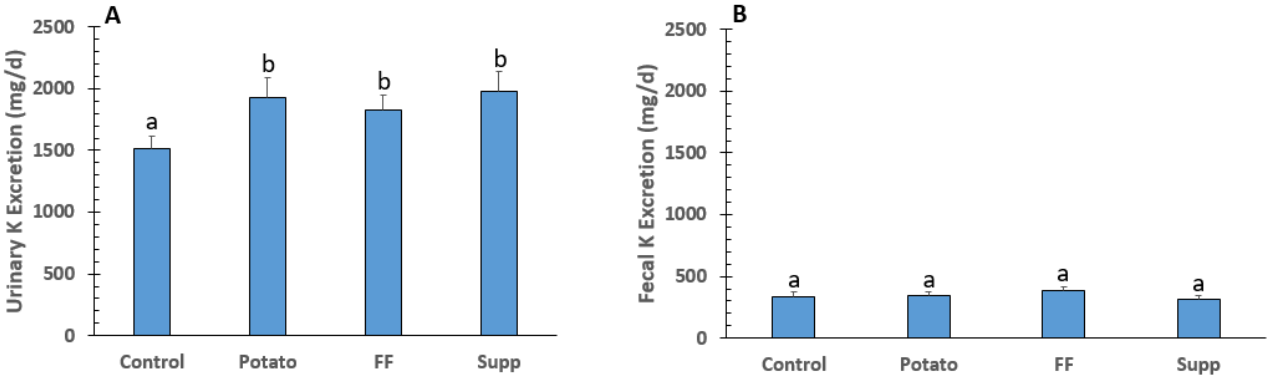

Figure 3.3. Urinary (N=28) and Fecal (N=20) Excretion of Potassium. There were significant differences among groups in urinary potassium (K) (A; P < 0.0001) excretion. Urinary K excretion during the control phase (1516 ± 101.9 mg/d) was significantly lower than the potato (1932.6 ± 153.1 mg/d; P = 0.0002), French fries (FF) (1824.4 ± 119.7 mg/d; P = 0.006), and supplement (1981.7 ± 162.4; P = 0.006) phases. There were no significant differences (P =0.18) in fecal K excretion (B) with losses of 336.5 ± 34.0, 348.5 ± 27.6, 382.7 ± 37.0, 317.6 ± 31.5 mg/d, for control, potato, FF, and supplement interventions. Different letters (a, b) denote a difference that is statistically significant (P < 0.05). Means ±SE.

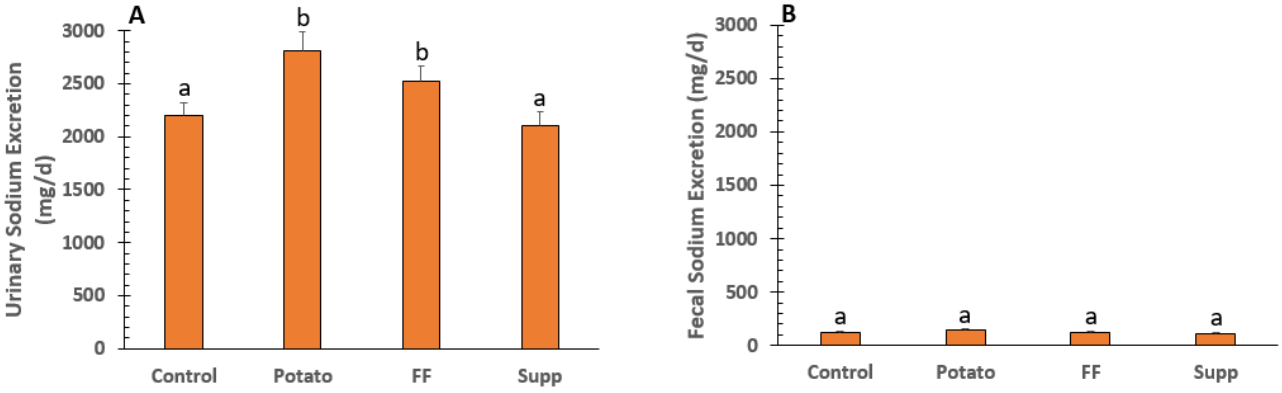

Figure 3.4. Urinary (N=28) and Fecal (N=20) Excretion of Sodium. There were significant differences among groups in urinary sodium (Na) (A; P < 0.0001) excretion. Urinary Na excretion during the potato phase (2817.0 ± 174.7 mg/d) was significantly higher than both the control (2202.3 ± 123.5 mg/d; P < 0.0001) and supplement (2106.0 ± 129.3 mg/d; P < 0.0001) interventions, and the French fries (FF) intervention was also significantly higher (2530 ± 143.8) compared to the control (P = 0.04) and supplement (P = 0.002) phases. There were also no significant differences (P =0.18) in fecal sodium excretion with losses of 123.8 ± 13.5, 141.3 ± 17.7, 122.8 ± 15.2, 111.8 ± 13.7 mg/d, for control, potato, FF, and supplement interventions. Different letters (a, b) denote a difference that is statistically significant (P < 0.05). Means ±SE.

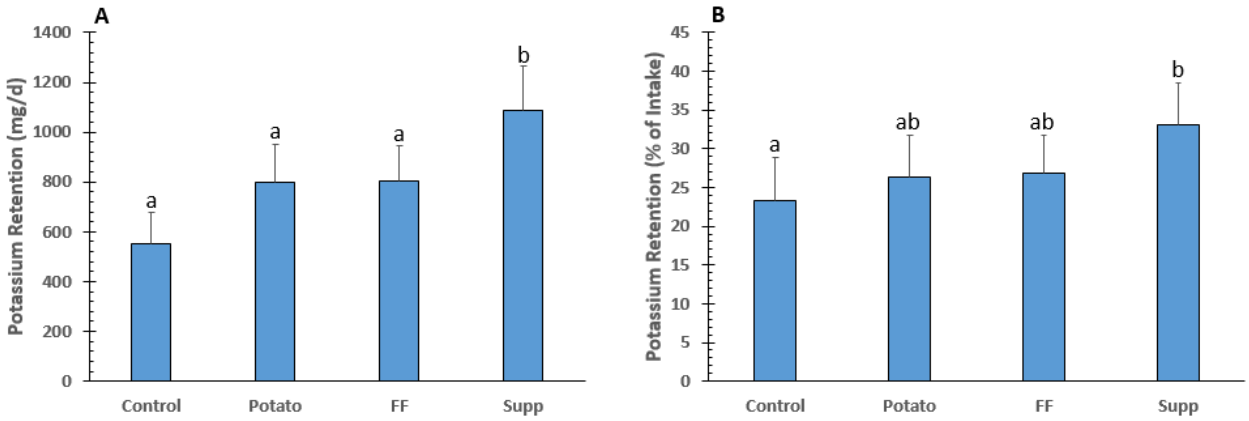

Figure 3.5. Differences in Potassium Retention in Hypertensives on a Controlled Diet. Differences in potassium (K) retention were assessed as absolute (A) and % of intake (B). Absolute K retention was significantly higher ($P < 0.0001$) during the supplement (1087.0 ± 176.5 mg/d) compared to the control (550.3 ± 126.7 mg/d; $P < 0.0001$), potato (797.0 ± 155.8 mg/d; $P=0.04$), and FF (804.0 ± 139.2 mg/d; $P = 0.05$) study phases. For interventions of control, potato, FF, and K-gluconate retention as percent (%) of intake was 23.3 ± 5.6%, 26.4 ± 5.3 %, 26.8 ± 4.9 %, and 33.1 ± 5.3 %, respectively, with only a significant difference between the supplement (33.1 ± 5.3 %) and the control (23.3 ± 5.6%; $P=0.04$) interventions remaining. Different letters (a, b) denote a difference that is statistically significant ($P < 0.05$). Means ±SE.

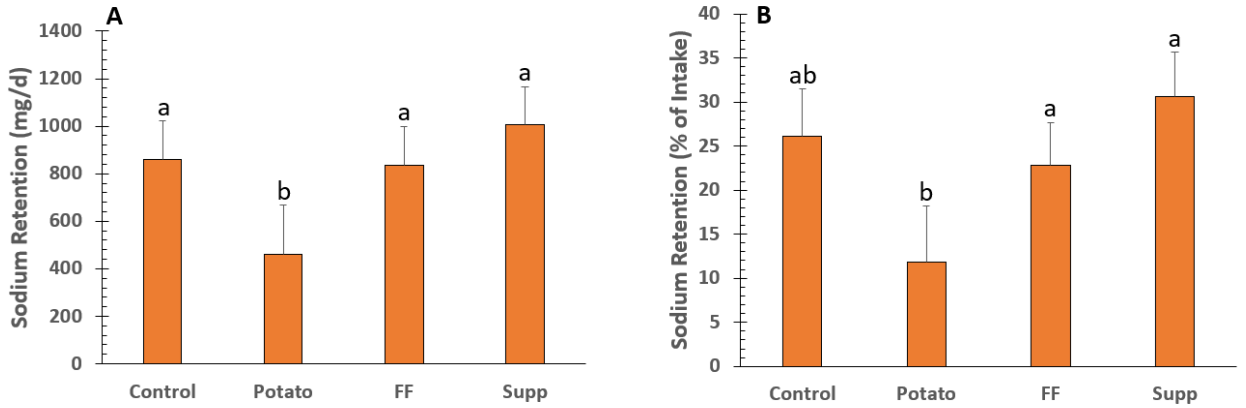

Figure 3.6. Differences in Sodium Retention in Hypertensives on a Controlled Diet. Differences in sodium (Na) retention were assessed as absolute (A) and % of intake (B). Absolute Na retention was significantly lower (P = 0.002) during the potato (458.9 ± 208.0 mg/d) compared to the control (858.5 ± 164.9 mg/d; P = 0.002), FF (834.8 ± 162.0 mg/d; P=0.047), and supplement (1005.1 ± 158.3 mg/d; P = 0.0015) groups. When assessed as % of intake, Sodium retention in the potato phase (11.8 ± 6.3%) was lower compared to the FF (22.8 ± 4.8) phase, and significantly lower (P = 0.0006) compared to the control (26.1 ± 5.4; P = 0.0004), and supplement (30.6 ± 5.0% P=0.01) interventions. Different letters (a, b) denote a difference that is statistically significant (P < 0.05). Means ±SE.

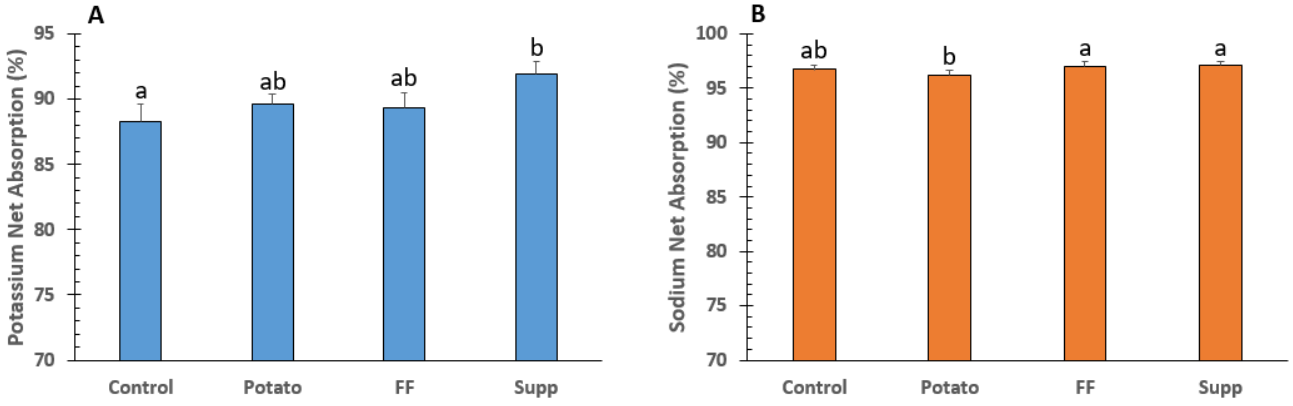

Figure 3.7. Differences in Sodium and Potassium Net Absorption. There were significant differences in percent net absorption (%ab) for both potassium (K; P = 0.005) and sodium (Na; P =0.025). For K, the %ab in the supplement phase (91.9 ± 1.0) was significantly higher compared to the control (88.3 ± 1.3; P = 0.003). For Na, %ab was significantly lower in the potato intervention (96.2 ± 0.5) compared to the FF (97.0 ± 0.4; P = 0.047) and supplement (97.1 ± 0.4; P = 0.03) interventions. Different letters (a, b) denote a difference that is statistically significant (P < 0.05), *P < 0.05. Means ±SE.

References

1. National Academies of Sciences, E., et al., *The National Academies Collection: Reports funded by National Institutes of Health*, in *Dietary Reference Intakes for Sodium and Potassium*, M. Oria, M. Harrison, and V.A. Stallings, Editors. 2019, National Academies Press (US) Copyright 2019 by the National Academy of Sciences. All rights reserved.: Washington (DC).

2. Aburto, N.J., et al., *Effect of increased potassium intake on cardiovascular risk factors and disease: systematic review and meta-analyses.* BMJ, 2013. **346**: p. f1378.

3. Vinceti, M., et al., *Meta-Analysis of Potassium Intake and the Risk of Stroke.* J Am Heart Assoc, 2016. **5**(10).

4. D'Elia, L., et al., *Potassium intake, stroke, and cardiovascular disease a meta-analysis of prospective studies.* J Am Coll Cardiol, 2011. **57**(10): p. 1210-9.

5. Larsson, S.C., N. Orsini, and A. Wolk, *Dietary potassium intake and risk of stroke: a dose-response meta-analysis of prospective studies.* Stroke, 2011. **42**(10): p. 2746-50.

6. Whelton, P.K., et al., *Effects of oral potassium on blood pressure. Meta-analysis of randomized controlled clinical trials.* JAMA, 1997. **277**(20): p. 1624-32.

7. Cappuccio, F.P. and G.A. MacGregor, *Does potassium supplementation lower blood pressure? A meta-analysis of published trials.* J Hypertens, 1991. **9**(5): p. 465-73.

8. Binia, A., et al., *Daily potassium intake and sodium-to-potassium ratio in the reduction of blood pressure: a meta-analysis of randomized controlled trials.* J Hypertens, 2015. **33**(8): p. 1509-20.

9. Geleijnse, J.M., F.J. Kok, and D.E. Grobbee, *Blood pressure response to changes in sodium and potassium intake: a metaregression analysis of randomised trials.* J Hum Hypertens, 2003. **17**(7): p. 471-80.

10. Newberry, S.J., et al., *Sodium and Potassium Intake: Effects on Chronic Disease Outcomes and Risks*. 2018, Rockville MD.

11. Hene, R.J., et al., *Adaptation to chronic potassium loading in normal man.* Miner Electrolyte Metab, 1986. **12**(3): p. 165-72.

12. Holbrook, J.T., et al., *Sodium and potassium intake and balance in adults consuming self-selected diets.* Am J Clin Nutr, 1984. **40**(4): p. 786-93.

13. Rosa, R.M., et al., *Gastrointestinal and renal excretion of potassium in African-Americans and White Americans.* J Hypertens, 2012. **30**(12): p. 2373-7.

14. Palacios, C., et al., *Racial differences in potassium homeostasis in response to differences in dietary sodium in girls.* Am J Clin Nutr, 2010. **91**(3): p. 597-603.

15. Consolazio, C.F., et al., *Excretion of sodium, potassium, magnesium and iron in human sweat and the relation of each to balance and requirements.* J Nutr, 1963. **79**: p. 407-15.

16. Costill, D.L., R. Cote, and W.J. Fink, *Dietary potassium and heavy exercise: effects on muscle water and electrolytes.* Am J Clin Nutr, 1982. **36**(2): p. 266-75.

17. Fukumoto, T., et al., *Differences in composition of sweat induced by thermal exposure and by running exercise.* Clin Cardiol, 1988. **11**(10): p. 707-9.

18. Armstrong, L.E., et al., *Voluntary dehydration and electrolyte losses during prolonged exercise in the heat.* Aviat Space Environ Med, 1985. **56**(8): p. 765-70.

19. Malhotra, M.S., et al., *Effect of restricted potassium intake on its excretion and on physiological responses during heat stress.* Eur J Appl Physiol Occup Physiol, 1981. **47**(2): p. 169-79.

20. Khaw, K.T. and E. Barrett-Connor, *The association between blood pressure, age, and dietary sodium and potassium: a population study.* Circulation, 1988. **77**(1): p. 53-61.

21. Chang, H.Y., et al., *Effect of potassium-enriched salt on cardiovascular mortality and medical expenses of elderly men.* Am J Clin Nutr, 2006. **83**(6): p. 1289-96.

22. Nowson, C.A., T.O. Morgan, and C. Gibbons, *Decreasing dietary sodium while following a self-selected potassium-rich diet reduces blood pressure.* J Nutr, 2003. **133**(12): p. 4118-23.

23. Chalmers, J., et al., *Australian National Health and Medical Research Council dietary salt study in mild hypertension.* J Hypertens Suppl, 1986. **4**(6): p. S629-37.

24. *The Hypertension Prevention Trial: three-year effects of dietary changes on blood pressure. Hypertension Prevention Trial Research Group.* Arch Intern Med, 1990. **150**(1): p. 153-62.

25. Squires, R.D. and E.J. Huth, *Experimental potassium depletion in normal human subjects. I. Relation of ionic intakes to the renal conservation of potassium.* J Clin Invest, 1959. **38**(7): p. 1134-48.

26. Kodama, N., et al., *Sodium and potassium balances in Japanese young adults.* J Nutr Sci Vitaminol (Tokyo), 2005. **51**(3): p. 161-8.

27. Benjamin, E.J., et al., *Heart Disease and Stroke Statistics—2018 Update: A Report From the American Heart Association.* Circulation, 2018. **137**(12): p. e67-e492.

28. Appel, L.J., et al., *A clinical trial of the effects of dietary patterns on blood pressure. DASH Collaborative Research Group.* N Engl J Med, 1997. **336**(16): p. 1117-24.

29. Palacios, C., et al., *Sodium retention in black and white female adolescents in response to salt intake.* J Clin Endocrinol Metab, 2004. **89**(4): p. 1858-63.

30. Institute of, M., *Dietary Reference Intakes for Water, Potassium, Sodium, Chloride, and Sulfate*. 2005, Washington, DC: The National Academies Press.

31. Sacks, F.M., et al., *Effects on blood pressure of reduced dietary sodium and the Dietary Approaches to Stop Hypertension (DASH) diet. DASH-Sodium Collaborative Research Group.* N Engl J Med, 2001. **344**(1): p. 3-10.

32. Soleimani, M., et al., *Potassium depletion increases luminal Na+/H+ exchange and basolateral Na+:CO3=:HCO3- cotransport in rat renal cortex.* J Clin Invest, 1990. **86**(4): p. 1076-83.

33. Veiras, L.C., et al., *Potassium Supplementation Prevents Sodium Chloride Cotransporter Stimulation During Angiotensin II Hypertension.* Hypertension, 2016. **68**(4): p. 904-12.

34. Sorensen, M.V., et al., *Rapid dephosphorylation of the renal sodium chloride cotransporter in response to oral potassium intake in mice.* Kidney Int, 2013. **83**(5): p. 811-24.

35. Malhotra, M.S., K. Sridharan, and Y. Venkataswamy, *Potassium losses in sweat under heat stress.* Aviat Space Environ Med, 1976. **47**(5): p. 503-4.

CHAPTER 4: SHORT-TERM SUPPLEMENTAL DIETARY POTASSIUM FROM POTATO AND POTASSIUM GLUCONATE: EFFECT ON CALCIUM RETENTION AND URINARY PH IN PRE-HYPERTENSIVE-TO-HYPERTENSIVE ADULTS

Abstract

Potassium is an essential nutrient, that has been labeled a shortfall nutrient by recent Dietary Guidelines for Americans Advisory Committees. Observational studies show a consistent bone benefit with increased, potassium rich, fruit and vegetable intakes in cohorts spanning adolescents to the elderly. In clinical trials, higher potassium intakes through supplementation have been associated with reduced urinary calcium (Ca) excretion and improvement in Ca balance. Clinical dietary intervention trials assessing the impact of potassium on bone are lacking. In this clinical intervention, we assessed the effects of potassium intake from potato sources and a potassium supplement on urinary Ca, urine pH, and overall Ca balance. Thirty pre-hypertensive-to-hypertensive (Systolic BP ≥ 120mmHg) men (N=15) and women (N=15) with a mean ± SD age, BMI, and BP (systolic(SBP)/ diastolic(DBP)) of 48.2 ± 15 y, 31.4 ± 6.1kg/m^2, and 136.3 ± 11.9/ 86.1 ± 7.1mmHg, respectively, were enrolled in a cross-over, randomized control feeding trial. Participants were assigned to a random order of four 16-day dietary potassium interventions including a basal diet (control) of 2300mg/d (~60mmol/d) of potassium, and three phases of an additional 1000mg/d (3300mg/d(~85mmol/d) total) of potassium in the form of potatoes (baked, boiled, or pan-heated with no additional fat), French fries (FF), or a potassium (K)-gluconate supplement. Calcium intake for all diets was between approximately 700-800mg/d. Each intervention was separated by two or more weeks of wash out. Twenty-four hour urine and stool samples were collected throughout each study phase, and assessed via ICP-OES for Ca content. Results for differences in mineral balance were assessed as 8-day averages as follows: retention = dietary intake – urine + stool losses. Using a mixed model ANOVA there was a significantly lower urinary Ca excretion in the supplement intervention (100.4 ± 9.3 mg/d; P = 0.009) compared to the potato (115.2 ± 10.4 mg/d; P=0.033) and the control (116.6 ± 9.9 mg/d; P=0.027). In addition, there was a significant difference in urinary pH between the control and supplement phases (6.08 ± 0.18 vs. 6.54 ± 0.16; P=0.0036). However,

there were no significant differences (P > 0.05) in Ca retention (mg/d) (control: 160.5 ± 58.1, French fries: 186.2 ± 57.1, potatoes: 162.5 ± 55.2, supplement: 260.5 ± 45.6; P=0.26. Mean ± SE). Although results show no difference in Ca metabolism due to K amount or diet type, an increased K intake via K-gluconate supplementation may favorably influence urinary pH and acid-base balance.

Introduction

Osteoporosis, or a severe reduction in bone mass leading to decreased bone health and increased fracture risk, is a global health problem with an estimated financial impact of ~100 billion [1]. Over 200 million people worldwide suffer from osteoporosis, including 30% of postmenopausal women in both the US and Europe [2]. Peak bone mass is achieved by the third decade of life, after which bone loss begins, accelerating with aging in both men and women. The bone mass present at any given point is determined by factors that influence the acquisition, maintenance, or loss of bone throughout the lifespan, some of which are modifiable lifestyle factors.

Adequate potassium intake may benefit overall bone health, and has been proposed to do so through its effect on acid-base balance [3, 4]. Western diets high in meats and cereal grains produce an environment of low-grade metabolic acidosis, and buffering of this increased acid load may rely on alkaline calcium (Ca) salts derived from the dissolution of bone tissue. Potassium salts, produced from the consumption and metabolism of fruits and vegetables or alkalizing supplements (e.g. potassium supplements), may also yield alkaline precursors that help maintain pH homeostasis (~7.35-7.45). The impact of excess systemic acid on bone is proposed to be mediated by two mechanisms: pH buffered through the dissolution of the bone matrix, and cell based mechanisms (e.g. up-regulation of bone resorbing cell (osteoclast) activity) [3, 4]. Despite its physiological plausibility, the acid-base balance theory has been met with challenge [5, 6], however, there are few other theories for the potential mechanism of potassium benefit to bone that have been proposed [7].

Cross-sectional, observational studies show a consistent bone benefit with increased dietary potassium associated with higher fruit and vegetable intakes, especially in adults and older men and women [8-13]. Dietary interventions accessing the impact of potassium on bone

are lacking, although in an ancillary study to the DASH trial osteocalcin and CTX were both significantly reduced from baseline by the DASH dietary pattern compared to control [14]. Evidence from clinical supplementation trials suggests that potassium intake decreases Ca excretion [15-17], and may increase overall Ca balance [18, 19], although retention findings have been inconsistent [20]. Studies have also examined the relationship between potassium intake and bone turnover markers, showing decreases in resorption markers of C- and N-telopeptide and increases in procollagen type I N-terminal propeptide, with potassium supplementation, along with decreases in calciuria [18, 21, 22]. Few randomized control trials have assessed the effect of potassium supplementation on bone mineral density (BMD) with mixed results [11, 23, 24], and none have assessed effects on osteoporotic fracture risk. Overall, the potential bone benefit of potassium intake has primarily been seen with potassium supplementation at high doses (2300-3500mg/d (60-90mmol/d)) [7]. It is yet to be determine if increases in dietary potassium can produce the same effect, and if so how this effect is being mediated.

Because of this gap in the potassium literature, the goal of this research was to utilize a controlled feeding study to examine the effect of increased dietary potassium from potato sources and a potassium-gluconate supplement on calcium economy, compared to a control diet. It was hypothesized that the interventions with an increased potassium intake would decrease calcium urinary excretion, and increase calcium retention and urinary pH, compared to the control.

Methods

Subjects

Subjects (N=30, age 21y and older) were recruited through a varitey of different techniques at Purdue University, and the surrounding area. Subjects were pre-hypertensive-to-hypertensive (SBP >120mmHg) men and women who were otherwise apparently healthy. Exclusion criteria included: medication to treat hyper or hypotension (hypertensive monotherapy was allowed later in recruitment), known to affect electrolyte metabolism or contain high levels of potassium or Na, history of myocardial infarction, diabetes mellitus, renal disease, GI disease, pancreatitis, cholestatic liver disease, cancer, smoking, use of illegal drugs, or excessive alcohol intake, subjects who were pregnant or lactating, allergy or intolerance to study foods,

unwillingness to refrain from dietary supplements, and weight loss > 3kg in the past 2 months. Screening consisted of two visits at least one week apart. Subjects came to the Purdue Nutrition Science clinical suite where they were consented, had their BP measured, a screening blood draw, and filled out screening questionnaires. They were also given additional screening forms to fill out before their second visit, which consisted of another BP measurement to ensure eligibility, and return of all screening documents. When eligible, subjects completed a 4-day run-in period (described below), continuing subjects were randomized to one of 24 possible sequences of the 4 phases including, control, potato, French fry (FF), and K-gluconate interventions. Subjects were enrolled in May 2016 to September 2017.. The study protocol was approved by the Purdue University Institutional Review Board, and all subjects provided written, informed consent.

Dietary Intervention

Study design was a randomized, cross-over, controlled feeding trial with primary measures of potassium balance and blood pressure (not reported on here), and secondary measures of calcium excretion and balance (Figure 4.1). After initial screening eligible subjects entered the run-in period. Each day of the 4 day run-in diet represented one of the four diet phases: control, potato, French fries, and supplement. During the run-in, subjects came to the clinical facility each morning, had their BP taken, either stayed to eat breakfast or had all their meals and snacks for the day packed out in a cooler to take with them. After the 4 day run-in period, participants were assigned to a random order of four 16-day dietary potassium interventions including a basal diet (control) of 2300mg/d (~60mmol/d), and three phases of an additional 1000mg/d (3300mg/d(~85mmol/d) total) of potassium in the form of potatoes (baked, boiled, or pan-heated with no additional fat), French fries, or a K-gluconate supplement. Each intervention was separated by two or more weeks of wash out. A 4-day menu cycle at three calorie levels (1800, 2200, and 2600 kcal/d) was designed to manipulate potassium levels while keeping other micro (e.g. Na, Ca) and macro (e.g. fat, protein, CHO) constant. Each menu was designed to include approximately 800mg/d of Ca. Subjects picked up study foods approximately every other day (on days 1, 4, 6, 8, 11, 13, 15, 16). For each day of controlled feeding, the subjects recorded their intake by checking off each item listed on daily menu sheets provided for each day. They were also instructed to indicate whether they ate any non-study

foods and/or whether they did not eat all the study foods. The food and beverages were prepared with deionized water and weighed to the nearest one tenth gram on digital scales. Deionized bottle water was also given to control water intake. Duplicates of each menu cycle day were homogenized and analyzed. Potassium gluconate supplements were sent out to a food chemistry lab for analysis (Eurofins Food Chemistry Testing US, Inc.). Researchers adjusted energy intakes with snack items that contained mostly sugar. Any uneaten items were instructed to be returned and analyzed for mineral content.

Calcium Balance and Net Absorption

Complete daily urine and feces were collected in acid washed containers for each 16-day intervention of the study, on a daily basis. Instructions and all necessary supplies (e.g. containers for urine and stool collections) were provided. Urine was pooled as 24-hr collections, which were analyzed for creatinine to assess urinary collection compliance. Urinary pH was assessed on 24-hr samples collected on days 14 and 15 of each intervention using a benchtop pH meter (OAKTON Instruments P.O. Box 5136, Vernon Hills, IL USA). Fecal samples were also pooled for each 24-h period, and completeness of collections was determined by using polyethylene glycol (PEG) recovery by turbidimetric assay. Urine for mineral analysis was acidified with 1% (by vol) HNO_3 and stored at −20 °C. Fecal samples were homogenized with ultra-pure water and concentrated HCl using a laboratory stomacher (Tekmar Co, Cincinnati, OH); they were then treated in a drying oven at 50 °C for a minimum of 24 h, ashed in a muffle furnace at 600 °C for 96 h, and diluted in 1N HNO_3 for total mineral analysis. Urine was analyzed for creatinine and minerals (potassium, sodium (Na), Ca). Stools were analyzed for PEG and minerals. PEG was administered as two 500 mg capsules, instructed to be taken three times per day with each meal. Dietary, urinary, and fecal minerals were measured by ICP-OES (5100 PC; Perkins Elmer, Waltham, MA). Unacidified urine was measured for creatinine by a kinetic modification of Jaffe's colorimetric assay (Cobas Mira Plus; Roche Diagnostic Systems, Nutley, NJ).

After a Ca intake equilibration period of the first 7 days and exclusion of day 16, which was an in clinic testing day, an 8-day period was assessed for Ca excretion and retention for each intervention. Balance and % absorption were determined for the 8-day average using the following equations:

Daily calcium balance (mg/d) = daily calcium intake (mg/d) - daily calcium excretion (mg/d) (urine and stools).

% Net absorption = daily calcium intake (mg/d) - daily calcium fecal excretion (mg/d) x 100.

Statistical Methods

Differences among interventions for Ca urinary loss, fecal loss, urine pH, and calcium retention (as % of intake), were analyzed using a mixed model ANOVA and Tukey post hoc adjustment for pairwise comparisons among diet interventions. Absolute Ca retention (mg/d) was analyzed using the same model with a contrast analysis for post hoc tests to examine mean differences between the control and K-gluconate phases. All statistics were performed using JMP (SAS Institute, Cary, NC, USA) software, and Alpha was set at 0.05. A sample size of 20 cross-over participants per group, provided 80% power with two-sided alpha=0.05 to detect differences in Ca retention of approximately 150 mg/d or 20% of daily intake, respectively. All values are reported as mean ± SE unless otherwise stated.

Results

Baseline Characteristics of Study Subjects

Overall data from 30 subjects were included in the final analysis of urinary pH (Table 4.1), 28 subjects were included for urinary Ca excretion, and 20 subjects for whom full balance measures were available were included in the fecal Ca excretion, and Ca retention analysis (Table 4.2). On average, subjects were middle-aged to older adults (48.2 ± 15.0y), had a BMI bordering overweight to obese (31.4 ± 6.1kg/m^2), and a SBP and DBP of 136.3 ± 11.9mmHg and 86.1 ± 7.1mmHg, respectively (all data mean ± SD).

Chemical Analysis of Controlled Diets

There was some unexpected variation in potassium, sodium, and calcium content of the diets (Figure 4.2). Potassium content for the phases of control, potato, French Fry (FF), and Supplement were 2238 ± 45, 3008 ± 4, 2977 ± 31, and 3299 ± 43mg/d, respectively. The potassium levels for the control and supplement phases were close to the target of 2300 and 3300mg/d, respectively, while the potato and FF were slightly lower at approximately 3000 mg/d

compared to the 3300mg/d target. Sodium levels were set at 3300mg/d for all diets, however analysis showed Na content to be slightly higher for the potato (3417 ± 83mg/d) and FF (3466 ± 117mg/d), and slightly lower for the control and supplement phases (2974 ± 61mg/d). For Ca, the target for all four interventions was set at 800 mg/d, however analysis showed Ca content to be slightly lower for the control and supplement diets (765 ± 29mg/d), as well as the FF phase (684 ± 30mg/d). The potato diet did achieve approximately 800mg/d of Ca (799 ± 22mg/d).

Urinary Calcium Excretion and pH

There were significant differences among groups in urinary Ca excretion (P = 0.009), the K-gluconate intervention (100.4 ± 9.3 mg/d) was significantly lower compared to the control (116.6 ± 9.9 mg/d; P = 0.027) and potato (115.1 ± 10.4mg/d; P = 0.033) study phases (Figure 4.3). There were also significant differences between interventions in urinary pH (control: 6.08 ± 0.2, potatoes: 6.36 ± 0.14, French fries: 6.33 ± 0.12, supplement: 6.54 ± 0.2; P = 0.008), with the K-gluconate intervention resulting in a higher urinary pH compared to control (6.08 ± 0.18 vs. 6.54 ± 0.16; P=0.0036) (Figure 4.3).

Calcium Fecal Excretion, Balance, and % Absorption

There were no significant differences (overall P = 0.19) in fecal Ca excretion with losses of 650.6 ± 77.9, 606.9 ± 66.5, 523.1 ± 62.3, 599.6 ± 53.0mg/d, for control, potato, FF, and supplement interventions, respectively (Figure 4.5). Differences in Ca retention were assessed as absolute and % of intake. There were no significant differences among groups for either absolute retention (all values mg/d: control: 160.5 ± 58.1, potatoes: 162.5 ± 55.2, French fries: 186.2 ± 57.1, supplement: 260.5 ± 45.6; overall P = 0.26) or retention based on percent of intake (control: 17.4 ± 7.3%, potatoes: 19.4 ± 6.8%, French fries: 21.6 ± 8.0%, supplement: 29.6 ± 5.4%; overall P = 0.37). However, Results did seem to indicate a trend toward higher Ca retention (absolute) between K-gluconate vs. the control (260.5 vs. 160.5mg/d) (Figure 4.4). There were also no significant differences among groups for percent Ca absorption (control: 31.2 ± 6.3%, potatoes: 31.6 ± 6.2%, French fries: 32.0 ± 7.1%, supplement: 36.8 ± 4.5%; P = 0.84). (Figure 4.5).

Discussion

The acid-ash hypothesis proposes that a diet higher in meats and cereal grains, precursors to acid metabolites (e.g. phosphates, sulfates), causes chronic acidemia over time. This can lead to the dissolution of bone tissue and the release of Ca for its alkalizing effects, which become necessary to combat the increased acid load [25, 26]. According to the hypothesis this would lead to increased urinary Ca loss from bone and, an overall decrease in Ca retention [27, 28]. In contrast, the consumption of fruits and vegetables, and other food sources or supplements that are more metabolically base producing (e.g. potassium, Ca), or provide other alkalizing organic molecules (e.g. bicarbonate) would decrease urinary Ca excretion and have a protective effect on bone [25, 27, 29]. A recent meta-analysis shows a linear relationship between urinary net acid excretion (NAE) and urinary Ca, with the increased Ca loss related to the modern acidogenic diet amounting to an excess of 66mg/d(1.6mmol/d) [30]. Despite this significant finding, in a follow-up meta-analysis, researchers from the same group found no relationship between NAE and Ca balance [27]. However, this review included only five studies, all primarily assessing increased protein load rather than intake of bicarbonate salts or fruit and vegetables as possible abating factors. Overall, the connection between increased (or decreased) dietary acid directly affecting bone is still unclear.

Observational studies show a consistent bone benefit with increased dietary potassium associated with higher fruit and vegetable intakes in adolescent boys and girls [8, 12, 31, 32], pre- and postmenopausal women [8-11] and elderly men and women [12, 13]. While clinical interventions assessing the effect of increased dietary potassium on Ca balance are lacking, evidence from clinical supplementation trials suggest that potassium intake decreases urinary Ca excretion [15-17], and may increase calcium retention [18, 19]. In postmenopausal women (N=18) potassium (K)-bicarbonate (60-120mmol/d) taken for 18 days decreased urinary Ca excretion by 76mg/d, and increased Ca retention by 56mg/d [18]. In a more recent randomized double-blind placebo-controlled study, Moseley and colleagues assessed the effect of potassium supplementation on Ca retention in older men and women (n= 52; >55y). Subjects who were randomly assigned to six months of 60 or 90mmol/d of potassium (K)-citrate supplementation had decreases in urinary Ca and net acid excretion, the highest dose (90mmol/d) resulting in positive Ca balance compared to control (0mmol/d) [19]. While these findings seem consistent

they are few, and overall research looking at the effects of increased potassium intake (via supplements or food) on Ca balance as a surrogate for bone health are lacking.

In this highly controlled feeding trial, we assessed the effects of dietary potassium from potato sources (bake or boiled and French fry (FF)) or a potassium supplement (K-gluconate) on outcomes of calcium balance, including urinary and fecal calcium excretion, calcium retention, and net percent calcium absorption. We also assessed urinary pH which has been cited as a surrogate for NAE [33]. Our findings confirm, to some degree, what has been reported in the literature in regards to the association between urinary Ca excretion and NAE. Differences in urinary calcium excretion between the supplement and control group are similar to previous findings from other groups, however the significant difference in urinary Ca between the supplement and potato interventions was unexpected and may be explained by the results from the chemical analysis of the study diets, and the actual mineral intake levels each intervention was able to achieve.

Chemical analysis of the diets showed a potassium content was closer to 3000mg/d for both potato groups (baked/boiled potatoes and FF), and approximately 3300mg/d for the supplement group. The difference in planned potassium intake vs. actual intake based on chemical analysis is similar to findings of other controlled feeding trials [34, 35], and could have been due to variations in potassium content of fruits and vegetables, which may differ based on season, location, and crop. Analysis also showed Na content to be slightly higher for the potato (3417 ± 83mg/d) and FF (3466 ± 117mg/d), and slightly lower for the control and supplement phases (2974 ± 61mg/d). High Na intakes have been shown to increase urinary Ca losses, with a loss of approximately 24-40mg of Ca for a Na intake of ≈2.3g [36]. This, along with 300mg/d potassium difference, may explain the differences seen been the K-gluconate and potato group for urinary Ca excretion. For Ca, the target for all four interventions was set at 800 mg/d which was confirmed in the potato diet (799 ± 22mg/d), however analysis showed Ca content to be slightly lower for the control and supplement diets (765 ± 29mg/d), as well as the FF phase (684 ± 30mg/d). Because of the inconsistency with calculated and analyzed mineral levels, we also looked at retention as a percent of intake.

While there were differences among groups in urinary calcium excretion and urine pH, these did not translate into differences in calcium retention. There were also no significant differences in % absorption among groups. Though our findings are somewhat consistent with

the previous literature assessing theories related to the acid-ash hypothesis, in this study, the results do not confirm any improvement in Ca balance, or bone benefit, based on these measures as surrogates for bone health.

The recently published 2019 Dietary Reference Intake recommendations for potassium and Na, assessed numerous chronic diseases in the context of adequate or increased potassium intake and risk reduction [37]. Bone health, or osteoporosis, was highlighted in this report while it has often been overlooked by others [38]. The committee focused their review on outcomes related to fractures and BMD, citing the fact that surrogate measures of bone health, including urinary Ca excretion, Ca retention, and bone markers of formation and resorption may suggest a potential effect to bone integrity, but their interdependence is not clear in the literature. To that end, there are no randomized control trials looking at potassium intake and fracture risk, and findings from the few trials which examine the relationship between potassium and BMD are mixed between BMD improvements [23, 39], and null results [11, 24]. Since the majority of the trials looking at the bone and bone related outcomes primarily manipulate potassium intake via supplementation, the committee also raised the question of the possibility of any effects being attributed to the non-potassium portions of the supplements. Jehle and colleagues found that postmenopausal women taking K-citrate showed significant increases in spine BMD after 12 months (0.89%; $P < 0.05$), whereas women in a potassium (K)-chloride(Cl) group had decreased spine BMD of (-0.98%; $P < 0.05$) [39]. Both supplements had the same potassium content (30 mmol/d). In addition, researchers using a rat model looking at the relationship between the inhibitory effect of vegetables on bone resorption and base excess, addition of K-citrate at levels that neutralized NAE from an acidogenic diet had no effect on bone turnover [5]. The authors suggested that bioactive compounds (e.g. flavonoids) in fruits and vegetables may be responsible for benefits to bone in the context of an acidogenic diet. However, in contrast to this evidence, in a short-term supplementation study in healthy men and women, Lemann et al. found that urinary Ca excretions decreased after 4-days of K-chloride or K-bicarbonate, which was not seen with identical supplementation of Na-chloride or Na-bicarbonate [16]. These findings, overall, suggest that the form of potassium supplementation, or the other constituents in potassium rich foods, are important factors in understanding the role of potassium in the acid-ash hypothesis, and whether or not potassium intake has any independent benefit to bone.

Further dietary interventions looking specifically at the effects of increasing potassium from food sources are needed to better understand the true effect of dietary potassium on Ca economy and ultimately bone health. Potatoes comprise ~20% of the vegetable intake in the American diet, with white potatoes and French fries representing ~7 and 3% of overall potassium intake [37]. Our findings agree in part with the previous literature that increased potassium through supplementation has a calciuric effect and decreases urinary pH, and Ca retention trended higher for the K-gluconate intervention vs. the control, perhaps with a larger sample (~N = 50, based on power analysis) or a longer duration significance would have been achieved. A limitation of this study is that only one, relatively moderate Ca intake was included. It is unclear whether or not this had any influence on our findings. It is possible that effects of potassium intake on calcium balance may have differing effects based on whether Ca intake is low, adequate, or high. It is also difficult to determine if any other factors present in each diet had any influence on the effects of potassium, such as any bioactives that were present in foods consumed in each diet, confounding the potential effects of the increased potassium in the potassium intervention arms.

Future research will first need to establish a specific role for potassium effecting Ca balance and bone health, independent of other supplement or dietary constituents that may have alkalizing, or other physiological, effects on their own. Dose-response evidence for potassium, especially potassium from foods, and how this influences Ca economy, as well as bone, also need to be examined. Here we confirm previously seen benefits on Ca excretion and NAE with a relatively low dose of additional supplemental potassium (~25 mmol/d), resulting in an overall intake of 3300mg/d, a practical modification to the everyday diet. Overall, research to date has established a vague relationship between potassium intake and bone benefit. Whether or not this association is exclusive to the context of the acid-ash hypothesis, or if increased potassium intake can independently improve Ca economy and bone health is still not well understood.

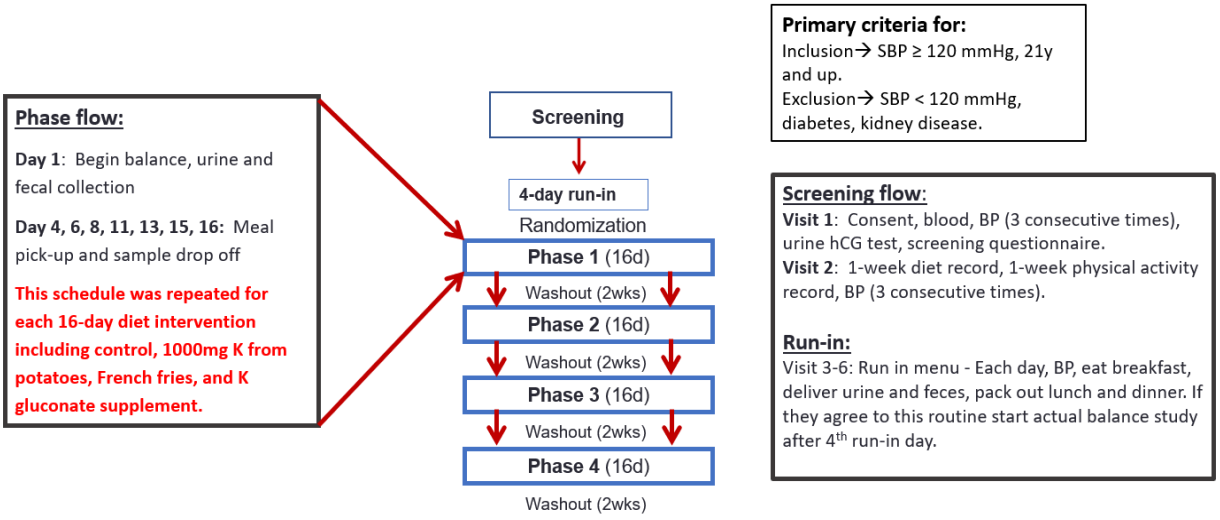

Figure 4.1 Overall study design and measures. Study design was a randomized, cross-over, controlled feeding study with primary measures of potassium balance and blood pressure (not reported here), and secondary measures of calcium excretion and retention (24-hr urine and stool collection).

Table 4.1 Baseline Characteristics of the Study Subjects.

	All Mean (SD)	Male Mean (SD)	Female Mean (SD)
n	30	15	15
Age (y)	48.2 (15.0)	43.8 (13.7)	52.7 (15.4)
Height (cm)	172.2 (10.2)	179.4 (7.1)	165.0 (7.2)
Weight (kg)	93.86 (22.9)	99.1 (20.7)	88.6 (24.4)
BMI (kg/m^2)	31.4 (6.1)	30.5 (4.8)	32.3 (7.2)
Systolic Blood Pressure (mmHg)	136.3(11.9)	134.6(12.7)	138.0(11.3)
Diastolic Blood Pressure (mmHg)	86.12(7.1)	85.9(7.0)	86.4(7.4)

Table 4.2 Baseline Characteristics of subjects included in Ca fecal excretion, retention, and percent absorption.

	All Mean (SD)	Male Mean (SD)	Female Mean (SD)
n	20	10	10
Age (y)	49.5 (15.3)	44.4 (15.2)	54.7 (14.2)
Height (cm)	172.0 (9.4)	177.9 (7.3)	166.0 (7.4)
Weight (kg)	93.73 (23.2)	96.7 (20.0)	90.8 (26.8)
BMI (kg/m^2)	31.5 (6.4)	30.4 (4.9)	32.7 (7.8)
Systolic Blood Pressure (mmHg)	137.3(13.3)	137.7(14.5)	137.0(12.7)
Diastolic Blood Pressure (mmHg)	85.9(6.9)	86.6(8.1)	85.3(5.8)

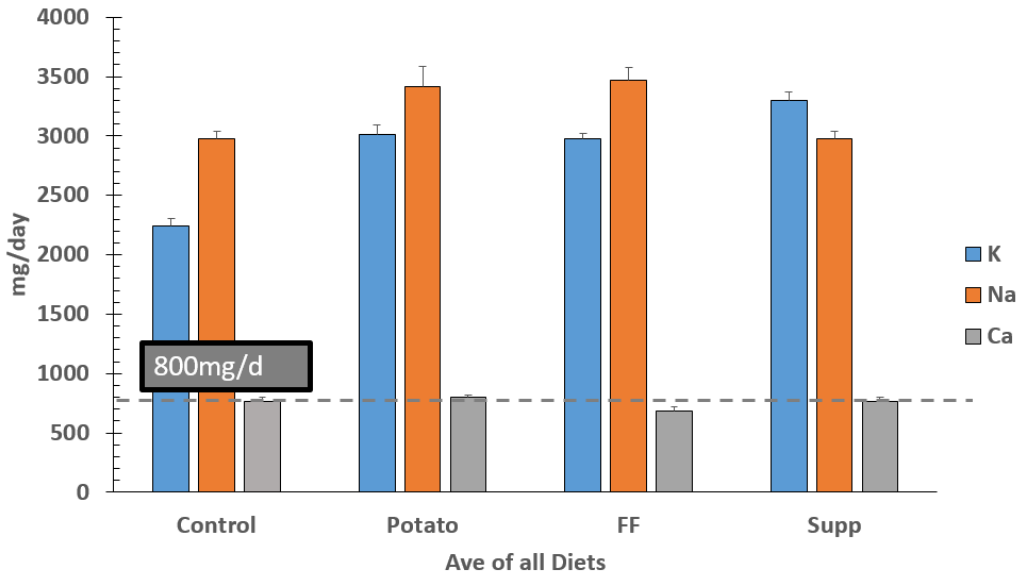

Figure 4.2 Chemical Analysis of Controlled Diets. Potassium (K) content for the interventions of control, potato, French Fry (FF), and Supplement (Supp) were 2238 ± 45, 3008 ± 4, 2977 ± 31, and 3299 ± 43 mg/d, respectively. Sodium levels were slightly higher for the potato (3417 ± 83 mg/d) and FF (3466 ± 117 mg/d) compared to the control and supplement phases (2974 ± 61 mg/d). Calcium (Ca) was designed to be 800 mg/d for all diets, although content was slightly lower for the control and supplement diets (765 ± 29 mg/d), as well as the FF phase (684 ± 30 mg/d). The potato diet did achieve approximately 800mg/d of Ca (799 ± 22 mg/d). Means ±SE.

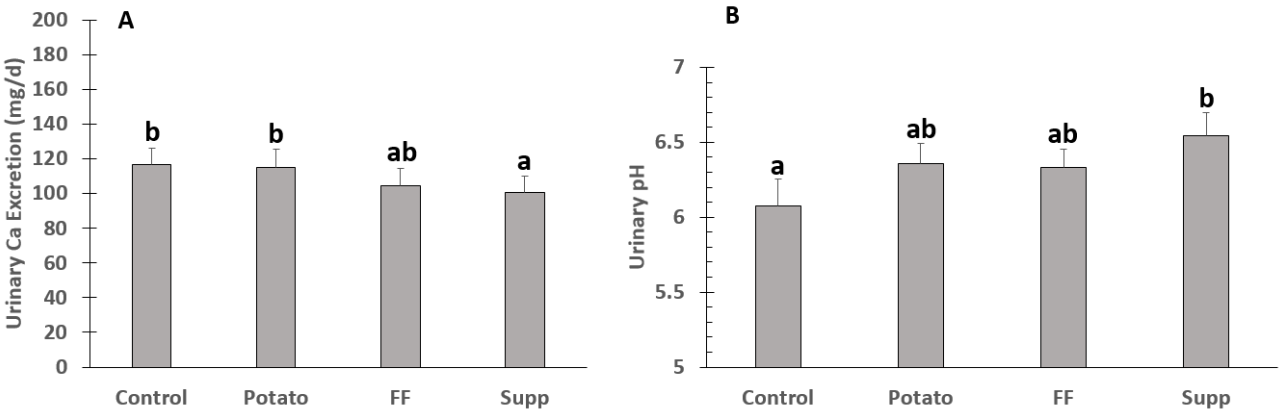

Figure 4.3 Urinary Ca Excretion (N=28) and pH (N=30). There were significant differences among groups in urinary calcium (Ca) excretion (A; P = 0.009), the potassium (K)-gluconate (100.4 ± 9.3 mg/d) was significantly lower compared to the control (116.6 ± 9.9 mg/d; P = 0.027) and potato (115.1 ± 10.4 mg/d; P = 0.033). There were also significant differences between interventions in urinary pH (B; P = 0.008) with the K-gluconate (Supp) phase resulting in a higher urinary pH compared to control (6.08 ± 0.18 vs. 6.54 ± 0.16; P=0.0036). Means ±SE. French Fries (FF).

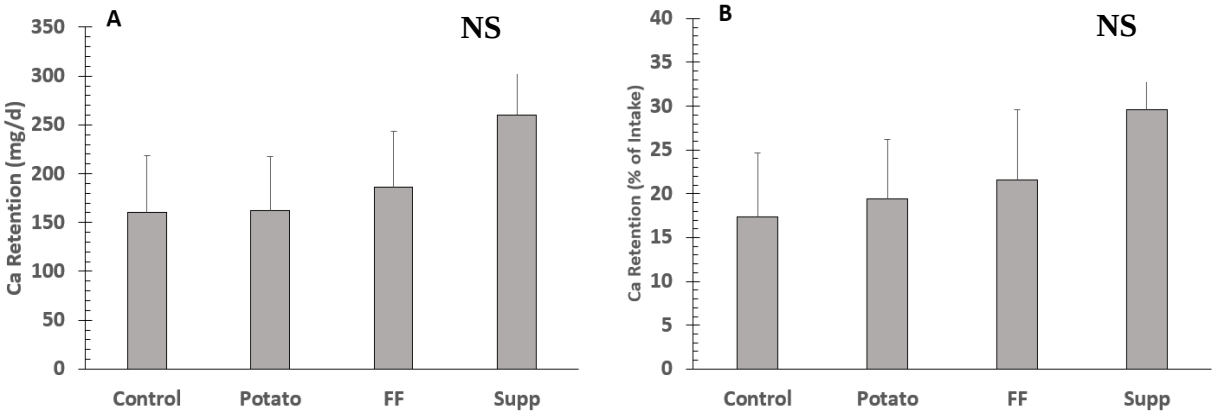

Figure 4.4 Differences in Calcium Retention (N=20). There were no significant differences (NS) among groups for either absolute calcium (Ca) retention (A; control: 160.5 ± 58.1, potatoes: 162.5 ± 55.2, French fries (FF): 186.2 ± 57.1, supplement (Supp): 260.5 ± 45.6; P = 0.26) of retention based on percent of intake (B; control: 17.4 ± 7.3%, potatoes: 19.4 ± 6.8%, French fries: 21.6 ± 8.0%, supplement: 29.6 ± 5.4%; P = 0.37). However, Results seem to indicate that there was a trend for higher Ca retention (absolute) between K-gluconate vs. the control (260.5 vs. 160.5 mg/d). Means ±SE.

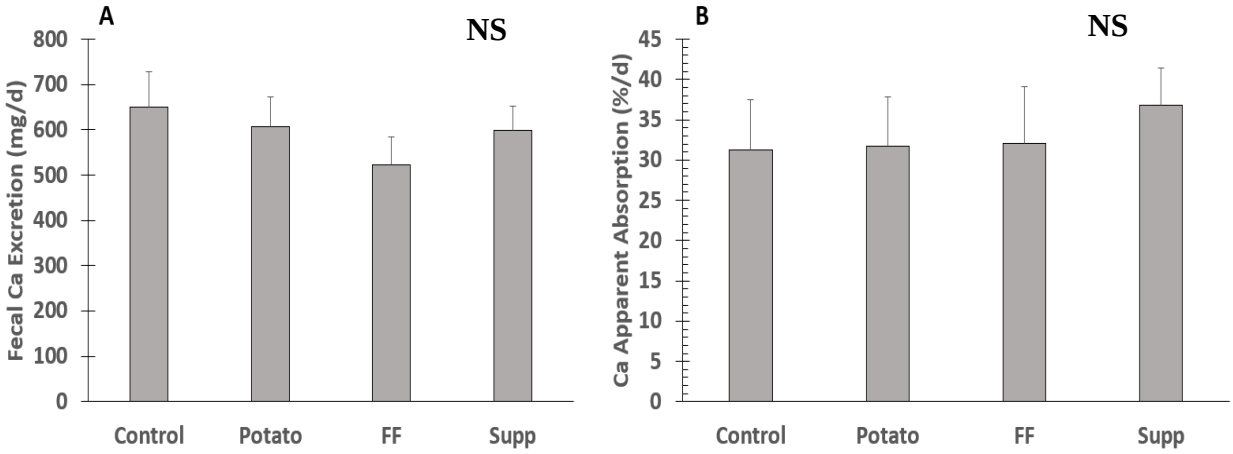

Figure 4.5 Differences in Ca Fecal Excretion and % Absorption (N=20). There were no significant differences (NS) in fecal calcium (Ca) excretion (A) with losses of 650.6 ± 77.9, 606.9 ± 66.5, 523.1 ± 62.3, 599.6 ± 53.0 mg/d, for control, potato, French fries (FF), and supplement (Supp) interventions. There were also no significant differences among groups for percent Ca absorption (B; control: 31.2 ± 6.3%, potatoes: 31.6 ± 6.2%, French fries: 32.0 ± 7.1%, supplement: 36.8 ± 4.5%; P = 0.84). Means ±SE.

References

1. Compston, J., *Osteoporosis: social and economic impact.* Radiol Clin North Am, 2010. **48**(3): p. 477-82.

2. Sözen, T., L. Özışık, and N.Ç. Başaran, *An overview and management of osteoporosis.* European Journal of Rheumatology, 2017. **4**(1): p. 46-56.

3. Barzel, U.S., *The skeleton as an ion exchange system: implications for the role of acid-base imbalance in the genesis of osteoporosis.* J Bone Miner Res, 1995. **10**(10): p. 1431-6.

4. Brandao-Burch, A., et al., *Acidosis inhibits bone formation by osteoblasts in vitro by preventing mineralization.* Calcif Tissue Int, 2005. **77**(3): p. 167-74.

5. Muhlbauer, R.C., A. Lozano, and A. Reinli, *Onion and a mixture of vegetables, salads, and herbs affect bone resorption in the rat by a mechanism independent of their base excess.* J Bone Miner Res, 2002. **17**(7): p. 1230-6.

6. Bonjour, J.P., *Dietary protein: an essential nutrient for bone health.* J Am Coll Nutr, 2005. **24**(6 Suppl): p. 526s-36s.

7. Weaver, C.M., *Potassium and health.* Adv Nutr, 2013. **4**(3): p. 368S-77S.

8. Li, J.J., et al., *Fruit and vegetable intake and bone mass in Chinese adolescents, young and postmenopausal women.* Public Health Nutr, 2013. **16**(1): p. 78-86.

9. New, S.A., et al., *Nutritional influences on bone mineral density: a cross-sectional study in premenopausal women.* Am J Clin Nutr, 1997. **65**(6): p. 1831-9.

10. Nieves, J.W., et al., *Nutritional factors that influence change in bone density and stress fracture risk among young female cross-country runners.* Pm r, 2010. **2**(8): p. 740-50; quiz 794.

11. Macdonald, H.M., et al., *Effect of potassium citrate supplementation or increased fruit and vegetable intake on bone metabolism in healthy postmenopausal women: a randomized controlled trial.* Am J Clin Nutr, 2008. **88**(2): p. 465-74.

12. Prynne, C.J., et al., *Fruit and vegetable intakes and bone mineral status: a cross sectional study in 5 age and sex cohorts.* Am J Clin Nutr, 2006. **83**(6): p. 1420-8.

13. Tucker, K.L., et al., *Potassium, magnesium, and fruit and vegetable intakes are associated with greater bone mineral density in elderly men and women.* Am J Clin Nutr, 1999. **69**(4): p. 727-36.

14. Lin, P.H., et al., *The DASH diet and sodium reduction improve markers of bone turnover and calcium metabolism in adults.* J Nutr, 2003. **133**(10): p. 3130-6.

15. Frassetto, L., R.C. Morris, Jr., and A. Sebastian, *Long-term persistence of the urine calcium-lowering effect of potassium bicarbonate in postmenopausal women.* J Clin Endocrinol Metab, 2005. **90**(2): p. 831-4.

16. Lemann, J., Jr., et al., *Potassium administration reduces and potassium deprivation increases urinary calcium excretion in healthy adults [corrected]*. Kidney Int, 1991. **39**(5): p. 973-83.

17. Lambert, H., et al., *The effect of supplementation with alkaline potassium salts on bone metabolism: a meta-analysis.* Osteoporos Int, 2015. **26**(4): p. 1311-8.

18. Sebastian, A., et al., *Improved mineral balance and skeletal metabolism in postmenopausal women treated with potassium bicarbonate.* N Engl J Med, 1994. **330**(25): p. 1776-81.

19. Moseley, K.F., et al., *Potassium citrate supplementation results in sustained improvement in calcium balance in older men and women.* J Bone Miner Res, 2013. **28**(3): p. 497-504.

20. Rafferty, K., K.M. Davies, and R.P. Heaney, *Potassium intake and the calcium economy.* J Am Coll Nutr, 2005. **24**(2): p. 99-106.

21. Dawson-Hughes, B., et al., *Treatment with potassium bicarbonate lowers calcium excretion and bone resorption in older men and women.* J Clin Endocrinol Metab, 2009. **94**(1): p. 96-102.

22. Marangella, M., et al., *Effects of potassium citrate supplementation on bone metabolism.* Calcif Tissue Int, 2004. **74**(4): p. 330-5.

23. Jehle, S., H.N. Hulter, and R. Krapf, *Effect of potassium citrate on bone density, microarchitecture, and fracture risk in healthy older adults without osteoporosis: a randomized controlled trial.* J Clin Endocrinol Metab, 2013. **98**(1): p. 207-17.

24. Gregory, N.S., et al., *POTASSIUM CITRATE DECREASES BONE RESORPTION IN POSTMENOPAUSAL WOMEN WITH OSTEOPENIA: A RANDOMIZED, DOUBLE-BLIND CLINICAL TRIAL.* Endocr Pract, 2015. **21**(12): p. 1380-6.

25. Remer, T. and F. Manz, *Estimation of the renal net acid excretion by adults consuming diets containing variable amounts of protein.* Am J Clin Nutr, 1994. **59**(6): p. 1356-61.

26. New, S.A., *Nutrition Society Medal lecture. The role of the skeleton in acid-base homeostasis.* Proc Nutr Soc, 2002. **61**(2): p. 151-64.

27. Fenton, T.R., et al., *Meta-analysis of the effect of the acid-ash hypothesis of osteoporosis on calcium balance.* J Bone Miner Res, 2009. **24**(11): p. 1835-40.

28. Buclin, T., et al., *Diet acids and alkalis influence calcium retention in bone.* Osteoporos Int, 2001. **12**(6): p. 493-9.

29. New, S.A., *Intake of fruit and vegetables: implications for bone health.* Proc Nutr Soc, 2003. **62**(4): p. 889-99.

30. Fenton, T.R., et al., *Meta-analysis of the quantity of calcium excretion associated with the net acid excretion of the modern diet under the acid-ash diet hypothesis.* Am J Clin Nutr, 2008. **88**(4): p. 1159-66.

31. Tylavsky, F.A., et al., *Fruit and vegetable intakes are an independent predictor of bone size in early pubertal children.* Am J Clin Nutr, 2004. **79**(2): p. 311-7.

32. Vatanparast, H., et al., *Positive effects of vegetable and fruit consumption and calcium intake on bone mineral accrual in boys during growth from childhood to adolescence: the University of Saskatchewan Pediatric Bone Mineral Accrual Study.* Am J Clin Nutr, 2005. **82**(3): p. 700-6.

33. Welch, A.A., et al., *Urine pH is an indicator of dietary acid-base load, fruit and vegetables and meat intakes: results from the European Prospective Investigation into Cancer and Nutrition (EPIC)-Norfolk population study.* Br J Nutr, 2008. **99**(6): p. 1335-43.

34. Appel, L.J., et al., *A clinical trial of the effects of dietary patterns on blood pressure. DASH Collaborative Research Group.* N Engl J Med, 1997. **336**(16): p. 1117-24.

35. Sacks, F.M., et al., *Effects on blood pressure of reduced dietary sodium and the Dietary Approaches to Stop Hypertension (DASH) diet. DASH-Sodium Collaborative Research Group.* N Engl J Med, 2001. **344**(1): p. 3-10.

36. Shils, M.E. and M. Shike, *Modern nutrition in health and disease*. 2006: Lippincott Williams & Wilkins.

37. National Academies of Sciences, E., et al., *The National Academies Collection: Reports funded by National Institutes of Health*, in *Dietary Reference Intakes for Sodium and Potassium*, M. Oria, M. Harrison, and V.A. Stallings, Editors. 2019, National Academies Press (US)Copyright 2019 by the National Academy of Sciences. All rights reserved.: Washington (DC).

38. Newberry, S.J., et al., *Sodium and Potassium Intake: Effects on Chronic Disease Outcomes and Risks*. 2018, Rockville MD.

39. Jehle, S., et al., *Partial neutralization of the acidogenic Western diet with potassium citrate increases bone mass in postmenopausal women with osteopenia.* J Am Soc Nephrol, 2006. **17**(11): p. 3213-22.

CHAPTER 5: DISCUSSION

Summary & Synthesis

Increased Dietary Potassium from Potato and Potassium Gluconate has no Effect on Blood Pressure and Microcirculation In Pre-Hypertensive-to-Hypertensive Adults

Including boiled/baked potatoes in the diet to increase potassium intake (~3000mg/d) may have some benefit to systolic blood pressure (SBP) in individuals with mild cardiometabolic risk, but this needs to be confirmed in larger trials. End of treatment SBP approached significance (P = 0.059) between the potato and control interventions, with a difference of 3.1 mmHg (126.2 vs. 129.3mmHg). Looking at change in SBP over the course of each intervention, there was a significant difference between the potato and the control phases (-6.01mmHg vs. -2.6mmHg; P = 0.017). There were no treatment differences for any measures of diastolic blood pressure (DBP) or microvasculature function. To our knowledge, this is the first controlled feeding trial to specifically look at the effect of potassium on blood pressure (BP) and vascular outcomes. Other trials looking at the effect of dietary potassium on BP have utilized dietary advice to manipulate intake, and found mostly null results. While this trial was more controlled, we may have been slightly under powered to detect significant differences in intervention effects, and the shorter study duration may have also been a limiting factor in completely differentiating outcomes.

Dietary Potassium from Potato and Potassium Gluconate Supplement Effect on Potassium and Sodium Retention

Increases in potassium intake seem to increase potassium retention regardless of source in pre-hypertensive-to-hypertensive adults. Potassium intakes of 2238, 3008, 2977, and 3299mg/d, for dietary interventions of control, potato, French fries (FF), and potassium (K)-gluconate, produced potassium retention of 550, 797, 804, and 1087mg/d, respectively, the K-gluconate intervention was significantly higher (P = 0.0001) than the other three treatments. However, when assessed as percent (%) of intake only differences between the control (23.3%) and supplement (33.1%; P = 0.04) interventions were apparent. Overall, these outcomes confirm findings from other potassium balance studies to some extent, with a potassium intake of ≥

2200mg/d resulting in a positive potassium retention, but it is difficult to draw absolute similarities, given how much our study differs in design and sample population compared to other trials that have been conducted. For sodium (Na), intakes of 2974, 3417, 3466, and 2974mg/d, for control, potato, French fries (FF), and K-gluconate, resulted in Na retention of 858, 459, 834, and 1005mg/d, respectively. Absolute Na retention was significantly lower for the potato compared to the other three treatments ($P = 0.002$), and remained significant between control (11.8 vs. 26.1%; $P = 0.01$) and K-gluconate (11.8 vs. 30.6%; $P = 0.0004$) as % of dietary intake. In animal models, potassium depletion increases Na reabsorption in the kidney, while potassium loading leads to decreases in reabsorption, and increases in urinary excretion [1-3]. Differences in Na retention may have also been the result of the non-mineral component of the potassium supplement: gluconate. Gluconic acid is an oxidized product of glucose and active Na absorption in the upper small intestine occurs through the Na+/glucose cotransporter, a process that is largely dependent on the presence of glucose [4]. This may explain the higher % absorption of Na in the K-gluconate group (97.1%), and the difference in Na retention. Urinary losses of both potassium and Na behaved in accordance with the controlled diets, serving as another tool for assessing compliance. Potassium urinary excretion averaged 63%, and Na excretion averaged 75% of total intake across all interventions, both similar to values that have been reported in other controlled diet studies. There were no significant differences in potassium or Na fecal excretion, with an average loss of ~12 and ~4%, respectively, across all interventions. Overall, this study adds to previous mineral retention research, and highlights the need for further assessment of potassium and Na retention across many different groups, especially unhealthy (e.g. hypertensive) populations, which may benefit from higher potassium intakes and a better understanding of potassium adequacy.

Dietary Potassium from Potato and Potassium Gluconate Effect on Calcium Retention and Urinary pH

The acid-ash hypothesis proposes that a diet higher in meats and cereal grains, precursors to acid metabolites (e.g. phosphates, sulfates), causes chronic acidemia, increased calcium (Ca) release from bone tissue over time and bone loss. Increased intake of potassium and other alkalizing minerals are proposed to abate this acidic load. Increased potassium intake via K-gluconate supplementation led to a significantly lower urinary Ca excretion (100.4 ± 9.3mg/d; P

= 0.009) compared to the potato (115.2 ± 10.4mg/d; P=0.033) and the control (116.6 ± 9.9mg/d; P=0.027) arms. Results also revealed significant differences between interventions in urinary pH (control: 6.08 ± 0.2, potatoes: 6.36 ± 0.14, French fries (FF): 6.33 ± 0.12, supplement: 6.54 ± 0.2; P = 0.008), follow-up analysis showed a lower urinary pH in the supplement compared to the control phase (6.08 ± 0.18 vs. 6.54 ± 0.16; P=0.0036). However, there were no significant differences (P > 0.05) in Ca retention in absolute units (mg/d) (control: 160.5 ± 58.1, French fries: 186.2 ± 57.1, potatoes: 162.5 ± 55.2, supplement: 260.5 ± 45.6; P=0.26. Mean ± SE), or as percent (%) intake of total diet, (control: 17.4 ± 7.3%, potatoes: 19.4 ± 6.8%, French fries: 21.6 ± 8.0%, supplement: 29.6 ± 5.4%; P = 0.37). While there were differences among groups in urinary Ca excretion and urine pH, these did not translate into differences in calcium economy, utilized in this study as a surrogate for bone benefit.

Strengths and Limitations

This study had several strengths and limitations, which are discussed within each individual chapter and will be further highlighted here. The study design was strong, a randomized, controlled feeding trial, which is often touted as the gold standard in clinical nutrition science research. Our study was also unique in the fact that it was a free-living balance study. Metabolic balance studies are often conducted in a hospital ward or medical center setting, where participants are closely monitored for extended periods of time. While participants in this study visited the clinical suite frequently (~every other day) they were not continuously monitored, and instead we relied on subjects to follow instructions and report any study protocol deviations. This made our study more generalizable, although not entirely, as the true free-living general population is more diverse and has far fewer constraints than this study required. The down side of less control in a controlled feeding retention study, or any study, is the error that may have been added to our results. As discussed previously in Chapters 3 and 4, even inadvertent noncompliance away from the clinical testing suite, where small amounts of sample (urine or fecal) were not collected, or small portions of food were discarded and not reported, could have added to inflation and error in our retention findings. Another major limitation to the mineral retention aspect of this study was the lack of sweat collection and analysis. Only three of the thirteen potassium and Na balance studies assessed by the 2019 DRI committee included

measures of whole body sweat [5]. Cited as the most rigorously controlled of these studies, Palacios and colleagues examined whole body potassium, Na, and Ca sweat losses in black and white adolescent girls in a summer camp setting [6, 7]. Sweat concentrations for potassium, Na, and Ca were approximately 200, 100, and 50mg/d, respectively. However, these were not different between diet intervention groups, which is an important distinction in a cross-over design such as in the Palacios study and our own. Nevertheless, these findings still represent a significant amount of mineral loss via sweat each day. We cannot directly extrapolate these sweat mineral loss estimates to our own study, given Palacios et al. studied an adolescent population, and the setting was a summer camp with many outdoor activities on hot days. Still, it is still important to acknowledge that lack of sweat mineral analysis may add error to our balance values. However, it would have been impractical to collect whole body sweat within the context of our study, given it was largely free-living, and would have been even more burdensome for the participants. While there were no differences between dietary interventions on sweat measures in the Palacios et al. adolescent summer camp study where only dietary Na was being manipulated (~4000 vs. 1200mg/d) and potassium held constant (~2200mg/d), other studies looking at effects of increasing dietary potassium intakes show differences in sweat potassium concentration [8, 9]. Overall it is difficult to completely understand how sweat mineral data would have changed our results, and it remains a large source of error when examining complete potassium, Na, or Ca mineral balance in this study and others.

For blood pressure outcomes, the study design was again a strength. The cross-over aspect eliminated the large interindividual variation that is inherent with BP measurements, and typically requires the recruitment of very large samples (N > 400) in parallel designs [10]. Despite the strength of our cross-over design, we were still unable to see differences between interventions for our primary outcomes, end of treatment systolic and diastolic blood pressure (SBP, DBP) measures. This is most likely due to our lack of power to detect the level of difference (~2 mmHg, N=71 needed) seen in similar studies such as the Dietary Approaches to Stop Hypertension (DASH) trials [10, 11]. With our sample (N = 30) we were powered to detect a difference in SBP of 3.7 mmHg, and our findings show a trend towards significance for the potato intervention, with a 3.1 mmHg decrease in SBP at end of treatment for the potato intervention compared to control (P = 0.059). The study duration may have also been a limitation. Other studies assessing differences in dietary potassium and effects on BP have been

longer duration ranging from 4 to 12 weeks [10, 12-14]. However, the majority of these studies utilized only dietary coaching for potassium intake manipulation and concluded with null results. A recent analysis by the DASH group showed diet-induced changes in BP were achieved within 2 weeks on a controlled DASH dietary pattern [15]. The DASH diet involves the manipulation of many nutrients, along with a higher potassium intake of ~4000mg/d, both of these factors may have contributed to their positive result after only 2 weeks. In the context of our study a longer duration may have allowed for full development of the intervention effects. Another strength of our study related to BP, was our measurement protocol and the number of measurements taken. There is a large amount of heterogeneity seen throughout the BP literature when it comes to BP assessment [16]. The DASH trials, again, represent the gold standard in this context, both for measurement protocol and frequency. Our study was designed to gather as much BP data as possible using a modified version of the DASH BP assessment protocol [17]. This makes our BP results very reliable and comparable to measurements from the DASH interventions. Another limitation to BP aspects of this study may be related to our potassium level of intake. Evidence exists from supplementation trials, for a moderate benefit of potassium between 3500 and 4700 mg/d for lowering BP [16]. For our interventions, the level of potassium intake was between 3000-3300 mg/d, while aiming for more of a practical increase in intake and still higher than the average population intake (~2600 mg/d), we may have been too low to see an effect on BP.

Future Directions

The Agency for Healthcare Research and Quality (AHRQ) and the 2019 DRI committee both concluded that more dietary interventions looking specifically at the effects of increasing potassium from foods are needed to understand the effects on BP and other CVD outcomes [5, 16]. Retention studies are extremely lacking as well, and a better understanding of how potassium, as well as Na, are retained and how they affect each other will help to determine potassium intake for adequacy and reduction of disease and mortality risk. Future research should aim to manipulate potassium intake source from foods (e.g. dairy, fruits, vegetables) as well as supplements, and to examine effects from various doses. Dose response evidence, especially from food sources, is very limited for potassium intake. While both potassium retention and BP are potentially related, it may be more beneficial for future trials to first assess

these outcomes separately, and with those findings design a study in which this relationship can be completely captured and understood. Assessment of potassium intakes effect on Ca economy and ultimately bone benefit is a bit more difficult. Surrogate measures of bone health (e.g. urinary Ca excretion, Ca balance) can be assessed but do not give concrete conclusions that are unequivocally indicative of what's occurring in the bone tissue. Longer studies (1-2 years) which assess BMD and fracture risk are needed to better understand the role of potassium in bone health, and if this relationship is in any way related to the acid-ash hypothesis. In addition to these specific health outcomes, studies need to be conducted in different subgroups to determine differential effects by sex, age, race/ethnicity, and those with vulnerable health conditions [18].

Conclusions

Currently the public health message for potassium is mixed. Potassium has been identified as a nutrient of concern by the 2015-2020 Dietary Guidelines for Americans [19], and the Food and Drug Administration recently (2016) passed new regulations that potassium content be displayed on nutrition facts labels (based on daily value of 4700 mg/d), citing the link between potassium intake and BP, as well as known inadequate intakes in the population [20]. Despite this, the 2019 Dietary Reference Intakes for Na and potassium set the adequate intake (AI) for potassium at the highest median intake of each population group(~3000mg/d for adult (≥19 y) men and women), essentially sending the opposite message of the two aforementioned public health agencies [5]. This is also a break from the 2005 DRI recommendations, in which the potassium AI was set at a seemingly inconsumable level of 4700mg/d [21]. The World Health Organization, in contrast, has set potassium recommendations at 3510mg/d for adults [22]. These differing views from various public health organizations are in the very least sending one clear message: a recognition of the lack of evidence related to potassium intake adequacy and disease. Recommendations from the most recent DRI review of essentially 'just keep doing what you've already been doing', may in fact be sufficient for maintaining adequacy in a healthy, normotensive population. However, higher intake levels may be optimal for more vulnerable groups. As mentioned, there is evidence for a benefit of potassium supplementation between 3500 - 4700mg/d for lowering BP in pre-hypertensive-to-hypertensive populations [16]. Currently it is difficult to determine how potassium retention relates to adequacy and/or any

potential health benefits. In our study, even at the lowest intake of ~2300mg/d, potassium retention was positive, again in line with previous balance findings. If positive potassium balance is to be utilized as the benchmark for adequacy, then it would seem from our results, while in a pre-hypertensive-to-hypertensive population, that lower intakes may be sufficient. It would also seem that lower intakes may benefit BP, with a potassium intake of ~3000mg/d showing a trend toward a significant reduction in SBP for our potato intervention vs. control. However, this finding was in the context of a controlled diet. The population, healthy or otherwise, in a free-living environment would most likely require a higher intake depending on intake of other nutrients. A higher intake, closer to the 3500 mg/d that has shown BP benefit in previous research, may have revealed more significant effects in our study as well.

Another important factor in evaluating what constitutes a potassium intake for adequacy and/or health benefits is Na intake. On average Americans consume 3440mg of Na per day, well above the current AI and CDRR (formerly upper limit (UL)) of 1500mg/d and 2300mg/d, respectively. It is well recognized that the intake of both potassium and Na are inter-related and together influence fluid balance and overall arterial pressure. Potassium has been shown to blunt the rise in BP due to excessive Na intake [23], and while some research shows positive associations between a Na:potassium(K) and BP outcomes [24, 25], overall, evidence linking a specific Na:K intake ratio BP or any health benefit is insufficient [5, 16]. Sodium intake in our study, after chemical analysis, was determined to be ~3400mg/d in our potato and FF arms, and closer to ~3000mg/d in our control and supplement phases. The Na:K ratios were relatively similar in all three increased potassium intake interventions (1.1 for potato and FF, 0.9 for K-gluconate) and slightly higher in the control phase (1.3), offering little further insight into potential significance. Similar to potassium, it is difficult to determine how Na retention relates to potassium adequacy, BP, or other health benefit. We found that the potato intervention with the lowest Na retention had the greatest BP benefit. This may highlight the importance of Na in relation to potassium intake and BP but, given intake levels of both potassium and Na were similar to other phases of the study, it is difficult to definitively determine what the underlying cause of the effect may have been. Overall, future dietary intake recommendations for potassium that consider differences due to energy needs, Na and other important micronutrients, and the context of the whole diet, may be more practical and translatable, and help guide future research [18].

There is still much to learn about the effect of dietary potassium, both alone and in the context of the whole diet, on overall health. A diet abundant in fruits and vegetables has been regarded for many years as optimal for maintaining overall health. Fruits and vegetables are rich in the minerals (e.g., potassium, Ca, etc) required for normal cardiovascular health and the development and maintenance of healthy bone [26, 27]. More research is needed to better understand the role of dietary potassium, how it relates to specific biomarkers and how those relate to health outcomes (e.g. BP-CVD, Ca retention-Osteoporotic fracture), and the overall physiological consequences of increased intake.

References

1. Soleimani, M., et al., *Potassium depletion increases luminal Na+/H+ exchange and basolateral Na+:CO3=:HCO3- cotransport in rat renal cortex.* J Clin Invest, 1990. **86**(4): p. 1076-83.

2. Veiras, L.C., et al., *Potassium Supplementation Prevents Sodium Chloride Cotransporter Stimulation During Angiotensin II Hypertension.* Hypertension, 2016. **68**(4): p. 904-12.

3. Sorensen, M.V., et al., *Rapid dephosphorylation of the renal sodium chloride cotransporter in response to oral potassium intake in mice.* Kidney Int, 2013. **83**(5): p. 811-24.

4. Wright, E.M. and D.D. Loo, *Coupling between Na+, sugar, and water transport across the intestine.* Ann N Y Acad Sci, 2000. **915**: p. 54-66.

5. National Academies of Sciences, E., et al., *The National Academies Collection: Reports funded by National Institutes of Health*, in *Dietary Reference Intakes for Sodium and Potassium*, M. Oria, M. Harrison, and V.A. Stallings, Editors. 2019, National Academies Press (US) Copyright 2019 by the National Academy of Sciences. All rights reserved.: Washington (DC).

6. Palacios, C., et al., *Racial differences in potassium homeostasis in response to differences in dietary sodium in girls.* Am J Clin Nutr, 2010. **91**(3): p. 597-603.

7. Palacios, C., et al., *Sodium retention in black and white female adolescents in response to salt intake.* J Clin Endocrinol Metab, 2004. **89**(4): p. 1858-63.

8. Costill, D.L., R. Cote, and W.J. Fink, *Dietary potassium and heavy exercise: effects on muscle water and electrolytes.* Am J Clin Nutr, 1982. **36**(2): p. 266-75.

9. Consolazio, C.F., et al., *Excretion of sodium, potassium, magnesium and iron in human sweat and the relation of each to balance and requirements.* J Nutr, 1963. **79**: p. 407-15.

10. Appel, L.J., et al., *A clinical trial of the effects of dietary patterns on blood pressure. DASH Collaborative Research Group.* N Engl J Med, 1997. **336**(16): p. 1117-24.

11. Sacks, F.M., et al., *Effects on blood pressure of reduced dietary sodium and the Dietary Approaches to Stop Hypertension (DASH) diet. DASH-Sodium Collaborative Research Group.* N Engl J Med, 2001. **344**(1): p. 3-10.

12. Chalmers, J., et al., *Australian National Health and Medical Research Council dietary salt study in mild hypertension.* J Hypertens Suppl, 1986. **4**(6): p. S629-37.

13. Berry, S.E., et al., *Increased potassium intake from fruit and vegetables or supplements does not lower blood pressure or improve vascular function in UK men and women with early hypertension: a randomised controlled trial.* Br J Nutr, 2010. **104**(12): p. 1839-47.

14. Miller, E.R., 3rd, et al., *A Dietary Intervention in Urban African Americans: Results of the "Five Plus Nuts and Beans" Randomized Trial.* Am J Prev Med, 2016. **50**(1): p. 87-95.

15. Juraschek, S.P., et al., *Time Course of Change in Blood Pressure From Sodium Reduction and the DASH Diet.* Hypertension, 2017. **70**(5): p. 923-929.

16. Newberry, S.J., et al., *Sodium and Potassium Intake: Effects on Chronic Disease Outcomes and Risks*. 2018, Rockville MD.

17. *The effects of nonpharmacologic interventions on blood pressure of persons with high normal levels. Results of the Trials of Hypertension Prevention, Phase I.* Jama, 1992. **267**(9): p. 1213-20.

18. Weaver, C.M., et al., *What Is the Evidence Base for a Potassium Requirement?* Nutr Today, 2018. **53**(5): p. 184-195.

19. *U.S. Department of Health and Human Services and U.S. Department of Agriculture. 2015-2020 Dietary Guidelines for Americans. 8th Edition.* 2015.

20. *Food Labeling: Serving Sizes of Foods That Can Reasonably Be Consumed at One Eating Occasion; Dual-Column Labeling; Updating, Modifying, and Establishing Certain Reference Amounts Customarily Consumed; Serving Size for Breath Mints; and Technical Amendments. Final rule.* Fed Regist, 2016. **81**(103): p. 34000-47.

21. Institute of, M., *Dietary Reference Intakes for Water, Potassium, Sodium, Chloride, and Sulfate*. 2005, Washington, DC: The National Academies Press.

22. *Guideline: Potassium Intake for Adults and Children*. 2012, Geneva: World Health Organization.

23. Morris, R.C., Jr., et al., *Normotensive salt sensitivity: effects of race and dietary potassium.* Hypertension, 1999. **33**(1): p. 18-23.

24. Stamler, J., et al., *Relation of Dietary Sodium (Salt) to Blood Pressure and Its Possible Modulation by Other Dietary Factors: The INTERMAP Study.* Hypertension, 2018. **71**(4): p. 631-637.

25. Weaver, C.M., et al., *Mineral Intake Ratios Are a Weak but Significant Factor in Blood Pressure Variability in US Adults.* The Journal of nutrition, 2018. **148**(11): p. 1845-1851.

26. Weaver, C.M., *Potassium and health.* Adv Nutr, 2013. **4**(3): p. 368S-77S.

27. Muhlbauer, R.C., A. Lozano, and A. Reinli, *Onion and a mixture of vegetables, salads, and herbs affect bone resorption in the rat by a mechanism independent of their base excess.* J Bone Miner Res, 2002. **17**(7): p. 1230-6.

APPENDIX A: REVIEW OF POTASSIUM TOXICITY/SAFETY

Potassium is an essential nutrient of concern according to recent Dietary Guidelines for Americans Advisory Committees [1-3]. Current potassium recommendations established by the Institute of Medicine (US and Canada) and the World Health Organization are given as an Adequate Intake (AI) of ~120mmol/d and 90mmol/d, respectively [2, 4]. The average potassium intake of U.S. adults participating in the National Health and Nutrition Examination Survey (NHANES) 2011-2012 was 2795+34 mg/d, with less than 3% of the population meeting the AI [5]. Blood pressure (BP) is currently the primary criterion for determining potassium requirements. Other benefits of increasing potassium consumption may include healthy kidney function, improvement in glucose control and prevention of diabetes, and possible benefit to bone [6].

The safety concerns for potassium (K) toxicity, hyperkalemia, are low for the general, healthy population due to the efficiency of the kidney maintaining K homeostasis. The normal kidney can respond to increases in dietary K load of up to ~15g/day (greater than 3-4x current recommended intakes) for extended durations (up to 20 days in some studies [7, 8]), maintaining K balance throughout[9]. Despite this, many international food and health regulatory bodies discourage the use of K salt replacers and K supplements due to the potential of hyperkalemia and risk of associated events, including arrhythmia and cardiac events, and neuromuscular manifestations [2, 10, 11]. These warnings are largely based on case reports of hyperkalemia and related symptoms due to excessive intake of K supplements [12, 13] and excessive use of K salt replacers[14-18]. In the 5 total cases of supplement abuse, 2 were fatal, although both were the result of extremely high K ingestion; 47 tablets of K chloride in one case, and a K dose of approximately 24000mg in the other [12, 13]. The case studies related to excessive use of K salt replacers include individuals with contraindications due to medications (beta-blocker [14], ACE inhibitor[16]), severe renal impairment [17, 18], and one apparently healthy individual [15]. It is generally accepted that in patients with severe chronic kidney disease (CKD) or other diseases with related renal impairment (type 1 diabetes, chronic renal insufficiency, end-stage renal disease, severe heart failure, and adrenal insufficiency), consumption of high levels of K may lead to hyperkalemia (serum K concentration >5.5 mmol/L) and, subsequently, an increased risk

of potentially lethal cardiac arrhythmias [9, 19]. Despite their utility in developing larger research questions, the use of case studies to justify any type of intake recommendation points to the lack of evidence from clinical trials in this area.

It is difficult to become hyperkalemic as a result of increased dietary potassium intake (via supplementation or food) without renal impairment. While clinically there is limited research on assessing the upper limit of K intake, some analyses and systematic reviews have examined the associations between different levels of K intake, kidney health, and mortality. In a recent systematic review with meta-analysis, researchers assessed the effect of oral K supplementation on plasma K levels and renal function [20]. In relatively healthy populations (N=1216 total) spanning 20 clinical trials, K supplementation, given within a dose range of 22-140mmol/day over a time period of 2-24 weeks, elicited a small increase in serum potassium level (0.14mmol/L) across all doses and durations, with no change in kidney function (measured via serum creatinine). Although some trials did include apparently healthy individuals, the majority of the populations assessed were actually individuals with hypertension (16 out of 20 trials), some taking anti-hypertensive medications (e.g ACE inhibitors). This population is often considered at higher risk for developing hyperkalemia and renal dysfunction as a result of increased K intake, however these results show that K intake levels ranging above the current recommendations (~90-120mmol/d) impose no increased health risk. Further, In June of 2018, the AHRQ panel noted that, out of 26 randomized control trials (RCTs) assessing the effects of potassium supplementation on blood pressure (BP) and related outcomes, the only adverse event reported in 6 of the trials was gastrointestinal distress [21]. Eighteen of the 21 trials assessing the effect of potassium on BP in adults included individuals with pre-hypertension or hypertension. Overall the panel rated the strength of evidence for potassium-related adverse outcomes in these populations as low. In another very recent (February 2018) and much larger (N=1, 217, 986) individual-level data meta-analysis of 27 international cohorts (10 general population, 10, chronic kidney disease, 7 high cardiovascular risk(CVD)) in the CKD Prognosis Consortium, investigators looked at the relationship between baseline serum potassium level and adverse outcomes (e.g. all-cause mortality, CVD-related mortality, etc.) over a 6.9 year follow-up [22]. Adjusting for numerous demographic and clinical characteristics, and stratified by estimated glomerular filtration rate (eGFR), compared with a reference serum potassium of 4.2mmol/L the hazard ratio for all-cause mortality was 1.22 (95% CI: 1.15-1.29) at a serum K level of

5.5mmol/L and 1.49 (95% CI: 1.26-1.76) at 3.0mmol/L, producing a U-shaped curve, with similar risk by eGFR across cohorts. A serum potassium level of 4-4.5mmol/L was associated with the lowest risk. Looking specifically at the concern of hyperkalemia across these populations, it is interesting to note that the prevalence of individuals in the non-CKD cohorts (N=1,175,816) with a serum potassium >5.0 and >5.5mmol/L was 3.31 and 0.49%, respectively, and 17.94 and 4.23%, respectively, in the CKD cohort (N=42,170). This may suggest that in those individuals with severe renal impairment monitoring K intake is warranted, however in individuals with normal kidney function, despite other possible comorbidities, limiting K intake may produce little to no benefit.

While overall clinical evidence in regard to potassium toxicity as a result of dietary intake is lacking for both healthy and unhealthy populations, a recent dose response trial did begin to address this question. In a RCT comparing the bioavailability of K from potatoes and K supplement in healthy men and women, investigators found that with an acute K intake of ~120mmol (60 mmol of K gluconate + 60mmol from a controlled diet) serum potassium increased from 3.6 to 4.1 mmol/L, lasting for approximately 4 hours, followed immediately by increased renal potassium excretion [23]. These findings again suggest that the body, with normal renal function, is able to efficiently adapt to high K intake.

Increasing potassium intake from food rather than supplements may be more beneficial to overall health (increasing fruit and vegetable intake would increase intake of other important nutrients as well), and may also limit the risk of hyperkalemia, though more research is needed in this area as well. It would be difficult to consume a level of K from food equivalent to the large doses that can be easily ingested via pills or a salt replacer. In vulnerable populations (CKD, heart failure, poly-medicated hypertension) increased intake of K via food may allow for a greater health benefit that would not be achievable otherwise.

References

1. *U.S. Department of Health and Human Services and U.S. Department of Agriculture. 2015-2020 Dietary Guidelines for Americans. 8th Edition.* 2015.

2. Institute of, M., *Dietary Reference Intakes for Water, Potassium, Sodium, Chloride, and Sulfate*. 2005, Washington, DC: The National Academies Press.

3. *Dietary Guidelines Advisory Committee. Report of the Dietary Guidelines Advisory Committee on the Dietary Guidelines for Americans, 2010.*, in *Washington, DC: U.S. Department of Agriculture, Agricultural Research Service.*2011.

4. *Guideline: Potassium Intake for Adults and Children*. 2012, Geneva: World Health Organization.

5. Bailey, R.L., et al., *Estimating Sodium and Potassium Intakes and Their Ratio in the American Diet: Data from the 2011-2012 NHANES.* J Nutr, 2016.

6. Stone, M.S., L. Martyn, and C.M. Weaver, *Potassium Intake, Bioavailability, Hypertension, and Glucose Control.* Nutrients, 2016. **8**(7).

7. Hene, R.J., et al., *Adaptation to chronic potassium loading in normal man.* Miner Electrolyte Metab, 1986. **12**(3): p. 165-72.

8. Rabelink, T.J., et al., *Early and late adjustment to potassium loading in humans.* Kidney International, 1990. **38**(5): p. 942-947.

9. Palmer, B.F. and D.J. Clegg, *Physiology and pathophysiology of potassium homeostasis.* Advances in Physiology Education, 2016. **40**(4): p. 480-490.

10. *Opinion of the Scientific Panel on Dietetic products, nutrition and allergies [NDA] on a request from the Commission related to the Tolerable Upper Intake Level of Potassium.* EFSA Journal, 2005. **3**(3): p. 193.

11. *Expert Group on Vitamins and Minerals. Safe upper levels of vitamins and minerals. Food Standard Agency.* 2003.

12. Wetli, C.V. and J.H. Davis, *Fatal hyperkalemia from accidental overdose of potassium chloride.* JAMA, 1978. **240**(13): p. 1339.

13. Illingworth, R.N. and A.T. Proudfoot, *Rapid poisoning with slow-release potassium.* Br Med J, 1980. **281**(6238): p. 485-6.

14. Hoyt, R.E., *Hyperkalemia due to salt substitutes.* JAMA, 1986. **256**(13): p. 1726.

15. Schim van der Loeff, H.J., R.J. Strack van Schijndel, and L.G. Thijs, *Cardiac arrest due to oral potassium intake.* Intensive Care Med, 1988. **15**(1): p. 58-9.

16. Ray, K., S. Dorman, and R. Watson, *Severe hyperkalaemia due to the concomitant use of salt substitutes and ACE inhibitors in hypertension: a potentially life threatening interaction.* J Hum Hypertens, 1999. **13**(10): p. 717-20.

17. Doorenbos, C.J. and C.G. Vermeij, *Danger of salt substitutes that contain potassium in patients with renal failure.* BMJ, 2003. **326**(7379): p. 35.

18. Dent, A., D. Walmsley, and S. Dhandapani, *Hyperkalaemia is a risk with low sodium salt in vulnerable patients.* BMJ, 2011. **343**.

19. Palmer, B.F., *A physiologic-based approach to the evaluation of a patient with hyperkalemia.* Am J Kidney Dis, 2010. **56**(2): p. 387-93.

20. Cappuccio, F.P., et al., *Systematic review and meta-analysis of randomised controlled trials on the effects of potassium supplements on serum potassium and creatinine.* BMJ Open, 2016. **6**(8).

21. Newberry, S.J., et al., *Sodium and Potassium Intake: Effects on Chronic Disease Outcomes and Risks*. 2018, Rockville MD.

22. Kovesdy, C.P., et al., *Serum potassium and adverse outcomes across the range of kidney function: a CKD Prognosis Consortium meta-analysis.* Eur Heart J, 2018. **39**(17): p. 1535-1542.

23. Macdonald-Clarke, C.J., et al., *Bioavailability of potassium from potatoes and potassium gluconate: a randomized dose response trial.* Am J Clin Nutr, 2016

APPENDIX B: REVIEW OF POTASSIUM BIOAVAILABILITY

Portions of this review have been published elsewhere:
Stone, M.S., L. Martyn, and C.M. Weaver, *Potassium Intake, Bioavailability, Hypertension, and Glucose Control.* Nutrients, 2016. **8**(7).

Potassium is intrinsically soluble and quickly dispersed in the luminal water of the upper digestive tract. The small intestine is the primary site of potassium absorption, with approximately 90% of dietary potassium being absorbed by passive diffusion [1]. Little is known about the bioavailability of potassium, with the majority of work being centered on the assessment of urinary potassium losses after potassium salt supplementation [2-4].

Many different models of potassium movement within the body have been proposed, each developed to fit various areas of biological interest. The complexity of each model varies, from early recommendations by the International Commission on Radiological Protection for evaluation of radiopotassium exposure limiting the body to one large mixed pool of potassium, to more complex anatomically related compartmentalization [5-7].. In one of the earliest schemes, Ginsburg and Wilde constructed a five compartment model, mathematically derived from murine data looking at tissue groupings (muscle/testes, brain/RBC, bone, lung/kidney/intestine, liver/skin/spleen) and their potassium exchange between a common compartment of extracellular fluid (ECF) [8, 9]. Utilizing $^{42}K+$ intravenous (IV) injections, a wide spectrum of tracer exchange rates between tissues, with kidneys being the fastest (equilibrium with plasma at 2 min) and muscle and brain being the slowest (≥600 min) was observed [8, 9]. Later, Leggett and Williams proposed a more anatomically specific model based on the quantitative movement of potassium through mathematically derived compartments within a physiologically relevant framework [10]. Their model, similar to previous depictions, identified plasma/ECF as the primary feeding compartment, with equilibrium distribution of potassium, regional blood flow rates, and potassium tissue extraction fractions, all influencing potassium exchange. The model also describes potassium exchange from plasma/ECF to tissues as a relatively rapid and uniform process; skeletal muscle being the only exception, with slower exchange due to its role as the main site of potassium storage [10].

In our recent study (Macdonald et al., 2016), the bioavailability of potassium from potato sources (non-fried white potatoes, French fries) and a potassium supplement (potassium gluconate) was compared. Thirty-five healthy men and women (29.7 ± 11.2 years, 24.3 ± 4.4 kg/m^2) were randomized to nine, five-day interventions of additional K+ equaling: 0 mEq (control at phase 1 and repeated at phase 5), 20 mEq (1500 mg), 40 mEq (3000 mg), 60 mEq (4500 mg) K+/day consumed as K+ gluconate or potato, and 40 mEq K+/day from French fries. Bioavailability of potassium was determined from serum AUC (serial blood draws) and 24 h urinary excretion assessed after a test meal of varying potassium dose given on the 4^{th} day. Investigators found increases in serum potassium AUC with increasing dose regardless of source, while potassium 24 h urine concentration also increased with dose but was greater with potato compared to supplement. These outcomes reveal the need for a full potassium balance study, looking at intakes from a variety of dietary sources and complete losses (urine and feces), to fully understand potassium bioavailability differences between dietary potassium and supplements and their subsequent health effects [11].

References

1. Demigne, C., et al., *Protective effects of high dietary potassium: nutritional and metabolic aspects.* J Nutr, 2004. **134**(11): p. 2903-6.

2. Melikian, A.P., et al., *Bioavailability of potassium from three dosage forms: suspension, capsule, and solution.* J Clin Pharmacol, 1988. **28**(11): p. 1046-50.

3. Bechgaard, H. and N.W. Shephard, *Bioavailability of potassium from controlled-release tablets with and without water loading.* Eur J Clin Pharmacol, 1981. **21**(2): p. 143-7.

4. Betlach, C.J., et al., *Bioavailability and pharmacokinetics of a new sustained-release potassium chloride tablet.* Pharm Res, 1987. **4**(5): p. 409-11.

5. *The 2007 Recommendations of the International Commission on Radiological Protection. ICRP publication 103.* Ann ICRP, 2007. **37**(2-4): p. 1-332.

6. *Chapter 3 Physiological data for reference man.* Annals of the ICRP/ICRP Publication, 1975. **23**: p. 335-365.

7. Valentin, J., *Basic anatomical and physiological data for use in radiological protection: reference values: ICRP Publication 89.* Annals of the ICRP, 2002. **32**(3–4): p. 1-277.

8. Ginsburg, J.M., *Equilibration of potassium in blood and tissues.* Am J Dig Dis, 1962. **7**: p. 34-42.

9. Ginsburg, J.M. and W.S. Wilde, *Distribution kinetics of intravenous radiopotassium.* Am J Physiol, 1954. **179**(1): p. 63-75.

10. Leggett, R.W. and L.R. Williams, *A model for the kinetics of potassium in healthy humans.* Phys Med Biol, 1986. **31**(1): p. 23-42.

11. Macdonald-Clarke, C.J., et al., *Bioavailability of potassium from potatoes and potassium gluconate: a randomized dose response trial.* Am J Clin Nutr, 2016.

APPENDIX C: REVIEW OF POTASSIUM, DIABETES, AND GLUCOSE CONTROL

Portions of this review have been published elsewhere:

Stone, M.S., L. Martyn, and C.M. Weaver, *Potassium Intake, Bioavailability, Hypertension, and Glucose Control.* Nutrients, 2016. **8**(7).

Blood glucose levels are tightly regulated within a range of 70-100 mg/dL. After ingestion of a meal the rise in circulating glucose levels, along with other factors, stimulates the release of the hormone insulin from the pancreas. Insulin is secreted from the islets of Langerhans from the β-cells of the pancreas and has action on target tissues (e.g. skeletal muscle, liver, adipose) to facilitate cellular glucose uptake. Antagonistically the hormone glucagon is secreted from the α-cells of the pancreas in response to low levels of blood glucose, stimulating the release of glucose from tissues and glucose production (gluconeogenesis) in the liver. Continually changing levels of insulin and glucagon are important signals in informing various physiological systems of the body's nutritional state.

Diabetes Mellitus (DM) is a degenerative disease associated with a lack of, or insufficient secretion, of insulin or an insensitivity to insulin stimulation in the cells of target tissues. DM comes in two forms: type 1, insulin dependent, DM, or type 2, non-insulin dependent DM. Type 1 DM is primarily characterized as an autoimmune disease in which the immune system attacks the cells of the pancreas leading to nearly complete β-cell destruction or extreme dysfunction. This results in essentially a complete inability to produce insulin, and the requirement of daily insulin injections to control blood glucose. Type 2 DM (T2DM) is more complex and is often the result of obesity coupled with poor dietary and lifestyle choices. In T2DM the pancreas may still produce insulin, often in increasing amounts in response to increases in glucose load, but this is often insufficient to maintain homeostatic glucose levels if intake becomes too high and frequent. Eventually β-cell insulin granules become depleted and target tissues exhibit resistance to insulin stimulation, leaving blood glucose levels unchecked. Prolonged elevated blood glucose levels can be damaging to small vessels, especially in the brain, kidneys, eyes, and extremities, and can eventually lead to nerve damage and tissue death. While the use of drugs to increase insulin secretion and improve tissue insulin sensitivity can be effective, lifestyle changes including

better dietary choices and increased physical activity will often lead to control of the disease [1] [2].

Potassium plays a role in blood glucose control by modulating the secretion of insulin from the pancreas. On the cellular level, K+ efflux from ATP sensitive K+ (K+/ATP) channels influences β-cell excitability and holds membrane potential at low levels (~-60mV) [3]. Increases in blood glucose lead to increased β-cell glucose uptake and subsequent ATP generation, which in turn inhibit K+/ATP channels. Decreased K+ efflux leads to stimulation of voltage gated Ca2+ (Ca2+V) channels, cellular depolarization via Ca2+ influx, and increased insulin secretion. Potassium efflux through voltage gated K+ channels leads to repolarization and an inhibition of Ca2+V channels, inhibiting insulin release. While experimentally supraphysiological concentrations of K+ (≥ 10mM) induce a depolarizing effect on β-cell membrane potential, the effects of extracellular K+ at the upper end of the physiological range (5.5 mM) are unknown [4].

Glucose intolerance can often be a result of severe hypokalemia due to a deficit in potassium balance that may occur in primary or secondary aldosteronism or prolonged treatment with diuretics [5]. The use of thiazide diuretics are widely considered the preferred initial pharmacological treatment for hypertension [6]. The tendency of thiazide diuretics to negatively influence glucose tolerance and increase the incidence of new onset diabetes is well known. In a recent quantitative review, researchers analyzed 59 clinical trials in which the relationship between the use of thiazide diuretics, hypokalemia, and glucose intolerance was strong [7]. Thiazide diuretics have a common side effect of lowering serum potassium and evidence shows that diuretic-induced hypokalemia may lead to impaired glucose tolerance via reduction in insulin secretion in response to glucose loads [8]. In healthy individuals, there is also evidence to support the role of potassium in glucose control. Studies involving potassium depletion (e.g., low potassium diets) show that low levels of potassium can lead to glucose intolerance via impaired insulin secretion [9, 10]. In addition, when patients with thiazide-induced hypokalemia are given potassium supplements, the defects in insulin release in response to glucose loads are corrected, thus indicating that hypokalemia may be a significant contributing factor to the glucose abnormality [11].

The relationship between potassium intake and diabetes was examined in a prospective cohort study conducted by Colditz and colleagues (1992) looking at women (n = 84,360; 34–59 years) from the Nurse's Health Study. After a six-year follow-up, investigators found that high K+ intake may be associated with a decreased risk for developing T2DM in women with a BMI of 29 or less [12]. When compared with women in the lowest quintile, women in the highest quintile for K+ intake had a relative risk of 0.62 (p trend = 0.008) for T2DM. More recently, Chatterjee et al. assessed the association between K+ intake and T2DM using data from the Coronary Artery Risk Development in Young Adults (CARDIA) study [8]. Researchers examined the relationship between urinary K+ and diabetes risk for 1066 participants. Use of multivariate models adjusted for potential confounders including BMI, fruit and vegetable intake, and other dietary factors revealed that those in the lowest quintile of potassium intake were more than twice as likely to develop diabetes compared to those in the highest quintile (HR 2.45; 95% CI 1.08, 5.59; p for trend 0.04). Investigators also found that those in the lowest quintile of K+ intake were significantly more likely to develop diabetes than those in the highest quintile of K+ intake (p = 0.008). Of the 4754 participants, 373 (7.8%) developed T2DM during the follow up period of 20 years, and, overall, the mean K+ intake of those who developed diabetes was significantly lower than those who did not (3393 mg/day vs. 3684 mg/day; p = 0.002). This same research group examined data from 12,209 individuals participating in the Atherosclerosis Risk in Communities (ARIC) cohort and found serum K+ to be independently associated with diabetes risk. Using multivariate cross-sectional analyses, a significant inverse relationship between serum K+ and fasting insulin levels was identified ($p < 0.01$) [13]. Dietary K+ intake was significantly associated with diabetes risk in unadjusted models, with adults having serum K+ levels lower than 4.0 mEq/L at highest risk for DM incidence. This relationship continued to hold true after covariate adjustment (e.g., age, sex, race, BMI, serum magnesium, serum calcium, physical activity, hypertension, etc.) in multivariate models, with lower K+ levels associated with higher BMI, larger waist circumference, lower serum magnesium levels, and higher fasting insulin levels as well.

The relationship between potassium and T2DM also extends to the kalemic effects of insulin. Higher plasma insulin levels are associated with increased K+ absorption into cells[14], and without a threshold as seen in glycemic response, these kalemic effects continue to increase as insulin levels rise [77]. DeFronzo et al. examined this relationship using the insulin clamp

technique and graded doses of insulin. Investigators found a dose-dependent decline in plasma K+ concentration with increasing insulin dose, independent of glucose uptake. This effect is likely to be mediated by an increased sensitivity to intracellular sodium, activation of Na+-K+ ATPase, and inhibition of potassium efflux [14].

References

1. Delli, A.J. and Å. Lernmark, *Chapter 39 - Type 1 (Insulin-Dependent) Diabetes Mellitus: Etiology, Pathogenesis, Prediction, and Prevention∗A2 - Jameson, J. Larry*, in *Endocrinology: Adult and Pediatric (Seventh Edition)*, L.J.D. Groot, et al., Editors. 2016, W.B. Saunders: Philadelphia. p. 672-690.e5.

2. Hupfeld, C.J. and J.M. Olefsky, *Chapter 40 - Type 2 Diabetes Mellitus: Etiology, Pathogenesis, and Natural History A2 - Jameson, J. Larry*, in *Endocrinology: Adult and Pediatric (Seventh Edition)*, L.J.D. Groot, et al., Editors. 2016, W.B. Saunders: Philadelphia. p. 691-714.e6.

3. Ekmekcioglu, C., et al., *The role of dietary potassium in hypertension and diabetes.* Journal of Physiology and Biochemistry, 2016. **72**(1): p. 93-106.

4. Meissner, H.P., J.C. Henquin, and M. Preissler, *Potassium dependence of the membrane potential of pancreatic B-cells.* FEBS Lett, 1978. **94**(1): p. 87-9.

5. He, F.J. and G.A. MacGregor, *Beneficial effects of potassium on human health.* Physiol Plant, 2008. **133**(4): p. 725-35.

6. Haddy, F.J., P.M. Vanhoutte, and M. Feletou, *Role of potassium in regulating blood flow and blood pressure.* Am J Physiol Regul Integr Comp Physiol, 2006. **290**(3): p. R546-52.

7. Zillich, A.J., et al., *Thiazide diuretics, potassium, and the development of diabetes: a quantitative review.* Hypertension, 2006. **48**(2): p. 219-24.

8. Chatterjee, R., et al., *Potassium intake and risk of incident type 2 diabetes mellitus: the Coronary Artery Risk Development in Young Adults (CARDIA) Study.* Diabetologia, 2012. **55**(5): p. 1295-303.

9. Rowe, J.W., et al., *Effect of experimental potassium deficiency on glucose and insulin metabolism.* Metabolism, 1980. **29**(6): p. 498-502.

10. Sagild, U., V. Andersen, and P.B. Andreasen, *Glucose tolerance and insulin responsiveness in experimental potassium depletion.* Acta Med Scand, 1961. **169**: p. 243-51.

11. Helderman, J.H., et al., *Prevention of the glucose intolerance of thiazide diuretics by maintenance of body potassium.* Diabetes, 1983. **32**(2): p. 106-11.

12. Colditz, G.A., et al., *Diet and risk of clinical diabetes in women.* Am J Clin Nutr, 1992. **55**(5): p. 1018-23.

13. Chatterjee, R., et al., *Serum and dietary potassium and risk of incident type 2 diabetes mellitus: The Atherosclerosis Risk in Communities (ARIC) study.* Arch Intern Med, 2010. **170**(19): p. 1745-51.

14. DeFronzo, R.A., et al., *Effect of graded doses of insulin on splanchnic and peripheral potassium metabolism in man.* Am J Physiol, 1980. **238**(5): p. E421-7.

APPENDIX D: EXTERNAL ABSTRACTS AND POSTERS FROM DISSERTATION PROJECT

Appendix D1: AHA Epi Lifestyle Annual Meeting 2019, Houston, TX

Effect of Short-term Increased Dietary Potassium from Potato and Potassium Gluconate on Blood Pressure and Microcirculation in Pre-Hypertensive-to-Hypertensive Adults.

Potassium is an essential nutrient of concern according to recent Dietary Guidelines for Americans Advisory Committees, with less than 3% of the population meeting the current adequate intake (AI) level of 4700mg/d. Increases in potassium intake have been linked to improvements in cardiovascular and other metabolic health outcomes. Blood pressure (BP) is currently the primary criterion for determining potassium requirements. In this clinical trial, we assessed the effects of increasing potassium intake on BP and microcirculation (endothelial function). Thirty pre-hypertensive-to-hypertensive (Systolic BP ≥ 120mmHg) men (N=15) and Women (N=15) with a mean ± SD age, BMI, and BP (systolic(SBP)/ diastolic(DBP)) of 48.2 ± 15 y, 31.4 ± 6.1, and 136.3 ± 11.9/ 86.1 ± 7.1 mmHg, respectively, were enrolled in a cross-over randomized control diet trial. Participants were assigned to a random order of four 16-day dietary potassium interventions including a basal diet (control) of 2300mg/d(~60mmol/d), and three periods of an additional 1000mg/d(3300mg/d(~85mmol/d) total) of potassium in the form of potatoes (baked, boiled, or pan-heated with no additional fat), French fries, or a potassium gluconate supplement. Each intervention period was separated by two or more weeks of wash out. Blood pressure was measured in triplicate using manual auscultation on days 1, 4, 6, 8, 11, 13, 15, 16, and 17 of each intervention. Microvascular and endothelial function were assessed via thermal hyperemia, utilizing laser Doppler flowmetry (LDF), at baseline and at the end of each intervention. Results for differences in both SBP and DBP ± SE were assessed at the end treatment (average of day 15, 16, and 17 measurements) using a mixed model with repeated measures and adjustment for baseline BP as a covariate, with no significant differences among periods (control: 129.3 ± 0.9/ 83.5 ± 1.3, potatoes: 126.2 ± 0.93/ 83.6 ± 1.3, French fries: 127.8 ± 0.94/ 83.6 ± 1.3, supplement: 128.5 ± 0.9/ 83.8 ± 1.6; SBP:P=0.07, DBP: P=0.97). However, differences between SBP for control and potato periods approached significance (P=0.059), and

secondary BP analysis of change in SBP over time was significantly reduced by feeding baked/boiled potatoes compared to control (-6.01mmHg vs. -2.6mmHg). Utilizing the same statistical analysis, there were also no significant differences in endothelial function (measured as percent of cutaneous vascular conductance max (%CVCmax) ± SE) among periods (control: 87.4 ± 1.5%, French Fries: 87.7 ± 1.4%, potatoes: 88.3 ± 1.1%, supplement: 88.9 ± 1.0%). In this small, controlled feeding study of short duration, increased potassium from potatoes or supplement achieved 70% of recommended intakes, but had no significant benefit to blood pressure or microvascular function in men and women with higher cardiometabolic risk.

Effect of Short-term Increased Dietary Potassium from Potato and Potassium Gluconate on Blood Pressure and Microcirculation in Pre-Hypertensive-to-Hypertensive Adults.

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ABSTRACT

Potassium is an essential nutrient of concern according to recent Dietary Guidelines for Americans Advisory Committees, with less than 3% of the population meeting the current adequate intake (AI) level of 4700mg/d. Increases in potassium intake have been linked to improvements in cardiovascular and other metabolic health outcomes. Blood pressure (BP) is currently the primary criterion for determining potassium requirements. In this clinical trial, we assessed the effects of increasing potassium intake on BP and microcirculation (endothelial function). Thirty pre-hypertensive-to-hypertensive [Systolic BP ≥ 120mmHg] men (N=15) and Women (N=15) with a mean ± SD age, BMI, and BP (systolic(SBP)/ diastolic(DBP)) of 48.2 ± 15 y, 31.4 ± 6.1, and 136.3 ± 11.9/ 86.1 ± 7.1 mmHg, respectively, were enrolled in a cross-over randomized control diet trial. Participants were assigned to a random order of four 16-day dietary potassium interventions including a basal diet (control) of 2300mg/d(~85mmol/d) total of potassium in the form of potatoes (baked, boiled, or pan-heated with no additional fat), French fries, or a potassium gluconate supplement. Each intervention period was separated by two or more weeks of wash out. Blood pressure was measured in triplicate using manual auscultation on days 1, 4, 6, 8, 11, 13, 15, 16, and 17 of each intervention. Microvascular and endothelial function were assessed via thermal hyperemia, utilizing laser Doppler flowmetry (LDF), at baseline and at the end of each intervention. Results for differences in both SBP and DBP ± SE were assessed at the end treatment (average of day 15, 16, and 17 measurements) using a mixed model with repeated measures and adjustment for baseline BP as a covariate, with no significant differences among periods (control: 129.3 ± 0.9/ 83.5 ± 1.3, potatoes: 126.2 ± 0.93/ 83.6 ± 1.3, French fries: 127.8 ± 0.94/ 83.6 ± 1.3, supplement: 128.5 ± 0.9/ 83.8 ± 1.6; SBP:P=0.07, DBP: P=0.97). However, differences between SBP for control and potato periods approached significance (P=0.059), and secondary BP analysis of change in SBP over time was significantly reduced by feeding baked/boiled potatoes compared to control (-6.01mmHg vs. -2.4mmHg). Utilizing the same statistical analysis, there were also no significant differences in endothelial function (measured as percent of cutaneous vascular conductance max (%CVCmax) ± SE) among periods (control: 87.4 ± 1.5%, French Fries: 87.7 ± 1.4%, potatoes: 88.8 ± 1.1%, supplement: 88.9 ± 1.0%). In this small, controlled feeding study of short duration, increased potassium from potatoes or supplement achieved 70% of recommended intakes, but had no significant benefit to blood pressure or microvascular function in men and women with higher cardiometabolic risk.

INTRODUCTION

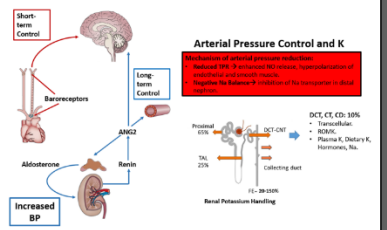


Figure 1. Arterial Pressure Control and Renal K Handling. Short-term arterial pressure control involves sensory input from baroreceptors and rapid response from CNS/autonomic neural integration. Long-term arterial pressure control relies on the kidneys ability to regulate blood volume and solute concentration. The role of potassium in arterial pressure control may involve enhanced nitric oxide release and vasodilation in the vasculature (short-term), as well as increased Na secretion and excretion via renal handling.

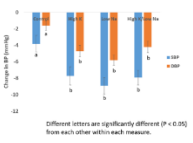


Figure 2. Increased K Through Dietary Advice Improves BP. Chalmers et al. (1986) assessed the effects of both an increase in dietary potassium and a reduction in dietary sodium on blood pressure in subjects (N=212, 52.3y ± 0.8 years; 181 males and 31 females) with hypertension (DBP between 90 and 100 mm Hg). The 12 week intervention included 4 diet groups: normal diet (control), high-potassium diet (>100 mmol/3900 mg K/d), reduced-sodium diet (50–75 mmol/1150–1725 mg Na/d), or high-potassium/low-sodium diet. Both SBP and DBP were significantly reduced in each treatment group compared with control. Reductions in the high-potassium group were 7.7 ± 1.1 and 4.7 ± 0.7 mm Hg for SBP and DBP, respectively (1).

Figure 3. K Dietary Interventions Have also Found Null Results. The effects of increased potassium intake from fruits and vegetables (F&V) and supplements on BP in untreated prehypertensive individuals (DBP 80–100 mm Hg). In a crossover design, subjects (n = 48, 22–65 years old) completed four 6-week dietary interventions including a control diet, an additional 20 or 40 mmol K/d (780 or 1560 mg/d) from F&V, and 40 mmol K-citrate per day. There were no significant changes in blood pressure between the control group and any of the dietary or supplement interventions (2).

PURPOSE

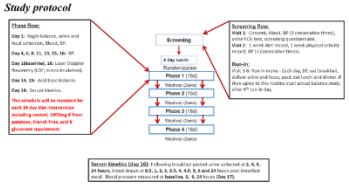
To assess the effects of increasing potassium intake on blood pressure and microcirculation (endothelial function).

MATERIALS AND METHODS

Experimental subjects

Subjects (N=30; age 21y and older) were recruited through a variety of different techniques. Subjects were pre-hypertensive-to-hypertensive (SBP >120mmHg) men and women who were otherwise apparently healthy. Exclusion criteria included: medication to treat hyper or hypotension (hypertensive monotherapy was allowed later in recruitment), known to affect electrolyte metabolism or contain high levels of K or Na, history of myocardial infarction, diabetes mellitus, renal disease, GI disease, pancreatitis, cholestatic liver disease, cancer, smoking, use of illegal drugs, or excessive alcohol intake. subjects who were pregnant or lactating, allergy or intolerance to study foods, unwillingness to refrain from dietary supplements, weight loss > 3kg in the past 2 months. Screening consisted of two visits at least one week apart. Subjects came to the Purdue Nutrition Science clinical suite where they were consented, had their BP measured, a blood draw, and filled out screening questionnaires. They were also given 1-week diet and activity logs to fill out before their second screening visit, which consisted of another BP measurement to ensure eligibility and return of the logs. When eligible subjects completed a 6 day run-in period (described below), continuing subjects were randomized to one of 24 possible sequences of the 4 phases including, control, potato, French fry, and K-gluconate interventions. The study protocol was approved by the Purdue University Institutional Review Board, and all subjects provided written, informed consent.

Study protocol



Dietary Intervention

Each day of the 4 day run-in diet represented one of the four diet phases: control, potato, French fries, supplement. During the run-in, subjects came to the clinical facility each morning, had their BP taken, either stayed to eat breakfast or had all their meals and snacks for the day packed out in a cooler to take with them. After the 4 day run-in period, participants were assigned to a random order of four 16-day dietary potassium interventions including a basal diet (control) of 2300mg/d(~85mmol/d), and three periods of an additional 1000mg/d(~85mmol/d) total of potassium in the form of potatoes (baked, boiled, or pan-heated with no additional fat), French fries, or a potassium gluconate supplement. Each intervention period was separated by two or more weeks of wash out. A four-day menu cycle at three calorie levels (1800, 2200, and 2600 kcal/d) was designed to manipulate potassium levels while keeping other micro (eg. Na, Ca) and macro (e.g. fat) constant. Subjects picked up study foods approximately every other day (on days 1, 4, 6, 8, 11, 13, 15, 16). For each day of controlled feeding, the subjects recorded their intake by checking off each item listed on daily menu sheets provided for each day. They were also instructed to indicated whether they ate any non-study foods and whether they did not eat all the study foods. The food and beverages were prepared with deionized water and weighed to the nearest one tenth gram on digital scales. Duplicates of each menu cycle day were homogenized and analyzed. Researchers adjusted energy intakes with snack items that contained mostly sugar. Any uneaten items were instructed to be returned and analyzed for mineral content.

Blood Pressure and Microvascular Outcomes

Blood pressure was measured by trained and certified study staff with a McKesson Deluxe Aneroid Sphygmomanometer (Richmond, Va), after the participant sat for 5 minutes at rest. Three readings (first and fifth Korotkoff's sounds) were recorded at each visit and averaged. We assessed blood pressure (BP) throughout the study approximately every other day (on days 1, 4, 6, 8, 11, 13, 15, 16, 17) during each phase. Results for differences in both systolic blood pressure (SBP) and diastolic blood pressure (DBP) were assessed at the end of treatment (average of day 15, 16, and 17 measurements).

Microvascular and endothelial function were assessed via thermal hyperemia, utilizing laser Doppler flowmetry (LDF), at baseline (Day 1 or 2 of first intervention) and at the end of each intervention (Day 15 or 16). Direct local heating of the skin, a physiological endothelial NO synthase (eNOS)-dependent dilator stimulus, is a standardized and reliable method to measure NO-dependent dilation and has been used to examine microvascular function in a variety of pathologies. Cutaneous red blood cell flux was continually measured with an integrated LDF probe placed in a local heating unit (Moor Instruments SHO2) position medially on the left forearm. Baseline measurements were collected for 15 min at a local skin temperature of 33° C. After a stable baseline period, the local skin temperature was increased to 42° C at a rate of 0.2° C every 5 s for 40min to achieve a 10min plateau, after which maximal vasodilation was induced by increasing skin temperature to 43° C (20 min). Mean arterial pressure (MAP) was measured at the brachial artery throughout the protocol using an automated blood pressure monitor (Welch-Allen). Cutaneous vascular conductance (CVC) was calculated as red blood cell flux divided by MAP and expressed as a percent of site-specific maximal vasodilation (%CVCmax).

Statistical methods

Results for differences in both SBP, DBP, and endothelial function (measured as percent of cutaneous vascular conductance max (%CVCmax) were analyzed using a mixed model with repeated measures and Dunnett adjustment ($P < 0.05$). Primary BP analysis assessed differences among periods in end of treatment BP (average of day 15, 16, 17), with adjustment for baseline BP (average of day 1 and 4) as a covariate ($P<0.0001$). Secondary BP analysis assessed change in BP over time (end of treatment – baseline). All computations were performed using JMP (SAS) statistical software. Alpha was set at 0.05.

RESULTS AND DISCUSSION

Table 1. Baseline Characteristics of the Study Subjects

Table 1. Baseline Characteristics (Mean ± SD)		Age (yr)	Ht (cm)	Wt (kg)	BMI (kg/m2)	SBP (mmHg)	DBP (mmHg)
Women (N=15)	Mean	52.7	164.9	88.6	32.3	138.0	86.4
	SD	15.4	7.2	24.5	7.2	11.3	7.4
Men (N=15)	Mean	43.8	179.4	99.1	30.5	134.6	85.9
	SD	13.7	7.1	20.7	4.8	12.7	7.0

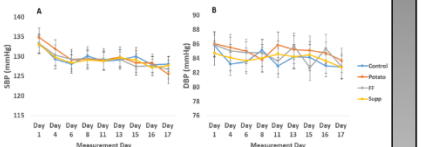


Figure 4. Blood Pressure throughout each intervention. Blood pressure (BP), both systolic (SBP) and diastolic (DBP), was measured approximately every other day throughout each intervention. Comparisons for differences among groups were assessed as an average of the last three days of each treatment (average of day 15, 16, 17) for both SBP (A) and DBP (B) (results below). Baseline was assessed as an average of the first two measurements during each treatment (average of day 1 and 4).

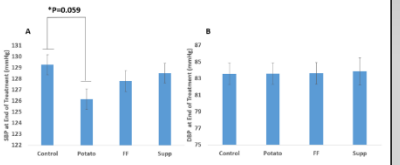


Figure 5. Primary BP Analysis: Comparison of end of treatment systolic (SBP) and diastolic (DBP) blood pressure for all intervention phases (N=30). Changes in systolic blood pressure (A; SBP) and diastolic blood pressure (B; DBP) for all intervention phases (N=30). Results for differences in systolic blood pressure were assessed at the end of treatment (average of day 15, 16, and 17 measurements) using a mixed model with repeated measures with adjustment for baseline BP as a covariate, with no significant differences among periods (control: 129.3 ± 0.9/ 83.5 ± 1.3, potatoes: 126.2 ± 0.93/ 83.6 ± 1.3, French fries: 127.8 ± 0.94/ 83.6 ± 1.3, supplement: 128.5 ± 0.9/ 83.8 ± 1.6; SBP:P=0.07, DBP: P=0.97). However, differences between SBP for control and potato periods approached significance (P=0.059). Means ±SE.

RESULTS AND DISCUSSION

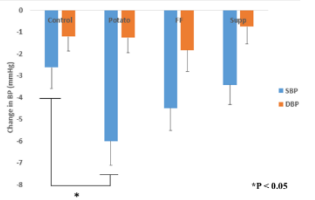


Figure 6. Secondary BP Analysis: Change in BP over time, reduction in SBP was Greater in the Potato vs. Control Group. Looking at change in BP over the course of each intervention, the potato arm resulted in a SBP change of -6.01 mmHg which, after contrast analysis, was significantly lower (P=0.02) when compared to the SBP change in the control group (-2.6 mmHg). Means ±SE.

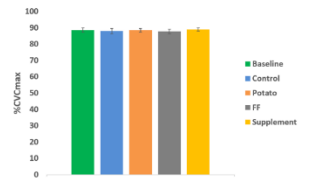


Figure 7. There were no Differences in Microvascular Function Among Groups. There were no significant differences in endothelial function (measured as percent of cutaneous vascular conductance max (%CVCmax) ± SE) among groups (control: 87.8 ± 1.5%, French Fries: 87.7 ± 1.4%, potatoes: 88.5 ± 1.1%, supplement: 88.9 ± 1.0%). Means ±SE.

CONCLUSIONS

- Including boiled/baked potatoes in the diet to increase potassium intake may have some benefit to SBP in mildly cardiometabolic individuals, but this needs to be confirmed in larger trials.
- Consuming potatoes as French fries had no effect on BP.
- There is still much to learn about the effect of dietary potassium, both alone and in the context of the whole diet, on overall health.
- More dietary interventions looking specifically at the effects of increasing potassium from food sources are needed to understand the effect of dietary potassium on BP and other CVD outcomes.

REFERENCES & ACKNOWLEDGMENTS

(1) Chamlers et al., Journal of Hypertension, 4 (suppl 6): S629-S637, 1986.
(2) Berry et al., British Journal of Nutrition, 104, 1839–1847, 2010.

Funding Source: Alliance for Potato Research and Education (APRE).

Appendix D2: ASN Annual Meeting 2019, Baltimore, MD

Short-term Increased Dietary Potassium from Potato and Potassium Gluconate Effect on Potassium Retention in Pre-Hypertensive-to-Hypertensive Adults

Potassium is an essential nutrient of concern according to recent Dietary Guidelines for Americans Advisory Committees. Current recommendations established by the Institute of Medicine (IOM) are given as an Adequate Intake (AI) of 3400 mg/d for men and 2600 mg/d for women. Little is known about potassium (K) tissue retention, less is understood on how retention may affect health outcomes. In this clinical trial, we assessed the effects of K intake from potato sources and a K supplement on overall K balance. Thirty pre-hypertensive-to-hypertensive (Systolic BP ≥ 120mmHg) men (N=5) and Women (N=10) with a mean ± SD age, BMI, and BP (systolic(SBP)/ diastolic(DBP)) of 55.1 ± 11.9 y, 31.5 ± 6.7, and 139.5 ± 14.6/ 86.8 ± 6.7 mmHg, respectively, were enrolled in a cross-over randomized control diet trial. Participants were assigned to a random order of four 16-day dietary K interventions including a basal diet (control) of 2300mg/d(~60mmol/d), and three periods of an additional 1000mg/d(3300mg/d(~85mmol/d) total) of K in the form of potatoes (baked, boiled, or pan-heated with no additional fat), French fries, or a K gluconate supplement. Each intervention period was separated by two or more weeks of wash out. Twenty-four hour urine and stool samples were collected through out each intervention phase, and assessed via ICP-OES for K and sodium (Na) content. Results for differences in mineral balance were assessed as 15-day sums as follows: retention (mg/d) = dietary intake – urine + stool losses. Using a mixed model with repeated measures and follow-up contrast analyses, initial analysis show significant differences ($P > 0.05$) in K mineral retention (± SE) with higher retention during the supplement (1156 ± 201 mg/d) compared to control (615 ± 133 mg/d; P=0.0001), supplement compared to potato (843 ± 144 mg/d; P=0.0406), and FF (932 ± 146 mg/d) compared to control (P=0.0375). Analysis also show Na retention in the potato period (483 ± 217 mg/d) was lower compared to the supplement (903 ± 194 mg/d; P=0.063) and FF periods (859 ± 176 mg/d; P=0.0302). Due to slight nutrient differences in the calculated vs. the chemical analysis of the controlled diets, retention was also assessed as a percent (%) of intake. Using the same statistical methods, there was a significant difference in K mineral retention (± SE) as % of intake with higher retention during the supplement (35.0 ± 6.0

%) compared to control (26.4 ± 5.9 %; P=0.03) intervention periods, as well significantly lower Na retention during the potato period (13.0 ± 6.5 %) compared to supplement (28.0 ± 6.3 %; P=0.03). After initial analysis, there does appear to be some difference in mineral retention due to source, although the significance of this possible difference is still difficult to characterize. These findings may challenge the current dogma that K and Na intake are equivalent to urinary output.

Short-term Increased Dietary Potassium from Potato and Potassium Gluconate Effect on Potassium Retention in Pre-Hypertensive-to-Hypertensive Adults.

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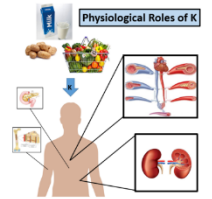
[1]Nutrition Science, Purdue University, West Lafayette, IN

PURDUE UNIVERSITY

ABSTRACT

Potassium is an essential nutrient of concern according to recent Dietary Guidelines for Americans Advisory Committees. Current recommendations established by the Institute of Medicine (IOM) are given as an Adequate Intake (AI) of 3400 mg/d for men and 2600 mg/d for women. Little is known about potassium (K) tissue retention, less is understood on how retention may affect health outcomes. In this clinical trial, we assessed the effects of K intake from potato sources and a K supplement on overall K balance. Thirty pre-hypertensive-to-hypertensive (Systolic BP ≥ 120mmHg) men (N=15) and Women (N=16) with a mean ± SD age, BMI, and BP (systolic[SBP]/ diastolic[DBP]) of 55.1 ± 11.2 y, 31.5 ± 6.7, and 139.5 ± 14.6/ 86.8 ± 6.7 mmHg, respectively, were enrolled in a cross-over randomized controlled-diet trial. Participants were assigned to a random order of four 16-day dietary K interventions including a basal diet (control of 2300mg/d(~60mmol/d), and three periods of an additional 1000mg/d(3300mg/d(~85mmol/d) total) of K in the form of potatoes (baked, boiled, or pan-heated with no additional fat), french fries, or a K gluconate supplement. Each intervention period was separated by two or more weeks of wash out. Twenty-four hour urine and stool samples were collected through out each intervention phase, and assessed via ICP-OES for K and sodium (Na) content. Results for differences in mineral balance were assessed as 15-day sums as follows: retention (mg/d) = dietary intake – urine + stool losses. Using a mixed model with repeated measures and follow-up contrast analyses, initial analysis show significant differences (P < 0.05) in K mineral retention (± SE) with higher retention during the supplement (1156± 201 mg/d) compared to control (615 ± 133 mg/d; P=0.0001), supplement compared to potato (843 ± 144 mg/d; P=0.0406), and FF (932 ± 146 mg/d) compared to control (P=0.0375). Analysis also show Na retention in the potato period (482 ± 217 mg/d) was lower compared to the supplement (902 ± 194 mg/d; P=0.062) and FF periods (859 ± 176 mg/d; P=0.0302). Due to slight nutrient differences in the calculated vs. the chemical analysis of the controlled diets, retention was also assessed as a percent (%) of intake. Using the same statistical methods, there was a significant difference in K mineral retention (± SE) as % of intake with higher retention during the supplement (35.0 ± 6.0 %) compared to control (26.4 ± 5.9 %; P=0.03) intervention periods, as well significantly lower Na retention during the potato period (13.0 ± 6.5 %) compared to supplement (28.0 ± 6.3 %; P=0.03). After initial analysis, there does appear to be some difference in mineral retention due to source, although the significance of this possible difference is still difficult to characterize. These findings may challenge the current dogma that K and Na intake are equivalent to urinary output.

INTRODUCTION



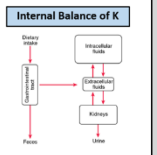


Figure 1.Physiological Roles and K Retention.
Adequate or increased dietary potassium intake has benefit to many physiological systems including the bone, insulin and glucose control, the heart and vasculature, and the kidneys. These two later systems work in concert to regulate arterial pressure and prevent pressure dysregulation, or hypertension (HTN). Little is known about the role of K intake and interaction with other nutrients, including Na on K homeostasis, and its relationship to health.

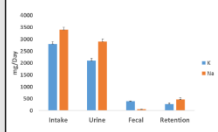
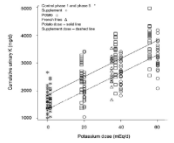


Figure 3. Potassium Bioavailability in Foods vs. Supplements. Thirty-five healthy men and women (29.7 ± 11.2 years, 24.3 ± 4.4 kg/m^2) were randomized to nine, five-day interventions of additional K+ equaling: 0 mEq (control at phase 1 and repeated at phase 5), 20 mEq (1500 mg), 40 mEq (3000 mg), 60 mEq (4500 mg) K+/day consumed as K+ gluconate or potato, and 40 mEq K+/day from French fries. Investigators found increases in serum potassium AUC with increasing dose regardless of source, while potassium 24 h urine concentration also increased with dose but was greater with potato compared to supplement. Stools were not collected to know if retention was affected (2).

Figure 2. Potassium and Na Balance in Normal Dietary Intake. Holbrook et al. (1984) observed K and Na retention in a 1-year dietary intake study conducted in American men and women (N=28, 20-53 years old). Participants were on a self-selected diet, and duplicate samples of all food and beverages were collected 4X, for 2 weeks during the study. Average intake, urinary loss, fecal loss, and retention for Na were 3400mg/d, 2900mg/d, 56mg/d, 470mg/d, respectively, and for K were 2800mg/d, 2100mg/d, 395mg/d, 280mg/d, respectively (1).



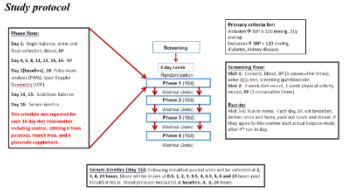
PURPOSE

Compare K retention from an intake of K from baked/boiled potatoes, French fries, and K-gluconate relative to a control diet in mildly hypertensive adults.

MATERIALS AND METHODS

Experimental subjects
Subjects (N=15) were recruited through a variety of different techniques. Subjects were pre-hypertensive-to-hypertensive(SBP >120mmHg) men and women who were otherwise apparently healthy. When eligible subjects completed a 4 day run-in period (described below), continuing subjects were randomized to one of 24 possible sequences of the 4 phases including, control, potato, French fry, and K-gluconate interventions. The study protocol was approved by the Purdue University Institutional Review Board, and all subjects provided written, informed consent.

Study protocol



Dietary Intervention
Each day of the 4 day run-in diet represented one of the four diet phases: control, potato, French fries, supplement. During the run-in, subjects came to the clinical facility each morning, had their BP taken, either stayed to eat breakfast or had all their meals and snacks for the day packed out in a cooler to take with them. After the 4 day run-in period, participants were assigned to a random order of four 16-day dietary potassium interventions including a basal diet [control] of 2300mg/d(~60mmol/d), and three periods of an additional 1000mg/d(3300mg/d(~85mmol/d) total) of potassium in the form of potatoes (baked, boiled, or pan-heated with no additional fat), French fries, or a potassium gluconate supplement. Each intervention period was separated by two or more weeks of wash out. A four-day menu cycle at three calorie levels (1800, 2200, and 2600 kcal/d) was designed to manipulate potassium levels while keeping other micro (e.g. Na, Ca) and macro (e.g. Fat) constant. Subjects picked up study foods approximately every other day (on days 1, 4, 6, 8, 11, 13, 15, 16). For each day of controlled feeding, the subjects recorded their intake by checking off each item listed on daily menu sheets provided for each day. They were also instructed to indicate whether they ate any non-study foods and whether they did not eat all the study foods. The food and beverages were prepared with deionized water and weighed to the nearest one tenth gram on digital scales. Duplicates of each menu cycle day were homogenized and analyzed. Researchers adjusted energy intakes with snack items that contained mostly sugar. Any uneaten items were instructed to be returned and analyzed for mineral content.

Potassium and Sodium Balance
Complete urine and fecal collections were performed in acid washed containers for each 16 day intervention period of the study, on a daily basis. Instructions and all necessary supplies (e.g. containers for urine and stool collections) were provided. Urine was pooled as 24-h samples, which were analyzed daily for creatinine to check compliance. Fecal samples were also pooled for each 24-h period, and completeness of collections was determined by using polyethylene glycol by turbidimetric assay. Balance was determined daily by the following equation:

Daily potassium balance = daily potassium intake - daily potassium excretion (urine and stools).

All 24hr excreta samples were processed and stored in a -20° C freezer for future analyses. Urine was analyzed for creatinine, K, and Na. Stools were analyzed for PEG and minerals. Dietary, urinary, and fecal minerals were measured by ICP-OES (5100 PC; Perkins Elmer, Waltham, MA). Unacidified urine was measured for creatinine by a kinetic modification of Jaffe's colorimetric assay (Cobas Mira Plus; Roche Diagnostic Systems, Nutley, NJ).

Statistical methods
Results for differences between intervention periods in K and Na for urinary loss, fecal loss, and retention (both absolute and as % of intake) were analyzed using a mixed model with repeated measures design. All computations were performed using JMP (SAS) statistical software. Alpha was set at 0.05.

RESULTS AND DISCUSSION

Table 1. Baseline Characteristics of the Study Subjects

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	Age (yr)	Ht (cm)	Wt (kg)	BMI (kg/m2)	SBP (mmHg)	DBP (mmHg)
Mean (N=15)	55.1	168.6	89.9	31.5	139.5	86.8
SD	11.9	7.7	22.5	6.7	14.6	6.7

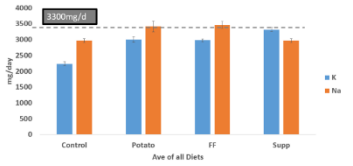

Figure 4. Chemical Analysis of Controlled Diets. There was some unexpected variation in both K and Na content of the diets. Potassium content for the intervention periods of control, potato, French Fry(FF), and Supplement were 2238 ± 43, 3008 ± 29, 2977 ± 4, and 3282 ± 43 mg/d, respectively. The K levels for the control and supplement periods were close to the target of 2300 and 3300 mg/d, respectively, while the potato and FF were slightly lower at approximately 3000 mg/d compared to the 3300 mg/d target. For Na, the target for all four intervention periods was set at 3300 mg/d, however analysis showed Na content to be slightly higher for the potato (3417 ± 77 mg/d) and FF (3466 ± 112 mg/d), and slightly lower for the control and supplement periods (2974 ± 60 mg/d). Means ±SE.

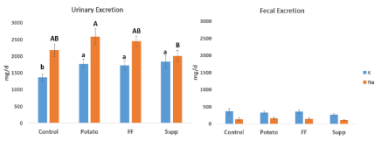

Figure 5. Urinary (N=14) and Fecal (N=15) Excretion of K and Na. Differences in urinary and fecal mineral loss were assessed using a mixed-model with repeated measures. For urinary excretion, there were significant differences between the three increased K intervention periods (potato (P=0.0034), FF(P=0.0107), and supplement(P=0.0061)) and the control period, with losses of, 1774 ± 145, 1728 ± 129, 1833 ± 176 compared to 1364 ± 107 mg/d, respectively. There were no significant differences in fecal excretion with losses of 367 ± 72, 334 ± 46, 355 ± 72, 272 ± 28 mg/d, for control, potato, FF, and supplement intervention periods. Different letters are significantly different from each other (P<0.05). Means ±SE.

RESULTS AND DISCUSSION

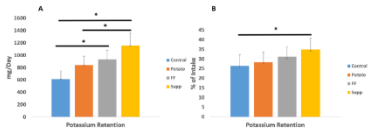

Figure 6. Differences in Potassium Retention in Hypertensives on a Controlled Diet. Differences in K retention were assessed as absolute (A) and % of intake (B), using a mixed-model with repeated measures with contrast analysis follow-up. There was significantly higher retention during the supplement intervention (1156 ± 201 mg/d) compared to control (615 ± 133 mg/d; P=0.0001), supplement compared to potato (843 ± 144 mg/d; P=0.0406), and FF (932 ± 146 mg/d) compared to control (P=0.0375). There was a significant difference in K mineral retention as % of intake with higher retention during the supplement (35.0 ± 6.0 %) compared to control (26.4 ± 5.9 %; P=0.03) intervention periods. *(P<0.05). Means ±SE.

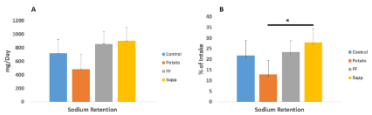

Figure 7. Differences in Sodium Retention in Hypertensives on a Controlled Diet. Differences in Na retention were assessed as absolute (A) and % of intake (B), using a mixed-model with repeated measures. The was significantly lower retention in the potato intervention (482.91 ± 217mg/d) compared to the supplement (902.98 ± 194mg/d; P=0.063) and FF periods (859.25 ± 176mg/d; P=0.0302). As % of intake Na retention was significantly lower during the potato period (13.0 ± 6.5 %) compared to supplement (28.0 ± 6.3 %; P=0.03). *(P<0.05). Means ±SE.

CONCLUSIONS

- Higher K intake regardless of source resulted in higher K retention.
- Increased intake of K via baked/boiled potatoes resulted in decreased Na retention compared to a similar K intake from a different source.
- There is still much to learn about the effect of dietary K, both alone and in the context of the whole diet, on overall health.
- More dietary interventions looking specifically at the effects of increasing K from food sources are needed to understand the effects of K and Na retention.

REFERENCES & ACKNOWLEDGMENTS

[1] Holbrook et al., Am J Clin Nutr, 40:786-793, 1984.
[2] Macdonald-Clarke, C. J., Am J Clin Nutr, 2016;104:346–53

Funding Source: Alliance for Potato Research and Education (APRE).
ClinicalTrials.gov #: NCT02697708

Appendix D3: ASBMR Annual Meeting 2019, Orlando, FL

Short-term Supplemental Dietary Potassium from Potato and Potassium Gluconate Effect on Calcium Retention and Urinary pH in Pre-Hypertensive-to-Hypertensive Adults

Potassium (K) is an essential nutrient of concern according to recent Dietary Guidelines for Americans Advisory Committees. Observational studies show a consistent bone benefit with increased, K rich, fruit and vegetable intakes in cohorts spanning adolescents to the elderly. In clinical trials, higher K intakes through supplementation have been associated with reduced urinary calcium (Ca) excretion and improvement in Ca balance. Clinical dietary intervention trials assessing the impact of K on bone are lacking. In this clinical intervention, we assessed the effects of K intake from potato sources and a K supplement on urinary Ca, urine pH, and overall Ca balance. Thirty pre-hypertensive-to-hypertensive (Systolic BP ≥ 120mmHg) men (N=15) and Women (N=15) with a mean ± SD age, BMI, and BP (systolic(SBP)/ diastolic(DBP)) of 48.2 ± 15 y, 31.4 ± 6.1, and 136.3 ± 11.9/ 86.1 ± 7.1 mmHg, respectively, were enrolled in a cross-over randomized control diet trial. Participants were assigned to a random order of four 16-day dietary K interventions including a basal diet (control) of 2300mg/d(~60mmol/d), and three periods of an additional 1000mg/d(3300mg/d(~85mmol/d) total) of K in the form of potatoes (baked, boiled, or pan-heated with no additional fat), French fries, or a K gluconate supplement. Calcium intake for all diets was between approximately 700-800mg/d. Each intervention period was separated by two or more weeks of wash out. Twenty-four-hour urine and stool samples were collected throughout each intervention phase and assessed via ICP-OES for Ca content. Results for differences in mineral balance were assessed as 15-day sums as follows: retention = dietary intake – urine + stool losses. Using a mixed model ANOVA with repeated measures there was a significantly lower urinary Ca excretion between the supplement intervention period (100.4 ± 9.3 mg/d) compared to the potato (115.2 ± 10.4 mg/d; P=0.033) and the control (116.6 ± 9.9 mg/d; P=0.027). In addition, there was a significant difference in urinary pH between the control and supplement intervention periods (6.08 ± 0.18 vs. 6.54 ± 0.16; P=0.0036). However, there were no significant differences (P > 0.05) in Ca retention (mg/d) (control: 160.5 ± 58.1, French fries: 186.2 ± 57.1, potatoes: 162.5 ± 55.2, supplement: 260.5 ± 45.6; P=0.26. Mean ± SE). Although

results show no difference in Ca metabolism due to K amount or diet type, an increased K intake via K-gluconate supplementation may favorably influence urinary pH and acid-base balance.

Short-term Supplemental Dietary Potassium from Potato and Potassium Gluconate Effect on Calcium Retention and Urinary pH in Pre-Hypertensive-to-Hypertensive Adults

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[1]Nutrition Science, Purdue University, West Lafayette, IN

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ABSTRACT

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INTRODUCTION

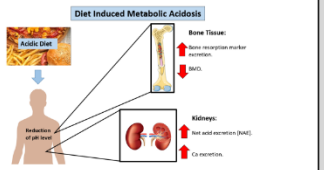


Figure 1. The Acid-Ash Hypothesis and Potassium.
The acid-ash hypothesis proposes that a diet higher in meats and cereal grains, precursors to acid metabolites (e.g. phosphates, sulfates), causes chronic acidemia leading to the dissolution of bone tissue. This leads to hypercalciuria, and if bone loss were occurring an overall decrease in Ca retention. In contrast, the consumption of fruits and vegetables, and other food sources or supplements that are more basic (e.g. K, Ca), or provide other alkalizing organic molecules (e.g. bicarbonate) would decrease urinary Ca excretion and have a protective effect on bone

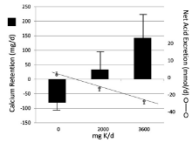
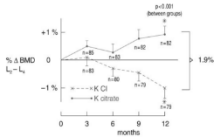


Figure 2. Potassium Supplementation Improves Ca Balance. In a randomized double-blind placebo-controlled study, men and women (n= 52) randomly assigned to six months of 60 or 90 mmol/d of K citrate supplementation had decreases in urinary Ca and net acid excretion resulting in positive Ca balance in the group on the highest dose (90 mmol/d) compared to control (0 mmol/d). Serum C-telopeptide, a marker of bone turnover, also decreased significantly in both K groups. (1).

Figure 3. Potassium may benefit BMD and be Dose/Form Dependent. In a randomized, prospective controlled trial Jehle and colleagues investigated the effect of 30mmol/d of K chloride (KCl) vs. K-citrate on spine (L2-L4) and hip BMD in postmenopausal women (N=161, 58.6 ± 4.8y) for 12 months. Women taking K-citrate showed significant increases in spine BMD from baseline at 3, 9, and 12 months, reaching an increase of 0.89% (P < 0.05) at month 12, whereas women in the KCl group showed a decreased spine BMD of -0.98% (P < 0.05) (2).



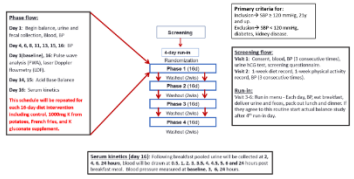
PURPOSE

Compare Ca retention from an intake of K from baked/boiled potatoes, French fries, and K-gluconate relative to a control diet in mildly hypertensive adults.

MATERIALS AND METHODS

Experimental subjects
Subjects (N=30, N=20 for fecal excretion and retention) were recruited through a variety of different techniques. Subjects were pre-hypertensive-to-hypertensive (SBP >120mmHg) men and women who were otherwise apparently healthy. When eligible subjects completed a 4-day run-in period (described below), continuing subjects were randomized to one of 24 possible sequences of the 4 phases including, control, potato, French fry, and K-gluconate interventions. The study protocol was approved by the Purdue University Institutional Review Board, and all subjects provided written, informed consent.

Study protocol



Dietary Intervention
Each day of the 4-day run-in diet represented one of the four diet phases: control, potato, French fries, supplement. During the run-in, subjects came to the clinical facility each morning, had their BP taken, either stayed to eat breakfast or had all their meals and snacks for the day packed out in a cooler to take with them. After the run-in period, participants were assigned to a random order of four 16-day dietary K interventions including a basal diet (control) of 2300mg/d (~60mmol/d), and three periods of an additional 1000mg/d(3300mg/d(~85mmol/d) total) of K in the form of potatoes (baked, boiled, or pan-heated with no additional fat), French fries, or a K-gluconate supplement. Each intervention period was separated by two or more weeks of wash out. A 4-day menu cycle at three calorie levels (1800, 2200, and 2600 kcal/d) was designed to manipulate K levels while keeping other micro (e.g. Na, Ca) and macro (e.g. fat, protein, CHO) constant. Each menu was designed to include approximately 800 mg/d of Ca. Subjects picked up study foods approximately every other day (on days 1, 4, 6, 8, 11, 13, 15, 16). The food and beverages were prepared with deionized water and weighed to the nearest one tenth gram on digital scales. Duplicates of each menu cycle day were homogenized and analyzed. Potassium gluconate supplements were sent out to a food chemistry lab for analysis (Eurofins Food Chemistry Testing US, Inc.). Researchers adjusted energy intakes with snack items that contained mostly sugar. Any uneaten items were instructed to be returned and analyzed for mineral content.

Calcium Balance and Net Absorption
Complete urine and fecal collections were performed in acid washed containers for each 16-day intervention period of the study, daily. Urine was pooled as 24-hr samples, which were analyzed daily for creatinine to check compliance. Urinary pH was assessed on 24-hr samples collected on days 14 and 15 of each intervention period using a benchtop pH meter (OAKTON Instruments P.O. Box 5138, Vernon Hills, Il USA). Fecal samples were also pooled for each 24-h period, and completeness of collections was determined by using polyethylene glycol (PEG) by turbidimetric assay. After a Ca intake equilibration period of the first 7 days and exclusion of day 16, which was an in-clinic testing day, a 8-day period was assessed for Ca excretion and retention for each intervention period. Dietary, urinary, and fecal minerals were measured by ICP-OES (5100 PC; Perkins Elmer, Waltham, MA). Balance and % absorption were determined daily by sums using the following equations:

Daily calcium balance (mg/d) = daily calcium intake (mg/d) - daily calcium excretion (mg/d) (urine and stools).

% Net absorption = daily calcium intake (mg/d) - daily calcium fecal excretion (mg/d) x 100.

Statistical methods
Results for differences between intervention periods in Ca for urinary loss, fecal loss, urine pH, and retention (both absolute and as % of intake), were analyzed using a mixed model ANOVA with repeated measures design. All computations were performed using JMP (SAS Institute, Cary, NC, USA) statistical software. Alpha was set at 0.05.

RESULTS AND DISCUSSION

Table 1. Baseline Characteristics of the Study Subjects

	All Mean (SD)	Male Mean (SD)	Female Mean (SD)
n	30	15	15
Age (y)	48.2 (15.0)	43.8 (13.7)	52.7 (15.4)
Height (cm)	172.2 (10.2)	179.4 (7.1)	165.0 (7.2)
Weight (kg)	93.86 (22.9)	99.1 (20.7)	88.6 (24.4)
BMI	31.4 (6.1)	30.5 (4.8)	32.3 (7.2)
Systolic Blood Pressure (mmHg)	136.3(11.9)	134.6(12.7)	138.0(11.3)
Diastolic Blood Pressure (mmHg)	86.12(7.1)	85.9(7.0)	86.4(7.4)

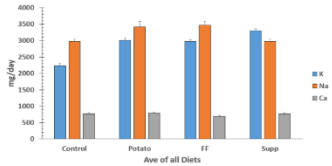


Figure 4. Chemical Analysis of Controlled Diets. There was some unexpected variation in K, Na, and Ca content of the diets. Potassium content for the intervention periods of control, potato, French Fry (FF), and Supplement were 2238 ± 45, 3008 ± 4, 2977 ± 31, and 3299 ± 43 mg/d, respectively. Target K levels were 2300 mg/d for the control, and 3300 mg/d for all intervention arms. Sodium levels were set at 3300 mg/d for all diets, however analysis showed Na content to be slightly higher for the potato (3417 ± 83 mg/d) and FF (3466 ± 117 mg/d), and slightly lower for the control and supplement periods (2974 ± 61 mg/d). For Ca, the target for all four study periods was set at 800 mg/d, however analysis showed Ca content to be slightly lower for the control and supplement diets (765 ± 29 mg/d), as well as the FF period (684 ± 30 mg/d). The potato diet did achieve approximately 800mg/d of Ca (799 ± 22 mg/d). Means ±SE.

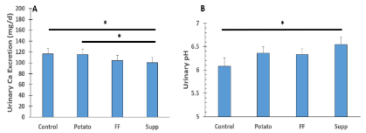


Figure 5. Urinary Ca Excretion (N=28) and pH (N=30). There were significant differences among groups in urinary Ca excretion (A; P = 0.009), the K-gluconate (100.4 ± 9.3 mg/d) was significantly lower compared to the control (116.6 ± 9.9 mg/d; P = 0.027) and potato (115.1 ± 10.4 mg/d; P = 0.033). There were also significant differences between intervention periods in urinary pH (B; control: 6.08 ± 0.2, potatoes: 6.36 ± 0.14, French fries: 6.33 ± 0.12, supplement: 6.54 ± 0.2; P = 0.008), with the K-gluconate phase resulting in a higher urinary pH compared to control (6.08 ± 0.18 vs. 6.54 ± 0.16; P=0.0036). Means ±SE.

RESULTS AND DISCUSSION

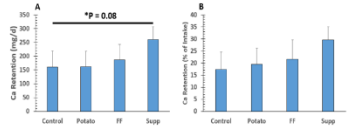


Figure 6. Differences in Calcium Retention (N=20).
Differences in Ca retention were assessed as absolute and % of intake. There were no significant differences among groups for either absolute retention (control: 160.5 ± 58.1, potatoes: 162.5 ± 55.2, French fries: 186.2 ± 57.1, supplement: 260.5 ± 45.6; P = 0.26) of retention based on percent of intake (control: 17.4 ± 7.3%, potatoes: 19.4 ± 6.8%, French fries: 21.6 ± 8.0%, supplement: 29.6 ± 5.4%; P = 0.37). However, Results seem to indicate that there was higher Ca retention (absolute) between K-gluconate vs. the control (260.5 vs. 160.5 mg/d), which approached significance after post hoc contrast analysis (P = 0.08). Means ±SE.

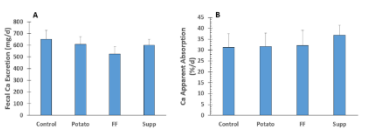


Figure 7. Differences in Ca Fecal Excretion and % Absorption (N=20).
There were no significant differences (P = 0.19) in fecal Ca excretion with losses of 650.6 ± 77.9, 606.9 ± 66.5, 523.1 ± 62.3, 599.6 ± 53.0 mg/d, for control, potato, FF, and supplement intervention periods. There were also no significant differences among groups for percent Ca absorption (control: 31.2 ± 6.3%, potatoes: 31.6 ± 6.2%, French fries: 32.0 ± 7.1%, supplement: 36.8 ± 4.5%; P = 0.84). Means ±SE.

CONCLUSIONS

- Higher K intake from a K-gluconate supplement resulted in lower urinary Ca and pH.
- Higher K from K-gluconate also seemed to benefit Ca retention over control, though was not a statistically significant finding(P = 0.08).
- There is still much to learn about the effect of dietary K, both alone and in the context of the whole diet, on overall health.
- More dietary interventions looking specifically at the effects of increasing K from food sources are needed to understand the effects on Ca economy and bone.

REFERENCES & ACKNOWLEDGMENTS

(1) Moseley, K.F., et al., J Bone Miner Res, 2013. 28(3): 497-504.
(2) Jehle et al, J Clin Endocrinol Metab, 2013. 98:207–17.

Funding Source: Alliance for Potato Research and Education (APRE).
ClinicalTrials.gov #: NCT02697708

APPENDIX E: BLOOD PRESSURE MEASUREMENT SOP

Protocol Adapted from current AHA guidelines for blood pressure measurement:
Muntner, P., et al., *Measurement of Blood Pressure in Humans: A Scientific Statement From the American Heart Association.* Hypertension, 2019. **73**(5): p. e35-e66.

Arm Measurement and Cuff Sizes

The proper cuff size must be used to avoid under- or over-estimating the correct blood pressure. To determine the proper cuff size, the observer must measure the arm circumference at the midpoint of the arm at each visit.

This measurement is taken on the right arm which has been bared from the shoulder.

With the participant standing, holding the forearm horizontal (90 degree angle), the arm length is measured from the acromion (or boney extremity of the shoulder girdle) to the olecranon (or tip of the elbow) with the Gullick II metric tape. The midpoint along with the acromion bone is marked with a washable marker.

The participant should then relax the arm straight down along the side of the body. The arm circumference is measured by drawing the tape snugly around the arm at the level of the midpoint marking. Care must be taken to keep the tape horizontal. Also, the tape should not indent the skin.

The chart of arm circumference measurements and corresponding cuff sizes (shown below) is consulted, and the indicated cuff size is checked on the study form and used.

The markings found on most blood pressure cuffs can be used for reference.

Arm Circumference Cuff Size (cm)

<24.0 cm Child, Pediatric, Small Adult
24.0 to < 33.0 cm Adult, Regular
33.0 to 41.0 cm Large Adult
>41.0 cm Thigh, Extra Large

Application of the Blood Pressure Cuff

A McKesson Aneroid Sphygmomanometer is always used. Use the same size cuff for every measure within a participant.

The participant should be seated with the elbow and forearm resting comfortably on a table with the palm of the hand turned upward. The area to which the cuff is to be applied must be bare.

Fold the cuff bladder in half mating each corner of the bladder to find and mark the midpoint on the cuff cover. Do not use the permanent marking on the cuff because it may become dislocated and off center.

The brachial artery is located by palpation and marked. Place the appropriate cuff (as determined in the arm measurement procedure) around the upper left arm so that the midpoint of the length of the bladder lies over the brachial artery and the mid-height of the cuff is at heart level.

The lower edge of the cuff, with its tubing connections, should be placed about 1 inch above the natural crease across the inner aspect of the elbow.

The cuff is wrapped snugly around the arm, with the palm of the participant's hand turned upward.

The wrapped cuff should be secured firmly by applying pressure to the locking fabric fastener over the area where it is applied to the cuff. You should not be able to fit more than two fingers under the wrapped cuff.

Stethoscope

A standard Littman stethoscope (or other comparable stethoscope) with a bell should be used. Korotkoff sounds are best heard with the bell because of their low pitch. Stethoscope tubing should be about 12 to 15 inches from the bell piece to "Y" branching. This length provides optimal acoustical properties and allows the observer to read the sphygmomanometer at eye level and in a comfortable position. Earpieces should fit comfortably and snugly in the ears. Four points should be observed in using the stethoscope.

The ear pieces should be directed forwards into the external ear canal.

The ear pieces should be tight enough to exclude outside sound but not so tight that they cause discomfort.

The valve between the bell and the diaphragm should be turned in the direction of the diaphragm.

The diaphragm of the stethoscope should be placed lightly on the skin overlying the brachial artery - immediately below, but not touching, the cuff. The brachial artery is usually found above the crease of the arm, slightly towards the body. Light pressure accentuates low-pitched sound and avoids compression murmurs. Pressing too heavily with the stethoscope over the brachial artery causes turbulent flow in the artery and a murmur can be heard which may prolong the apparent duration of fourth-phase Korotkoff sounds.

Blood Pressure Measurement

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Criteria for Systolic and Diastolic Blood Pressure

To correctly identify the 1st-phase (systolic) and 5th-phase (diastolic) Korotkoff values, the observer must listen carefully via the stethoscope while reading and interpreting the aneroid dial.

The systolic value can be identified as the pressure level where the first of 2 or more consecutive beats are heard in appropriate rhythm.

The diastolic value can be identified as the pressure level where the last of two consecutive beats heard.

The aneroid dial should be made to drop at 2-3 mm Hg per second, from the maximum pressure until 10 mm Hg below that of the last regular sound heard.

The control of the deflation rate is essential for accurate readings and depends on handling of the bulb and its control valve.

NOTE: A single sound heard in isolation (i.e., not in rhythmic sequence) before the first of the rhythmic sounds (systolic) or following the last of the rhythmic sounds (diastolic) does not alter the interpretation of the blood pressure.

Measuring Blood Pressure with McKesson Aneroid Device

The participant should refrain from caffeine, smoking, and exercise at least one half hour prior to and until completion of blood pressure measurement.

The elbow and forearm should rest comfortably on the table.

The seated arm blood pressure is measured three times at each clinic visit.

Blood pressure equipment should be checked prior to seeing the participant. Once a participant is given instructions and explanations, blood pressure measurement begins.

Next, the observer should proceed to carry out the first blood pressure reading. Detailed instructions are given below for measuring blood pressure.

Wait at least 30 seconds after complete deflation of the cuff following any preceding inflation.

Place the ear pieces of the stethoscope into the ears, with the tips turned forward.

Apply the diaphragm of the stethoscope over the brachial artery, just below but not touching the cuff or tubing. The brachial artery is usually found at the crease of the arm, slightly toward the body.

Rapidly inflate the cuff to 160-180 mm/Hg. The eyes of the observer should be focused on the dial of the aneroid sphygmomanometer.

By slightly adjusting the valve, deflate and maintain a constant rate of deflation at approximately 2-3 mm Hg per second. Allow the cuff to deflate, listening throughout the entire range of deflation, from the maximum pressure past the systolic reading (the pressure where the first of two consecutive beats is heard), until 10 mm Hg below the level of the diastolic reading (that is, 10 mm Hg below the level where the last of two consecutive beats is heard).

Open the valve to deflate fully.

Record the systolic and diastolic reading.

Repeat two more times, waiting at least 30 seconds after complete deflation of the cuff following any preceding inflation. These are the Second and Third Blood Pressure Values.

NOTE: Any marked irregularity observed during this period should be called to the attention of the Principal Investigator.

Forgotten Blood Pressure Readings

If for any reason the observer is unable or has forgotten to complete any portion of the exam, and the participant is gone, leave the items blank on the paper form. If a blood pressure value is missed or forgotten, completely deflate the cuff and start over with a replacement reading after the proper interval.

Do not re-inflate the blood pressure cuff during a reading. However, under no other circumstances may a replacement reading be obtained. Do not repeat a reading that looks unusual to you.

Reporting the Blood Pressure Results to the Participant

The participant may wish to know his or her results before the form is entered into the database. If so, average the second and third readings and give the results to the participant. State clearly the systolic and diastolic pressures and offer to write down these values for the participant.

APPENDIX F: EXAMPLE OF JMP SCRIPTS FOR PRIMARY AND SECONDARY OUTCOMES

Systolic Blood Pressure (SBP_End):

```
Fit Model(
	Y( :SBP_End ),
	Effects( :Intervention, :Baseline SBP ),
	Random Effects( :Subject ),
	NoBounds( 1 ),
	Personality( "Standard Least Squares" ),
	Method( "REML" ),
	Emphasis( "Minimal Report" ),
	Run(
		:SBP_End << {Summary of Fit( 1 ), Analysis of Variance( 0 ),
		Parameter Estimates( 1 ), Lack of Fit( 0 ), Scaled Estimates( 0 ),
		Plot Actual by Predicted( 0 ), Plot Regression( 0 ),
		Plot Residual by Predicted( 0 ), Plot Studentized Residuals( 0 ),
		Plot Effect Leverage( 0 ), Plot Residual by Normal Quantiles( 0 ),
		{:Intervention << {LSMeans Tukey HSD(
			0.05,
			Ordered Differences Report( 1 )
		)}}}
	),
	SendToReport(
		Dispatch( {"Response SBP_End"}, "Effect Details", OutlineBox, {Close( 0
)} )
	)
);
```

Diastolic Blood Pressure (DBP_End):

```
Fit Model(
	Y( :DBP_End ),
	Effects( :Intervention, :Baseline DBP ),
	Random Effects( :Subject ),
	NoBounds( 1 ),
	Personality( "Standard Least Squares" ),
	Method( "REML" ),
	Emphasis( "Minimal Report" ),
	Run(
		:DBP_End << {Summary of Fit( 1 ), Analysis of Variance( 0 ),
		Parameter Estimates( 1 ), Lack of Fit( 0 ), Plot Actual by Predicted( 0
),
		Plot Regression( 0 ), Plot Residual by Predicted( 0 ),
		Plot Studentized Residuals( 0 ), Plot Effect Leverage( 0 )}
	)
);
```

Potassium Balance (Kbal):

```
Fit Model(
	Y( :Kbal ),
	Effects( :Intervention ),
	Random Effects( :Subject ),
	NoBounds( 1 ),
	Personality( "Standard Least Squares" ),
	Method( "REML" ),
	Emphasis( "Minimal Report" ),
	Run(
		:Kbal << {Summary of Fit( 1 ), Analysis of Variance( 0 ),
		Parameter Estimates( 1 ), Lack of Fit( 0 ), Plot Actual by Predicted( 0
),
		Plot Regression( 0 ), Plot Residual by Predicted( 0 ),
		Plot Studentized Residuals( 0 ), Plot Effect Leverage( 0 ),
{:Intervention
		 << {LSMeans Tukey HSD( 0.05, Ordered Differences Report( 1 ) )}}}
	),
	SendToReport(
		Dispatch( {"Response Kbal"}, "Effect Details", OutlineBox, {Close( 0 )}
)
	)
);
```

Sodium Balance (Nabal):

```
Fit Model(
	Y( :Nabal ),
	Effects( :Intervention ),
	Random Effects( :Subject ),
	NoBounds( 1 ),
	Personality( "Standard Least Squares" ),
	Method( "REML" ),
	Emphasis( "Minimal Report" ),
	Run(
		:Nabal << {Summary of Fit( 1 ), Analysis of Variance( 0 ),
		Parameter Estimates( 1 ), Lack of Fit( 0 ), Plot Actual by Predicted( 0
),
		Plot Regression( 0 ), Plot Residual by Predicted( 0 ),
		Plot Studentized Residuals( 0 ), Plot Effect Leverage( 0 ),
{:Intervention
		 << {LSMeans Tukey HSD( 0.05, Ordered Differences Report( 1 ) )}}}
	),
	SendToReport(
		Dispatch( {"Response Nabal"}, "Effect Details", OutlineBox, {Close( 0 )}
)
	)
);
```

Calcium Balance (Ca balance 8d):

```
Fit Model(
	Y( :Ca balance 8d ),
	Effects( :Intervention ),
	Random Effects( :Subject ),
	NoBounds( 1 ),
	Personality( "Standard Least Squares" ),
	Method( "REML" ),
	Emphasis( "Minimal Report" ),
	Run(
		:Ca balance 8d << {Summary of Fit( 1 ), Analysis of Variance( 0 ),
		Parameter Estimates( 1 ), Lack of Fit( 0 ), Plot Actual by Predicted( 0
),
		Plot Regression( 0 ), Plot Residual by Predicted( 0 ),
		Plot Studentized Residuals( 0 ), Plot Effect Leverage( 0 )}
	)
);
```

VITA

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EDUCATION

2019(expected) Nutrition Science, PhD, Department of Nutrition Science, College of Health and Human Sciences, Purdue University, West Lafayette, IN. Expected December 2019. GPA: 3.76.

2013 Nutrition Science, MS, Department of Exercise and Nutritional Sciences, College of Health and Human Services, San Diego State University, San Diego, CA. Completed December 2013. GPA: 3.80.

2013 Exercise Physiology, MS, Department of Exercise and Nutritional Sciences, College of Health and Human Services, San Diego State University, San Diego, CA. Completed December 2013. GPA: 3.80.

2012 Didactic Program In Dietetics, Department of Exercise and Nutritional Sciences, College of Health and Human Services, San Diego State University, San Diego, CA. Completed December 2012.

2007 Kinesiology, BS, Department of Exercise and Nutritional Sciences, College of Professional Arts and Sciences, San Diego State University, San Diego, CA. Cum Laude. Concentration: Fitness, Nutrition, and Health.

RESEARCH INTERESTS:

My long-term research goals are to explore and understand the relationship between micronutrients and various biological and physiological stresses (e.g. CVD, hypertension, osteoporosis, etc.) via clinical and epidemiological studies, which could facilitate an impact at the population level. I am also interested in examining how some physiological stresses (e.g. physical activity, exercise) may act synergistically and paradoxically with proper nutrition/nutrients to attenuate degenerative disease progression.

PROFESSIONAL EXPERIENCE

2014-present Research Assistant, Department of Nutrition Science, Purdue University, West Lafayette, IN.

2014-2016 Teaching Assistant, Course: Food Chemistry, Department of Nutrition Science, Purdue University, West Lafayette, IN.

2014 Research Physiologist, Bio-behavioral Lab, Naval Health Research Center, San Diego, CA.

2014 Faculty Lecturer, Advanced Nutrition Lab, Department of Exercise and Nutritional Sciences, San Diego State University, San Diego, CA.

2012- 2013 Teaching Assistant, Course: Exercise Physiology Lab, Department of Exercise and Nutritional Sciences, San Diego State University, San Diego, CA.

2011- 2013 Research Assistant, San Diego State University, Department of Exercise and Nutritional Sciences, San Diego, CA.

2011- 2012 Nutrition Consultant, UC San Diego, Santec, Inc., ISCHEMIA Trial, San Diego, CA.

2004- 2012 Inventory Technician/Logistics/Shipping and Receiving, Nordstrom: Fashion Valley, San Diego, CA. Full-time position.

RESEARCH PROJECTS

2016-present *The Effect of Potatoes on Potassium Retention, Acid Base Balance, and Blood Pressure.*
Principal Investigator: Dr. Connie Weaver, Ph.D.
Role: Co-Investigator, Data Collection, Entry, Analysis, Manuscript writing and publication submission.

2015-present *The Effect Of Oral Vs Non-Oral Contraceptive Therapy On Bone Turnover Using Ca^{41} Methodology.*
Principal Investigator: Dr. Connie Weaver, Ph.D., Dr. Mary Jane De Souza, Ph.D.
Role: Co-Investigator, Data Collection, Entry, Analysis, Manuscript writing and publication submission.

2014-2016 *Primary Mechanisms Underlying the Effects of Oral vs Non-oral Contraceptives on GH/IGF-1 Axis and Bone Metabolism in Young Women.*
Principal Investigators: Dr. Connie Weaver, Ph.D., Dr. Mary Jane De Souza, Ph.D.
Role: Co-Investigator, Data Collection, Entry, Analysis, Manuscript writing and publication submission.

2014 *Sex Differences in Allostatic Load.*
Principal Investigator: Dr. Marcus K. Taylor, Ph.D.
Role: Co-Investigator, Data Collection, Entry, Analysis, Manuscript writing and publication submission.

2013 *The Effect of Exercise Intensity on the Sweat Amino Acid Concentration and the Sweat Amino Acid Excretion Rate.*
Principal Investigators: Dr. Mark Kern, Ph.D., Dr. Michael J. Buono, Ph.D.
Role: Co-Investigator, Data Collection, Entry, Analysis, Manuscript writing and publication submission.

2011-2013 *Leaching from the Stratum corneum cannot explain the previously reported elevated potassium ion concentration in sweat*.
Principal Investigator: Dr. Michael J. Buono, Ph.D.
Role: Co-Investigator, Data Collection, Entry, Analysis, Manuscript writing and publication submission.

PUBLICATIONS

Lobene AJ, McCabe LD, **Stone MS**, Kindler JM, Bailey RL, Mosfegh AJ, Rhodes DG, Goldman JD, McCabe GP, Weaver CM. Ch. 6 Dietary minerals, mineral ratios, and bone. In: Nutritional Influences of Bone Health. International Congress Series Proceedings of the 10th International Symposium on Nutritional Aspects of Osteoporosis, Hong Kong. Weaver CM, Bischoff-Ferrari H, Daly, R, Wong M-S, Eds. Springer, Pg. 53-67, 2018.

Weaver CM, **Stone MS**, Lobene AJ, Cladis DP, Hodges JK. What is the evidence base for a potassium requirement? Nutrition Today, 2018, 53(5): 184-195.

Taylor, M.K., Hernández, L.M., Kviatkovsky, S.A., Schoenherr, **M., Stone**, M., Sargent, P. The "Yin and Yang" of hypothalamic-pituitary-adrenal and hypothalamic-pituitary-gonadal systems in elite military men. Stress, 2017, 20(3):258-264.

Stone MS, Martyn L, Weaver CM. Potassium Intake, Bioavailability, Hypertension, and Glucose Control. Nutrients. 2016 Jul 22;8(7). pii: E444. doi: 10.3390/nu8070444. Review. PubMed PMID: 27455317.

Buono MJ, **Stone M**, Cannon DT. Leaching from the stratum corneum does not explain the previously reported elevated potassium ion concentration in sweat. J Basic Clin Physiol Pharmacol. 2016 Mar;27(2):171-3. doi: 10.1515/jbcpp-2015-0097.

Taylor MK, Carpenter J, **Stone M**, Hernandez LM, Rauh MJ, Laurent HK, Granger DA. Genetic and environmental modulation of neurotrophic and anabolic stress response: Counterbalancing forces. Physiol Behav. 2015 Nov 1;151:1-8. doi: 10.1016/j.physbeh.2015.06.027. Epub 2015 Jun 28.

Taylor MK, **Stone M**, Laurent HK, Rauh MJ, Granger DA. Neuroprotective-neurotrophic effect of endogenous dehydroepiandrosterone sulfate during intense stress exposure. Steroids. 2014 Sep;87:54-8. doi: 10.1016/j.steroids.2014.05.011. Epub 2014 Jun 2.

Submitted

Allaway, Heather CM, Misra, Madhusmita, **Stone, Michael S**, Weaver, Connie M, Petkus, Dylan L, Southmayd, Emily A, De Souza, Mary Jane. Oral and vaginal contraceptive use decrease rhGH stimulated hepatic IGF-1 secretion and circulating P1NP in healthy, young women.

Stone MS, Weaver CM. Chapter 1. Improving Human Nutrition: A Critical Objective for Potassium Recommendations for Agricultural Crops. International Plant Nutrition Institute.

In Preparation

Stone MS, Martin BR, McCabe LD, McCabe GP, Weaver CM. Effect of Short-term Increased Dietary Potassium from Potato and Potassium Gluconate on Blood Pressure and Microcirculation in Pre-Hypertensive-to-Hypertensive Adults.

Stone MS, Martin BR, Weaver CM. Short-term Supplemental Dietary Potassium from Potato and Potassium Gluconate Effect on Potassium Retention in Pre-Hypertensive-to-Hypertensive Adults.

NON-PEER REVIEWED PUBLICAITONS

Stone, MS, Weaver, CM. Potassium Intake, Metabolism, and Hypertension. *SCAN's Pulse* 2018. 37: 5-10.

Stone, M., Kern, M. Nutritional Considerations for Dementia and Alzheimer 's disease. *SCAN's Pulse* 2013. 32: 13-15.

PRESENTATIONS

Oral:
Stone, M.S., Weaver, C.M., Potassium and Human Health, Frontiers of Potassium Science Conference, International Plant Nutrition Institute. January 2017, Rome, Italy.

Stone, M.S., Buono, M.J., Kern, M.J., SDSU Student Research Symposium 2014, San Diego, CA: The effect of exercise intensity on sweat amino acid excretion. March 2014.

Poster:
Stone MS, Martin BR, Weaver CM. Short-term Supplemental Dietary Potassium from Potato and Potassium Gluconate Effect on Calcium Retention and Urinary pH in Pre-Hypertensive-to-Hypertensive Adults. American Society of Bone Mineral Research. September 2019.

Stone MS, Martin BR, Weaver CM. Short-term Increased Dietary Potassium from Potato and Potassium Gluconate Effect on Potassium Retention in Pre-Hypertensive-to-Hypertensive Adults. American Society of Nutrition. June 2019.

Stone MS, Martin BR, McCabe LD, McCabe GP, Weaver CM. Short-term Increased Dietary Potassium From Potato and Potassium Gluconate Has No Effect on Blood Pressure and Microcirculation in Pre-hypertensive-to-hypertensive Adults. AHA EPI/Lifestyle Scientific Sessions. March 2019.

Taylor, M.K., Hernández, L.M., Kviatkovsky, S.A., Schoenherr, M.R., **Stone, M.S**., Sargent, P. (2017). The "Yin and Yang" of the adrenal and gonadal systems in elite military men. Medicine and Science in Sports and Exercise, 49(5S). June 2017.

H.C.M. Allaway, **M.S. Stone**, D.L. Petkus, C.M. Weaver, M. Misra, B.R. Martin, E.A. Southmayd, NI. Williams, M.J. De Souza. Oral Contraceptives Decrease Recombinant Human GH-stimulated Hepatic IGF-1 Secretion in Young Women Following Two Months of Use. Endocrine Society. April 2017.

Fuller, S.A., Hernández, L.M., **Stone, M**., Taylor, M.K. Dose-dependent increases in electrodermal activity during a graded exercise test in military men (2016). Medicine and Science in Sports and Exercise. June 2016.

Hernández, L.M, Fuller, S.A., **Stone, M.**, Carpenter, J, Taylor, M.K. (2016). Catabolic/Anabolic Hormone and Inflammatory Marker Responses to a Graded Exercise Test in Military Men. Medicine and Science in Sports and Exercise, 48(5), Supplement. June 2016.

Michael Stone, Michael J. Buono, Mark Kern. The effect of exercise intensity on sweat amino acid excretion. FASEB J. April 2014.

SERVICE ACTIVITES

2016-2017	Vice President, Nutrition Science Graduate Student Organization, Department of Nutrition Science, College of Health and Human Sciences, Purdue University, West Lafayette, IN.
2013- 2014	Research Volunteer, Naval Health Research Center, San Diego, CA. Project: Investigating the findings of a neurological growth factor study and the possible correlation to anabolic/catabolic hormones (eg. DHEA). Principal Investigator: Dr. Marc Taylor, Ph.D., Dept 163, Behavioral Sciences & Epidemiology, Naval Health Research Center, San Diego, CA.
2013-2016	SCAN Notables Editor: editor of the notables section of the publication of the Sports, Cardiovascular, and Wellness Nutrition (SCAN) dietetic practice group of the Academy of Nutrition and Dietetics.
2011- 2012	Research Assistant Volunteer, San Diego State University, Department of Exercise and Nutritional Sciences, San Diego, CA. Project: Investigated the effects of eating a carbohydrate based breakfast vs. an egg based breakfast, coupled with consistent resistance training, on body composition and cholesterol levels, participated in the supervision, coordination, and guidance of subjects in all gym activities and exercises. Principal Investigator: Dr. Mark Kern, Ph.D.
2012	Instructor Volunteer, Fitness Specialist Certificate Program, San Diego Mesa College, San Diego, CA. Role: Supervised and instructed students in the understanding of VO_2 kinetics and ventilatory measures, body composition measurements, the use of metabolic carts for VO_2 testing, and the use of a hydrostatic weighing tank and a Bod Pod for body composition measurements.
2003	Volunteer, Chula Vista Olympic Training Center, San Diego, CA. Role: Participated in the making of an instructional video that was to be used in the aiding of female shot put, discus, and hammer throwers, to better improve their specific event skills.

HONORS AND AWARDS

Bilsland Dissertation Fellowship: 2018-2019, Department of Nutrition Science, College of Health and Human Sciences, Purdue University, West Lafayette, IN.

Graduate Student of the Year (nominated): 2012-2013, Department of Exercise and Nutritional Sciences, College of Health and Human Services, San Diego State University, San Diego, CA.

CERTIFICATIONS AND MEMBERSHIPS

2019-Present	American Heart Association.
2016-Present	American Society of Nutrition.