SODIUM AND RELATED MINERAL INTAKE IN CHRONIC DISEASE

by

Andrea J. Lobene

A Dissertation

Submitted to the Faculty of Purdue University

In Partial Fulfillment of the Requirements for the degree of

Doctor of Philosophy

Department of Nutrition Science

West Lafayette, Indiana

May 2020

THE PURDUE UNIVERSITY GRADUATE SCHOOL
STATEMENT OF COMMITTEE APPROVAL

Dr. Kathleen Hill Gallant, Chair

Department of Nutrition Science

Dr. Regan Bailey

Department of Nutrition Science

Dr. George McCabe

Department of Statistics

Dr. Nana Gletsu-Miller

Department of Applied Health Science, Indiana University

Approved by:

Dr. Amanda H. Seidl

To my major professor, Dr. Kathleen Hill Gallant.

Thank you for your guidance, mentorship, and support throughout this process.

Thank you for fighting for me, for instilling your passion for nutrition research, for serving as a role model for women in academia, and for reminding me why I wanted to do this.

This would not have been possible without you.

ACKNOWLEDGMENTS

To my major professor, Dr. Hill Gallant, and all my committee members, Dr. Regan Bailey, Dr. George McCabe, and Dr. Nana Gletsu-Miller—thank you for your continued support, your advice, and your encouragement. You all have been incredibly helpful to me, both individually and as a group. I have learned so much from all of you. I could not have asked for a better committee to guide me through the PhD process.

To others who have provided help and support: Linda McCabe, statistical wizard; Dr. Sharon Moe, my CTSI fellowship co-mentor; and Amy Wright, menu planning wizard and friend—thank you all for your help. Your support and kind words are sincerely appreciated.

To all my officemates, labmates, and all the other friends I have made over the past four years—thank you. This has been a long journey, but we had a lot of laughs and made a lot of memories together along the way. Thank you for reminding me to take breaks and goof off once in a while. I know I will leave here with friendships and memories that will last a lifetime.

Lastly, thank you to all of my family and friends who have supported me especially my parents and my boyfriend, Jake. Mom and Dad, thank you for supporting me on my path to becoming a "professional student." Thank you for your love, your support, and for reminding me that there is more to life than work. To Jake, thank you for being there for me through the ups and downs. Thank you for supporting me, encouraging me, and being my adventure buddy. I look forward to many more adventures together.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

The intake of sodium, potassium, and phosphorus has important implications for chronic disease risk. Excess sodium intake is shown to be associated with elevated blood pressure, which in turn is a risk factor for cardiovascular disease (CVD) and chronic kidney disease (CKD). Potassium intake, on the other hand, is shown to be beneficial for lowering blood pressure and reducing the risk of CVD and CKD. Once an individual develops CKD, they experience alterations in mineral metabolism, especially phosphorus, and must closely monitor mineral intake and biochemical laboratory values in order to avoid complications. Thus, monitoring mineral intake is important in both healthy and CKD individuals in both research as well as clinical practice settings. It is therefore also important to have a method for estimating mineral intake that is both accurate as well as easy to administer. Two commonly used methods are self-report and 24-hour urinary mineral excretion. however, both methods have pros and cons. An alternative option that has been explored for all three minerals of interest is to collect a spot urine sample, then use one of several published equations to calculate an estimate of 24-hour urinary mineral excretion. While this method is relatively easy to administer, much remains unexplored regarding the accuracy of estimated 24-hour mineral excretion. My aim for my dissertation was to explore how estimated 24-hour sodium (e24hUNa), potassium (e24hUK) and phosphorus (e24hUP) compared to true mineral intake in healthy participants as well as those with CKD. We conducted secondary analyses from two controlled feeding studies, in which true mineral intake was known. Our results show that e24hUNa and e24hUK are not reliable indicators of true sodium and potassium intake, respectively, in healthy participants nor those with CKD, and e24hUP is not a reliable indicator of phosphorus intake in CKD participants. Though these findings should be confirmed by larger studies, these findings suggest that currently available equations may need to be revised and estimated 24-hour mineral excretion from spot urine samples should be interpreted with caution.

CHAPTER 1: INTRODUCTION AND LITERATURE REVIEW

Cardiovascular disease (CVD) and chronic kidney disease (CKD) are two chronic diseases of major public health concern both in the U.S. and globally. Dietary factors are important contributors to the development and progression of both diseases, and sodium, potassium, and phosphorus are three minerals of particular concern. Understanding the physiological mechanisms underlying these diet-disease relationships, designing effective dietary interventions, and developing appropriate guidelines requires accurate and convenient methods for estimating sodium, potassium, and phosphorus intake. This chapter presents an overview of each mineral's physiology, relationship to chronic disease risk, and available methods for estimating intake.

Sodium Functions, Intake, and Role in Chronic Disease

Sodium Physiology and Functions in the Body

Sodium is an essential nutrient whose primary function is to regulate fluid balance. Sodium resides primarily in the extracellular fluid (e.g. plasma) and is maintained within narrow limits—any alteration in plasma sodium concentration will trigger compensatory physiological mechanisms. Sodium is readily absorbed at the intestine and is excreted primarily in the urine. Sodium is absorbed in both the small (1) and large (2) intestine. Balance studies have shown that under normal circumstances, more than 97% of sodium consumed is absorbed, with small amounts excreted in the feces (3, 4). Under normal conditions when excessive sweating does not occur, minimal sodium is lost via sweat (4). Therefore, because sodium is readily absorbed and little is lost via sweat, the kidneys play a crucial role in maintaining plasma and body sodium balance (5). This is accomplished by regulating sodium excretion and water reabsorption. At the kidney, sodium is first filtered by the glomeruli and then may be reabsorbed in the proximal tubule and loop of Henle (6, 7). Evidence suggests that sodium retention and excretion may be racially dependent (4), and these racial disparities will be discussed later in this review. This regulation at the level of the kidney is important because sodium levels in the body must be maintained within very narrow limits (8).

Various factors have been shown to affect sodium excretion, with one of the main factors being sodium intake. When sodium intake is low, aldosterone and angiotensin II are increased, and sodium and water are reabsorbed, thus reducing sodium excretion (3). When sodium intake is abruptly increased, these hormones are decreased, resulting in increased urinary sodium excretion (3). Thirst and vasopressin also increase when sodium intake is high, resulting in increased water intake and retention in order to maintain normal plasma osmolality (8). After about 3 days, sodium balance is restored, meaning sodium intake is matched by sodium excretion (3, 8). Given the important interplay between sodium and water in maintaining sodium balance, water intake is another factor that affects sodium excretion. Indeed, a significant positive association between hydration status and sodium excretion has been shown (9). Potassium intake has also been shown to promote natriuresis, which may have a protective effect against CVD (10, 11). Genetics also play a role in sodium homeostasis; for instance, the no-lysine kinase 1 (*WNK1*) has been identified as a gene known to regulate sodium reabsorption and affect blood pressure response to dietary sodium intervention (12). Chronic diseases also affect normal sodium excretion, which will be discussed in more detail later in this paper.

Our understanding of sodium homeostasis has come from studies using radioisotopes of sodium as well as full balance studies. Older studies that used radioisotopes of sodium (^{22}Na and ^{24}Na) found that exchangeable sodium in the body may be altered by changes in sodium intake (13), and changes in exchangeable sodium in the body are related to blood pressure (13, 14). Short-term balance studies have shown that 24-hour urinary sodium excretion represents about 90% of sodium intake (15, 16). Recent ultra-long-term balance studies have allowed the characterization of long-term sodium balance (17, 18). In these studies, the researchers found that on fixed salt intakes, 90-95% of salt consumed was excreted in the urine on average, which is in line with previous literature. However, these studies altered our traditional understanding of sodium balance because 24-hour sodium excretion rarely matched that day's intake due to rhythmic day-to-day variability in sodium excretion, even though intake was fixed. This rhythmicity in sodium excretion suggests that sodium accumulation and excretion may be regulated by a neuro-endocrine clock (8). This rhythmicity in sodium excretion, even with a constant intake, may affect our ability to characterize sodium intake from urinary sodium excretion. This limitation will be discussed later in the paper.

Sodium Intake and Chronic Disease

Hypertension, a major risk factor for cardiovascular disease (CVD), is highly prevalent in the United States. A recent report from the American Heart Association found that 34% of American adults have hypertension (19). One nutrient that has been linked to increased blood pressure is sodium. While the exact mechanism by which sodium intake raises blood pressure is unclear, one widely accepted hypothesis is that increased sodium intake increases plasma sodium concentration, resulting in increased extracellular fluid volume and elevated blood pressure. Indeed, clinical studies have demonstrated an increase in plasma sodium concentration with large, sudden increases in sodium intake (20), and even acute increases in sodium intake have been shown to cause increased plasma sodium concentration and increased blood pressure (21). The resultant increase in extracellular volume, as well as the plasma sodium itself, exert a pressor effect, resulting in increased blood pressure (20).

The link between sodium intake and blood pressure has been demonstrated in both epidemiological and clinical studies. Perhaps one of the most well-known international epidemiological studies aimed at assessing the relationship between electrolyte excretion (as a surrogate of intake) and blood pressure is the INTERSALT study. This study found significant positive associations between blood pressure and 24-hour urinary sodium excretion, and the linear slope of blood pressure with age was positively related to median 24-hour sodium excretion (22, 23). The relationship between sodium intake and blood pressure has been observed in clinical studies as well. Indeed, a meta-analysis of randomized controlled salt-reduction trials found that a modest reduction in salt intake can significantly lower blood pressure (24). One study that was referenced in this meta-analysis was the DASH-Sodium Trial. The DASH-Sodium Trial was a randomized-controlled trial aimed at assessing the effect of three different levels of sodium intake, with both a control diet and the DASH diet, on blood pressure in adults (25). The results of this study showed that reducing sodium intake resulted in a significant reduction in those with and without hypertension, in all races, and in both men and women in a dose-dependent manner regardless of concurrent dietary pattern (26). These data together demonstrate the positive association between sodium intake and blood pressure, and the blood pressure lowering effects of reduced sodium intake.

The vast body of evidence linking sodium intake to elevated blood pressure and CVD served as the foundation for establishing the Dietary Reference Intakes (DRIs) for sodium. The

first iteration of the sodium DRIs were published in 2005 and established an Adequate Intake (AI) of 1500 mg/d for young adults and a Tolerable Upper Intake Level (UL) of 2300 mg/d (27). The sodium DRIs were updated in 2019 to better align with current research findings (28). Prior to this update, a new category of DRI was established to accommodate the unique challenge of establishing an "upper limit" for sodium. The currently defined UL typically establishes a level above which an individual may experience acute toxicity effects. For sodium, research shows that excess intakes over a long period of time increase the risk of chronic disease—a paradigm that did not match any of the DRI categories. Thus, the "Guiding Principles" report defined the new Chronic Disease Risk Reduction Intakes (CDRR), the intake level below which the risk of chronic disease development is reduced (29). The 2019 updated sodium DRIs eliminated the UL and established the CDRR for sodium at 2300 mg/d based on a high strength of evidence suggesting that reducing sodium intake reduces blood pressure and subsequent risk of CVD (28).

Despite the large body of evidence linking sodium to CVD, typical U.S. intakes of sodium exceed these recommended levels. A recent report from the American Heart Association found that the average consumption of sodium in adults is about 3.5 g/d, with less than 8% of adults consuming less than the 2300 mg/d limit (19). The 2015-2020 Dietary Guidelines for Americans reports similar findings, with usual intakes in males reaching up to 4500 mg/d, and reports that the main sources of dietary sodium are mixed dishes, protein foods, and grains (30). While some argue that modest sodium reduction is appropriate for all, others note that those at greatest risk will benefit the most from a reduction in sodium intake, such as older adults, blacks, and hypertensives (31). This sentiment is echoed in the 2015-2020 Dietary Guidelines for Americans, which recommend a sodium intake of < 1500 mg/d in those with hypertension (30).

Aside from CVD, sodium intake has important implications for other chronic diseases, particularly chronic kidney disease (CKD). Hypertension is one of the most common underlying etiologies for CKD (32), and CKD in turn exacerbates hypertension (33). Ultimately, CVD is the number one cause of death in those with CKD and is even more common than progression to end stage kidney disease (32). Given the link between sodium intake and hypertension, it makes sense that this mineral would also be a risk factor for CKD. Indeed, increased sodium intake is associated with an increased risk of CKD (34), and in those who already have CKD, increased sodium intake is shown to be positively associated with CVD risk (35) and CKD progression (36). In clinical studies of CKD, reducing sodium intake is shown to be effective a reducing

blood pressure (37), and is also shown to reduce signs of kidney damage (38, 39). The importance of reducing sodium intake in CKD has been recognized by international organizations such as Kidney Disease|Improving Global Outcomes (KDIGO), which includes a recommendation to keep sodium intake below 2 g/day in their CKD clinical guidelines (40).

Though a large body of evidence supports the conclusion that reducing sodium intake is important for reducing chronic disease risk, there is also a body of evidence that suggests reducing sodium intake too much could also be harmful. Indeed, the findings of many epidemiological studies suggest that the relationship between sodium intake and disease risk is not linear, but actually J- or U-shaped. J- or U-shaped relationships have been observed when examining the relationship between sodium intake and risk of CVD event (41-44) and all-cause mortality (41, 44), and inverse linear relationships between sodium intake and risk of end-stage kidney disease have been observed (45). While these findings might call into question the dietary guidance and recommendations to reduce sodium intake, it is important to keep in mind that this evidence is largely observational and is likely influenced by reverse causality and the presence of comorbidities. Most importantly, recent analyses have shown that these J- or U-shaped findings may be largely due to inaccurate methods used to estimate sodium intake. Indeed, two recent analyses by He et al (46, 47) sought to debunk these studies by reanalyzing data from large cohort studies using the "inaccurate" method of estimating sodium intake as well as the "gold standard." Both studies found that, when using the "inaccurate" method, the relationship between sodium intake and mortality was J-shaped, but when using the "gold standard" the relationship was positive and linear. These findings highlight the importance of using accurate methods for estimating sodium intake (and the intake of all nutrients) to properly characterize the relationship between dietary intake and chronic disease. This sentiment was echoed in a position statement published by the International Consortium for Quality Research on Dietary Sodium/Salt (TRUE), which advised against the use of a spot urine samples for estimating sodium intake, and instead recommends using multiple 24-hour urine collections (48).

To further address the link between sodium intake and chronic disease, and to help in developing accurate dietary policies and clinical guidelines, we need an accurate, reliable, and low-burden method for assessing sodium intake. This remainder of this section will discuss the common methods of assessing sodium intake and will discuss the utility of spot urine samples as an alternative method for assessing sodium intake.

Common Methods for Estimating Sodium Intake

Self-reported methods of assessing sodium intake, including dietary recall, diet records and food frequency questionnaires, are widely used both in research and in practice. While these methods are convenient, they are often inaccurate. Underreporting of intake, especially with diet records and dietary recall, is common (49). Self-reported methods are especially inaccurate in individuals with overweight and obesity because they are more likely to underreport their intake (49, 50). As an alternative to the traditional interviewer-administered 24-hour dietary recall, the USDA Automated Multiple-Pass Method was developed, which provides a more thorough, structured approach to collecting dietary recall data. While the accuracy of reported sodium intake using this method was found to be 93% and 90% in normal weight men and women, respectively, reporting accuracy was only 78% in obese subjects (50). This is problematic because estimating sodium intake is important for assessing disease risk, and obese individuals are at even greater risk for chronic disease compared to normal weight individuals.

In addition to underreporting, self-reported intake may be inaccurate because it is difficult to quantify sodium content in foods prepared at restaurants, at home, and in processed and manufactured foods (49, 51). Discretionary salt use, or salt added at the table, as well as sodium in medications and tap water are also nearly impossible to quantify with these methods (49). Food Frequency Questionnaires (FFQs) may provide some benefit over diet records and diet recalls, but it is extremely difficult to quantify daily sodium intake with this method as well (49). Overall, self-reported methods have several noticeable limitations including intrinsic inaccuracy and difficulty in quantifying sodium content in foods and are therefore unable to provide an accurate estimate of sodium intake. This establishes the need for an alternative method for use in both research and in clinical practice.

Twenty-four-hour urine collections are another method commonly used to estimate sodium intake. Approximately 90% of ingested sodium is excreted in the urine over the same period (17, 49, 51). This means that nearly all sodium consumed, whether it is sodium in our food, sodium added at the table, or sodium in our medications, will appear in our urine, making it more advantageous for estimating intake than self-report. As a result, 24-hour urine collection is regarded as the gold standard for assessing intake (49, 51), and was shown to be feasible enough to be implemented in The National Health and Nutrition Examination Survey (NHANES) 2014 in adults (52). Indeed, 24-hour urinary sodium excretion is often used as the standard to validate

other methods of assessing sodium intake, including diet questionnaires and diet records, in both adults and children (53-56). Twenty-four-hour urinary sodium excretion is a valid biomarker for assessing sodium intake in adolescents as well (57). Importantly, urinary sodium excretion has been shown to be associated with blood pressure, especially in hypertensives (58), and a reduction in 24-hour sodium excretion is associated with a reduction in blood pressure in hypertensives (59). This is important because it suggests that 24-hour urinary sodium excretion can be used as a marker of disease risk, much like sodium intake is used.

Despite the advantages of using 24-hour urinary sodium excretion, this method is not without limitations. Collection of 24-hour urine is burdensome for participants, which could affect whether or not a complete collection is obtained (49, 51, 60). A recent review found that in population-based studies that use urinary sodium excretion to estimate intake, most studies had a response rate, or a rate of complete collections obtained, of approximately 40% (51). Because of the burden on participants and the prevalence of incomplete data collections, alternative methods of assessing sodium intake using urinary biomarkers have been explored.

As an alternative to a 24-hour urine collection, the use of a single spot urine sample to estimate sodium intake has been explored. In contrast to the low response rate with a 24-hour urine collection, the response rate in studies that use spot urine samples is between 73-100% (3). The advantage of collecting a single spot urine sample rather than a 24-hour urine collection is that it is collected in a single encounter (49). This review will further discuss how spot urine samples are (or should be) used to estimate sodium intake, the strengths and limitations of using spot urine samples, and important gaps in the literature regarding their utility that still need to be filled.

Spot Urine Estimates of Sodium Intake

Estimating 24-hour Urinary Sodium Excretion from Spot Urine Samples

Several studies have assessed the utility and accuracy of a single urine sample for estimating 24-hour urinary sodium excretion. A recent systematic review published in 2012 explored 20 such studies (61). While the authors determined that the evidence on the use of spot urine for estimating 24-hour urinary sodium excretion is inconclusive due to the heterogeneity of outcomes and protocols, most studies discussed in the systematic review reported a positive

association between sodium in the spot urine sample and 24-hour urinary sodium excretion. More recent studies have been published that further explore the relationship between sodium in spot urine samples and 24-hour urinary sodium excretion at the population level. While some of these studies found that a spot urine sample can be a useful alternative for estimating 24-hour urinary sodium excretion (62, 63), others concluded that a spot urine sample is not accurate for estimating 24-hour sodium excretion (64). Though the evidence is still mixed, spot urine samples show promise as a practical alternative to a 24-hour urine collection.

There are at least two possible explanations for the disparate findings on the utility of spot urine samples for predicting 24-hour urinary sodium excretion. First, sodium from a single urine sample must be put into the context of the daily urinary sodium excretion for interpretation (51). Not all studies have done this—many simply correlate sodium in the spot urine sample with sodium in the 24-hour urine collection. Correlations simply tell us if spot urine sodium is related to 24-hour sodium excretion and therefore do not contextualize sodium excretion. In order to quantitatively use spot urine sodium to predict 24-hour urine excretion, equations have been developed (62, 63, 65-72) as described in **Table 1.1.** One set of prediction equations were developed and validated from the INTERSALT study using western adults from North America and Europe (62, 73). Whereas some equations, such as Tanaka's prediction equations, are shown to provide biased estimates of 24-hour sodium excretion (i.e. under- or over-estimating 24-hour excretion) (64), the INTERSALT equations have been shown to provide the least biased estimates of 24-hour excretion (73, 74). Therefore, the INTERSALT equations are some of the most widely used equations for estimating 24-hour sodium excretion. A second possible explanation for the disparate findings in the literature could be the differences in statistical methods used for comparison. Some studies use correlations to assess the relationship between spot urine samples, or 24-hour sodium excretion predicted from a spot urine sample, and actual 24-hour sodium excretion. However, other studies assess agreement between the two measures using methods such as Bland-Altman analysis. This presents a major inconsistency in the literature because we cannot answer the same sort of research questions or draw the same type of conclusions with these different statistical analyses. Indeed, one study that utilized both correlation and Bland-Altman analysis to compare sodium in a 24-hour urine collection and a spot urine sample found that the samples were significantly correlated, but were not in agreement (75), indicating that correlation does not always indicate agreement in different urinary sodium

measurements. When exploring the utility of spot urine samples for estimating 24-hour excretion, future studies should utilize prediction equations and statistical methods that assess agreement in order to draw consistent and accurate conclusions.

Table 1.1 Prediction equations developed for estimating 24-hour urinary sodium excretion

Equation	Mathematical Formula	Urine Sample	Population	Reference
Kawasaki	Predicted 24hUNa= 23*(16.3*$XNa^{0.5}$), where XNa = {spot Na (mmol/L)/[spot Cr (mg/dL)*10]}*Pr24hCr (mg/d)[1]	Second morning void	Japanese adults	Kawasaki et al 1993
Tanaka	Predicted 24hUNa= 23*(21.98*$XNa^{0.392}$), where XNa = {spot Na (mmol/L)/[spot creatinine (mg/dL)*10]}*Pr24hCr (mg/d)[2]	Casual urine sample	Japanese adults (Japanese INTERSALT)	Tanaka et al 2002
INTERSALT	**Male:** Predicted 24hUNa= 23*{25.46 + [0.46*spot Na (mmol/L)] - [2.75*spot Cr (mmol/L)] – [0.13*spot K (mmol/L)] + [4.10*BMI (kg/m2)] + [0.26*age (y)]} **Female:** Predicted 24hUNa= 23*{5.07 + [0.34*spot Na (mmol/L)] - [2.16*spot Cr (mmol/L)] - [0.09*spot K (mmol/L)] + [2.39*BMI (kg/m2)] + [2.35*age (y)] – [0.03*age^2 (y)]}	Casual urine sample	North American and European adults	Brown et al 2013
Nerbass RRID	Predicted 24hUNa (mmol/L) = -68.625 + [weight (kg)*1.824) + [EM UNa (mmol/L)*0.482]	Early morning void	UK adults with CKD	Nerbass et al 2014
Nerbass SALTED	**Male:** Predicted 24UNa (g/day) = 0.96 + (weight in kg x 0.03) + (sodium in the urine specimen in g/L x 0.63) **Female:** Predicted 24hUNa (g/day) = 0.15 + (weight in kg x 0.03) + (sodium in the urine specimen in g/L x 0.63)	Second morning void	Brazilian adults with CKD	Nerbass et al 2017

Spot Urine Sodium Excretion Compared to Intake

In order to truly assess the utility of spot urine samples, it is necessary to understand not only how well they can predict 24-hour excretion, but how well this predicted sodium excretion is related to intake. However, few studies have assessed the relationship between sodium

excretion in a spot urine sample and sodium intake. A randomized, crossover, controlled feeding study by Luft et al (76) found strong correlations between sodium excretion in an overnight urine collection, or the period of collection between bedtime and waking, and 24-hour sodium excretion at varying levels of known intake. In this study, participants consumed a controlled diet with fixed sodium intake at three different levels for 7 days. They found a significant relationship between overnight urinary sodium excretion, 24-hour excretion, and intake. Luft and colleagues (77) recognized a limitation in their study design, which was the day-to-day variation in sodium intake in a free-living population and conducted a follow-up study to address this gap. In this study, participants consumed a controlled diet for 10 days in which the sodium intake was randomly varied each day. They found that mean 24-hour sodium excretion was highly correlated with mean intake over the 10 days, while mean overnight sodium excretion was weakly correlated with mean intake over the 10 days. On a randomly selected day, they also found significant correlations between that day's sodium intake and both 24-hour sodium excretion and overnight sodium excretion. While both studies demonstrate a relationship between spot urine samples, 24-hour urine collection, and actual sodium intake, an important limitation of both studies is that the overnight urine sample was not put into the context of 24-hour excretion (i.e. it was not used to predict 24-hour excretion). A more recent study utilized NHANES data to assess the trend in 24-hour sodium excretion in the U.S. over time using prediction equations developed from INTERSALT (strength), but with less robust random sampling of spot urines and estimates of sodium intake from a 24-hour dietary recall from the day before the sample was collected (78). They found weakly significant correlations between spot urine sodium concentrations and sodium intake, and moderate significant correlations between estimated 24-hour sodium excretion and sodium intake.

A major gap in the literature is the lack of studies connecting predicted 24-hour urinary sodium excretion to a known sodium intake. This is important because the purpose of collecting a spot urine sample is to ultimately use it to better understand sodium intake. There are a number of studies that have compared predicted 24-hour sodium excretion from a spot urine sample to actual 24-hour sodium excretion (49, 61, 62, 73, 74). In addition, many studies have compared actual 24-hour sodium excretion to sodium intake, though in some studies intake was assessed by self-report (17, 18, 53, 56). While some studies have compared predicted 24-hour sodium excretion to sodium intake, intake was estimated using self-report (78) which is known to be

inaccurate. Virtually no studies have connected the dots from spot urine samples to 24-hour sodium excretion to known sodium intake, likely because this can only be accomplished with a controlled feeding study. To our knowledge, only two studies conducted by the same research group have explored all three variables in the context of a controlled feeding study (76, 77). Both studies were discussed previously. While both studies demonstrated a relationship between spot urine sodium and known sodium intake, these studies had two major limitations: 1) the spot urine samples were not put into the context of 24-hour excretion (i.e. it was not used to predict 24-hour excretion), and 2) agreement between spot urine sodium and sodium intake was not truly assessed because correlations were used to assess the relationships between the two variables.

Timing of Spot Urine Sample Collection

The accuracy of spot urine samples for estimating intake may in part depend on the time of day that the sample is collected. An important limitation in the literature on spot urine samples is the lack of consistency in when a spot urine sample is collected. In studies that utilize spot urine samples for estimating sodium intake, methods for collecting samples are highly variable and may include overnight collection, casual spot urine collection, fasting urine collection, or a timed collection. Indeed, the meta-analysis by Ji and colleagues (61) discussed the heterogeneity in protocols and methods of urine sample collection in the studies they reviewed, which ultimately precluded a firm conclusion on the utility of spot urine samples for estimating 24-hour sodium excretion.

To date few studies have explored the ideal strategy for collecting a spot urine sample to most accurately estimate 24-hour sodium excretion. One study by Mann and colleagues (79) aimed to answer this question by comparing the sodium/creatinine ratio in spot urine samples collected as part of a 24-hour urine collection. In this study participants were instructed to collect a 24-hour urine sample, and as part of the collection they were asked to turn in three spot urine samples separate from the rest of their collection: an “AM” sample (i.e. the second morning void, or the second occasion of urination), a “PM” sample (i.e. an evening sample) and a random urine sample (i.e. collected at any time). Each spot urine sample was then used to predict 24-hour sodium excretion by adjusting for 24-hour creatinine excretion. They found that with the PM sample, but not with the random or AM samples, both the sodium/creatine ratio and the predicted

24-hour excretion were significantly correlated with actual 24-hour sodium excretion. These results indicate that the time of day that a urine sample is collected is important.

Further studies have explored the variability in sodium excretion assessed using different timed spot urine samples. A study by Wang and colleagues (80) utilized a study design similar to that by Mann et al to assess the variability in timed spot urine samples collected as part of a 24-hour urine collection. In this study, participants were instructed to collect their urine for 24-hour with each void collected in a new container. From each participant four timed collections were selected for analysis: a morning sample, an afternoon sample, an evening sample, and an overnight sample. They found that the sodium concentration in the overnight urine collection was significantly lower than the other timed collections. As a follow-up to this study, Cogswell and colleagues (74) found that the 24-hour excretion predicted from the overnight sample was generally lower than that predicted from the other timed collections, and the afternoon and evening samples provided a better approximation of actual 24-hour sodium excretion compared to the morning or overnight samples. Taken together, these data suggest that the time of day that a urine sample is collected is important and should be considered in designing a study using spot urine samples. Another follow-up study used each of these timed urine collections, in either one void or two void combinations, to estimate the population distribution of 24-hour sodium excretion (69). They found that the estimated usual distribution of 24-hour sodium excretion using two void combinations, regardless of which two voids were used, was more consistent with the observed usual distribution compared to estimates using one void. This finding is important because it may indicate that if spot urine samples are unable to be timed due to the nature of the study design, collecting two spot urine samples could still provide an accurate estimate of 24-hour excretion.

Importantly, no study has taken timing of meals into account when collecting spot urine samples. Given that most of the sodium consumed is excreted in the urine quickly, time of collection relative to the last meal is an important factor that should be considered in future studies. In addition, evidence has shown that urinary creatinine increases during the first few hours after a meal (81). This is important because creatinine is often used as a marker of a complete urine collection, and many equations used to predict 24-hour sodium excretion from a spot urine sample include creatinine as a variable in their models. Until studies consider both

time of day and timing of meals in their design, the ideal time is to collect a spot urine sample for the most accurate estimate of 24-hour sodium excretion cannot be determined.

Spot Urine Sodium Estimates at the Population vs. Individual Level

While urinary sodium excretion is an accurate method for estimating sodium intake at the population level, much debate remains about the utility of urinary sodium excretion for estimating sodium intake at the individual level. The primary argument against using urinary samples to estimate intake at the individual level is the extreme variability in an individual's sodium excretion from day to day. This variation could in part be due to variability in the amount of sodium consumed, as it has been shown that sodium intake varies from day to day depending on the amount and types of foods consumed (51). However, it has been shown that even on a fixed, constant sodium intake, daily sodium excretion exhibits rhythmic patterns (18). This suggests that, regardless of sodium intake, urinary sodium excretion exhibits a high level of day-to-day variability. This rhythmic variability of sodium excretion from day to day calls into question the accuracy of even one day of 24-hour urine collection for estimating sodium intake (17). Indeed, because of the high within-person variability in sodium excretion, to accurately assess sodium intake using urinary sodium in an individual, multiple days of 24-hour urine collections would be necessary (17, 51, 82). This is problematic because urinary sodium excretion is the preferred method of assessing sodium intake and, as one study pointed out, the variability in sodium excretion could affect our ability to assess relationships between sodium intake and blood pressure (83).

Given that 24-hour urinary sodium excretion is variable from day to day, a spot urine sample is also subject to extreme intraindividual variability. In addition to the day to day variability in sodium excretion, evidence also shows that sodium excretion is variable within a day (80). Thus, many argue that spot urine samples are not accurate for estimating intake at the individual level. Indeed, prediction equations that use spot urine samples to estimate 24-hour sodium excretion are shown to be positively biased at low levels and negatively biased at high levels of actual 24-hour excretion in individuals (74). A further study by Zhou et al (84) directly evaluated the validity of spot urine samples and published prediction equations to estimate 24-hour urinary sodium excretion at the individual level. The results showed a high rate of misclassification of sodium intake at the individual level when estimated excretion was

compared to actual 24-hour excretion, suggesting spot urine samples cannot accurately estimate sodium intake at the individual level with the methods currently available. To improve estimates at the individual level, some have suggested collecting multiple spot urine samples on different days (72, 79).

In contrast to use of spot urine samples for assessing an individual's sodium intake, spot urine samples are a useful tool for assessing sodium intake at the population level. Within-person variation in sodium excretion does not bias estimates of a population's average intake (51). Indeed, spot urine samples have been used to assess trends in sodium intake in nationally representative samples (78). Evidence suggests that collecting multiple spot urine samples may improve the accuracy at the population level even more. Wang et al (69) found that a combination of two urine voids may provide a better estimate of the population distribution of 24-hour sodium excretion compared to estimates using one urine collection, which may suggest an even better way to utilize spot urine samples to estimate population sodium intake.

Spot Urine Sodium Estimates in Diseased Populations

Certain disease states that affect sodium retention or sodium excretion may affect the accuracy of urinary sodium for estimating sodium intake. As a recent review explained, in a healthy individual, sodium intake is typically matched by sodium excretion, but in cases of rapid, excessive sodium intake or in diseases such as congestive heart failure or renal failure, sodium excretion may not sufficiently match sodium intake, leading to sodium excess (51). Diseases such as congestive heart failure and chronic kidney disease (CKD) that result in fluid retention will result in decreased sodium excretion and thus excess sodium retention (3). Alterations in sodium excretion with certain disease states make urinary sodium excretion an unreliable marker of sodium intake in these populations. This is of concern because these populations are at highest risk of complications due to excess sodium intake. Therefore, an accurate method for assessing sodium intake in diseased populations is needed.

High sodium intake is problematic in patients with CKD and could lead to increased progression of the disease. Therefore, having an accurate method for estimating intake is crucial. Unfortunately, evidence on the accuracy of estimates of sodium intake from spot urine samples in CKD is mixed. A study conducted by Kang et al (85) found that spot urine sodium was significantly positively correlated with both 24-hour urine excretion and intake estimated by

dietary recall in patients with CKD. While this study suggests spot urine samples can be used for estimating intake in CKD patients, two limitations are that the researchers assessed correlation rather than agreement, and spot urine samples were not put into the context of daily excretion (i.e. a prediction equation wasn't used). To overcome these limitations, a valid formula for estimating 24-hour sodium excretion from a spot urine sample that is specifically designed for CKD patients is necessary. Nerbass and colleagues (67) used a sample of CKD patients from a large cohort study to estimate 24-hour urinary sodium excretion from a morning urine sample. Using this new formula, the authors noted that estimated and actual 24-hour excretion were significantly correlated; however, the accuracy of the formula for estimating 24-hour excretion was poor, suggesting the formula may only be appropriate for population studies rather than individual assessment. A study by Dougher et al (86) utilized this new formula developed by Nerbass as well as other published equations to assess their accuracy in estimating 24-hour urinary sodium excretion from a spot urine sample in CKD patients. The authors found that all equations investigated demonstrated poor precision and accuracy, suggesting that spot urine samples cannot accurately estimate sodium intake in CKD patients. Recently, Nerbass et al (68) developed a new formula for estimating 24-hour sodium excretion from the second morning void sample in CKD patients. While this formula was sensitive in detecting individuals with high sodium intake, the accuracy of the formula was low. Taken together, these data suggest that spot urine samples are not an accurate method for assessing sodium intake in CKD.

Hypertension is another disease that is known to be associated with sodium intake. Evidence suggests that sodium kinetics are altered in salt-sensitive hypertension (87). Indeed, hypertensive, salt-sensitive subjects were shown to have a longer half-life of sodium elimination than the hypertensive, salt-resistant subjects. Given these alterations in sodium balance in hypertension, studies have examined the utility of spot urine samples for estimating sodium intake in hypertensives. A recent review concluded that spot urine samples, especially the second morning void, can be used to estimate sodium intake in hypertensives (88). The authors point out that a potential complicating factor in using urinary sodium excretion in hypertensives is the natriuretic effect of many antihypertensive drugs. However, they concluded that spot urine can still be used to estimate sodium intake in patients on antihypertensive drugs. Looking at patients with hypertension taking medications, a recent study characterized the variability in spot urine sodium (89). They concluded that, due to the high intraindividual variability in spot urine

sodium, multiple spot urine samples may be necessary to assess sodium intake in hypertensive patients. Another complication is the lack of prediction equation for estimating 24-hour sodium excretion from a spot urine sample in hypertensives. Indeed, Allen et al (90) reported that existing equations to estimate 24-hour excretion in older, hypertensive adults may not be valid.

Gaps in the Literature: Children and Non-White Races

Much of the research investigating the utility of spot urine samples for estimating sodium intake has focused on adults; very few studies have investigated their utility in children. This is an important gap in the literature because the incidence of high blood pressure in children is increasing (91), which tracks into adulthood (92). As in an adult population, one factor that could affect the agreement between sodium intake and urinary sodium is the pattern of variability in sodium excretion. When looking at 24-hour urinary sodium excretion in children, day-to-day variability has been observed (93), even in a controlled feeding study (82). Despite this, a recent controlled feeding study concluded that 24-hour urinary sodium excretion is a valid biomarker of sodium intake in adolescents (57). When looking at spot urine samples, specifically overnight samples, significant intraindividual variability has been observed (93), though one study showed that 4 days on a controlled diet could reduce this variability (94). Although overnight urine sodium and 24-hour urine sodium excretion were correlated in children and adolescents, Bland-Altman analysis showed poor agreement between the two measures (56). There are no prediction equations for estimating 24-hour urine sodium excretion from a spot urine sample in children.

Racial differences have not been considered in developing prediction equations from spot urines. This is an important oversight because the risk of hypertension differs by race, with blacks being at higher risk than other racial groups. According to the most recent report by the American Heart Association, the prevalence of hypertension among blacks in the United States is among the highest in the world, with 45% of black males and over 46% of black females having hypertension (19). Blacks demonstrate a higher half-life for sodium excretion with increasing levels of sodium intake compared to whites (95), and greater sodium retention than whites, even on a controlled diet (4). In addition, blacks exhibit greater day-to-day variability in sodium excretion than whites (82, 90). Good agreement between spot urine sodium and 24-hour urinary sodium excretion as assessed by Bland-Altman was found in an adult African population (96), though spot urine samples were not put into the context of 24-hour excretion (i.e. a prediction

equation was not used). In a study in which prediction equations for estimating 24-hour sodium excretion were used, all equations significantly over or under estimated 24-hour excretion in older, hypertensive African Americans (90). Race specific prediction equations are likely needed to evaluate the relationship between sodium intake and disease risk.

Potassium Function, Intake, and Roles in Chronic Disease

Potassium Physiology and Functions in the Body

Potassium is the main intracellular cation and plays an important role in maintaining electrochemical gradients across cell membranes. This electrochemical gradient maintains the normal resting membrane potential, which is crucial for triggering action potentials for muscle contraction and transmission of nerve impulses (97). Apparent absorption is about 85% of consumed potassium (15); absorption occurs in the small intestine with net secretion of potassium in the colon (97). Absorbed potassium appears in the plasma, where insulin plays an important role in the cellular uptake of potassium, particularly into skeletal muscle (98). Ultimately, 77% of dietary sodium is excreted in the urine (15), making the kidneys the primary regulators of potassium balance. Potassium is filtered by the glomerulus and >90% is reabsorbed in the proximal tubule and loop of Henle, though reabsorption may occur at the distal tubule and collecting duct when potassium is low (97, 99). Potassium secretion occurs in the distal convoluted tubule and the collecting duct, and the amount of potassium secreted is determined by aldosterone levels as well as sodium (99). Aldosterone release is triggered by a low plasma sodium or a high plasma potassium concentration (97). Aldosterone can act by directly increasing the potassium permeability of the luminal membrane and indirectly by stimulating sodium reabsorption in the lumen, which creates an electrochemical gradient that favors potassium secretion (99).

Potassium Intake and Chronic Disease

Potassium intake has important implications for CVD risk. Indeed, epidemiological evidence has shown that potassium intake is negatively associated with blood pressure (22) and clinical studies have shown that increasing potassium intake, often achieved with supplementation, is effective at lowering blood pressure (100, 101). There are multiple

mechanisms by which potassium exhibits blood pressure-lowering effects. Potassium is an important vasodilator (102); thus, increasing plasma potassium concentration induces vasodilatory effects, and lowering plasma potassium concentration induces vasoconstriction. In addition, potassium can indirectly affect blood pressure by altering renal sodium excretion (103, 104). Because of this, sodium and potassium are often examined together in both observational and intervention studies of blood pressure and CVD risk. Observational studies show that a higher sodium-to-potassium intake ratio (Na/K) is associated with greater blood pressure and CVD risk (22, 105-108), and clinical studies show that increasing potassium can mitigate the effects of a high sodium diet on blood pressure (109, 110).

The evidence linking potassium intake to blood pressure and CVD risk provided the foundation for the DRIs in the U.S. as well as the potassium recommendations published by the World Health Organization (WHO). The first DRIs for potassium were published in 2005, at which time an AI for adults was set at 4700 mg/d (27). The DRI used available evidence that suggested insufficient potassium intakes could lead to increases in blood pressure, though the specific level of 4700 mg/d was established based off one study in salt sensitive black and white adult men (111). However, this recommendation was controversial, given the scarcity of clinical studies that have evaluated the effect of potassium intake at or above this level (112). In 2012 the WHO published their own guidelines for potassium intake, which recommends an intake of at least 3500 mg/d for adults, citing evidence that shows the largest reduction in blood pressure occurs at intakes between 3500-4700 mg/d (113). Notably, usual potassium intakes in the U.S. fall below the current AI. Indeed, usual potassium intake in adults from foods and supplements is about 2700 mg/d, with less than 3% of the population achieving an intake above the AI (114). Main sources of dietary potassium include milk, other beverages, potatoes, and fruit (115). Given the strong link between potassium intake and CVD risk, and the largely inadequate intakes in the U.S., the 2015-2020 Dietary Guidelines for Americans have identified potassium as a shortfall nutrient, citing low intakes of dairy, fruits, and vegetables as the reason for inadequate potassium intakes (30). In addition, in an effort to help Americans increase their potassium intake, the Food and Drug Administration now requires potassium content to appear on all nutrition facts labels (116). The DRIs for potassium were updated in 2019, and the AI for potassium was set at 3400 mg/d for adult men and 2600 mg/d for adult women (28). Citing a lack of consistent and accurate data for estimating potassium requirements, the committee used median usual intakes of a

healthy population for establishing the AI. In addition, no UL was established given the lack of evidence suggesting toxicity effects of increased potassium intake in a healthy population.

Potassium intake has important implications for other chronic diseases, most notably CKD. Higher potassium intake seems to be beneficial for reducing CKD risk; observational evidence has found that lower potassium intake is associated with a higher risk of CKD (117, 118). However, potassium may do more harm than good once a patient develops CKD. Studies have shown that, in those with CKD, higher potassium intake is associated with increased risk of CKD progression (36), and dietary potassium restriction is associated with a reduced risk of mortality (119). Potassium homeostasis is altered in CKD, which can lead to hyperkalemia or hypokalemia, both of which can lead to CVD and/or mortality (120, 121). Hyperkalemia in particular is very prevalent in patients with CKD: a recent analysis found that about 7% of all CKD patients have hyperkalemia, with the prevalence of hyperkalemia increasing with increasing CKD severity (122). Because of the prevalence and risks associated with hyperkalemia in CKD, many clinical guidelines include recommendations about potassium intake in CKD, though the recommendations are mixed. Reducing potassium intake to below 2400 mg/d is recommended for patients with CKD stages 3-5, with additional adjustments as needed based on serum potassium levels and other considerations, by the Academy of Nutrition and Dietetics (123) yet the KDIGO guidelines do not include a potassium intake recommendation (40). These discrepancies in guidelines likely stem from the fact that data on the risks of potassium intake for health outcomes in CKD in are mixed. Some studies show that, in fact, higher potassium intake is associated with a reduced risk of mortality in non-dialysis CKD (124). In addition, dietary management of potassium intake in patients with CKD is challenging, as is the overall management of hyperkalemia (125) and reducing potassium intake may inadvertently cause a reduced intake of other beneficial foods and nutrients (126). More work needs to be done to assess the benefit and practicality of reducing potassium intake in CKD in order to develop consistent and effective guidelines. Work on the potential renoprotective effects of potassium supplementation in moderate stage CKD patients with hypertension is ongoing (127).

Methods of Estimating Potassium Intake

Accurate and convenient methods of estimating potassium intake are necessary in both research and clinical practice settings. One commonly used method is self-report, which includes food records, 24-hour recalls, and food frequency questionnaires. While these tools are relatively easy to administer, they may not always be accurate, and the level of inaccuracy is inconsistent among validation studies. Some analyses report little difference between self-reported potassium intake and potassium measured from recovery biomarkers (128). However, other have shown that all three methods of self-report underestimate potassium intake when compared to estimates from multiple 24-hour urine collections (129, 130), while still others report overestimates of potassium intake compared to 24-hour urinary potassium excretion (131). While self-report is certainly still a valuable tool for understanding dietary intake and informing policy (132) other methods that provide a more accurate measurement of actual potassium intake may be desired. One such method that is often used is 24-hour urinary potassium excretion. Approximately 77% of dietary potassium is excreted in the urine (15, 133), which makes urinary potassium excretion a reliable biomarker of intake. However, as discussed in the sodium section of this review, 24-hour urine collections are burdensome, and the burden may affect the completeness of the urine collection and the accuracy of the measurement (49). Because of this, an alternative method that has been developed and explored is collecting a spot urine sample and using a prediction equation to calculate estimated 24-hour urinary potassium excretion. Two equations have been developed or this purpose: the Kawasaki equation (66) and the Tanaka equation (65). Both equations were developed using a Japanese population, and both were developed for use in a general population. Since they were developed, studies have sought to validate estimated 24-hour potassium excretion against measured 24-hour potassium excretion, and have generally found poor agreement between estimated and measured 24-hour urinary potassium excretion (134, 135). One study that examined multiple timed spot urine collections found that, regardless of equation and time of spot urine collection, estimated 24-hour potassium excretion produced biased estimates of measured 24-hour potassium excretion (136). The study found that the equations overestimated 24-hour potassium excretion at low levels and underestimated at high levels of actual 24-hour potassium excretion. Notably, no previous study has examined the relationship between estimated 24-hour potassium excretion and actual potassium intake. Given

that potassium intake is the true exposure of interest, this is a large gap in the literature on potassium assessment methods that needs to be filled.

Phosphorus Functions, Intake, and Role in Chronic Disease

Phosphorus Physiology and Functions in the Body

Phosphorus is important for a variety of functions in the body, including bone mineralization, cell signaling, energy metabolism, and the formation of the phospholipid bilayers in cell membranes, among others (137). Phosphate metabolism and homeostasis is regulated by complex hormonal processes. Under normal conditions, 60-70% of dietary phosphate is absorbed via both active and passive mechanisms in the small intestine (137, 138). Intestinal phosphorus absorption is regulated by 1,25-dihydroxyvitamin D, or $1,25(OH)_2D$. When phosphorus intake is low, $1,25(OH)_2D$ expression is increased, which leads to increased production of sodium-phosphate cotransporter 2b (NPT2b), which is the transport protein responsible for most of the active phosphate absorption in the small intestine (138). A low phosphate diet can also upregulate NPT2b independent of $1,25(OH)_2D$. Upregulation of NPT2b ultimately leads to an increase in phosphorus absorption. On the other hand, with high levels of dietary phosphorus, NPT2b is internalized into the cell, thus decreasing active phosphate absorption (138).

In addition to $1,25(OH)_2D$, parathyroid hormone (PTH) and fibroblast growth factor 23 (FGF23) are important hormones involved in the regulation of phosphorus metabolism. In response to high dietary or serum phosphate levels, FGF23 is produced by bone and PTH is produced by the parathyroid gland (137, 138). Both hormones act to decrease expression of sodium-phosphate cotransporters 2a and 2c (NPT2a and NPT2c) in renal tubular cells, resulting in decreased renal phosphate reabsorption and increased urinary phosphate excretion (137, 138). While PTH also acts to increase the formation of active $1,25(OH)_2D$ in the kidney, FGF23 inhibits this effect, resulting in a net decrease in serum $1,25(OH)_2D$ and a decrease in $1,25(OH)_2D$-induced intestinal phosphorus absorption.

Phosphorus Intake and Chronic Disease

The DRIs for phosphorus were established in 1997 (139). The Recommended Dietary Allowance (RDA) for adults was set at 700 mg/d based on the intake level required to maintain a

normal serum phosphate level. In addition, the UL for most adults was set at 4.0 g/d due to the potential risk of hyperphosphatemia with intakes above this level. Usual phosphorus intakes are much higher than the RDA; indeed, usual intake of phosphorus in adults is >1300 mg/d (114). Phosphorus is highly prevalent in the food supply both as naturally occurring phosphate as well as in the form of inorganic phosphorus-containing food additives. Top sources of dietary phosphorus include milk, cheese, meat, bread products, processed foods, and soft drinks (115).

Phosphorus intake becomes problematic in the context of CKD. Evidence suggests that high dietary phosphorus intake may be a risk factor for developing CKD in those with diabetes, who are already at increased risk for CKD (140). Phosphorus intake and serum phosphorus levels become even more problematic once a person develops CKD. Indeed, in those with CKD, elevated serum phosphorus levels are shown to be associated with increased risk of kidney failure and mortality (141). CKD causes disruption in the hormonal regulation of phosphorus metabolism, and research has shown this begins early in the disease process, before elevations in serum phosphorus levels are detected (142). Indeed, elevated FGF23 levels are observed early on, leading to decreasing levels of $1,25(OH)_2D$, which in turn results in uninhibited PTH production (i.e. secondary hyperparathyroidism) (143). All of these hormonal alterations are observed long before hyperphosphatemia develops, which often does not occur until advanced CKD (142, 143). These hormonal alterations in phosphorus (as well as calcium) metabolism are characteristic of CKD-mineral and bone disorder (CKD-MBD) (144). CKD-MBD is additionally characterized by bone disease and vascular calcification, which can ultimately lead to morbidity and mortality in patients with CKD (144).

Given the alterations in phosphorus homeostasis that occur, reducing phosphorus intake and/or absorption is an important treatment goal for patients with CKD. As such, the K/DOQI clinical guidelines recommend that phosphorus intake should be restricted to 800-1,000 mg/d based on CKD stage and serum phosphorus level (145). While this restriction is notably higher than the phosphorus RDA, it is also notably lower than the usual phosphorus intake of the general population. Prescribing phosphate binders is another common strategy for reducing overall phosphorus absorption (146), and research suggests these medications are effective at reducing serum phosphorus levels as well as urinary phosphorus excretion (147). Though not reflected in current dietary recommendations, targeting phosphorus absorption may be especially important earlier in the disease process. Given the decreasing levels of $1,25(OH)_2D$ observed

with disease progression, it would be expected that intestinal phosphorus absorption would be consequently decreased. For patients with advanced stage CKD, that seems to be the case (148). However, research using rodent models suggest that phosphorus absorption in moderate stage CKD is similar to that in healthy controls (149). Further research in this area, using controlled feeding studies and direct measures of phosphorus absorption, are needed.

Methods of Estimating Phosphorus Intake

Having accurate and convenient methods of estimating phosphorus intake is important to help patients with CKD and their clinicians monitor their intake and make dietary and pharmacological adjustments accordingly. Self-report (i.e. 24-hour recalls, food records, and food frequency questionnaires) are one monitoring strategy. However, these methods require comparing reported intake to a database in order to estimate intake. This may be problematic for phosphorus—databases may be unable to accurately estimate phosphorus intake contributed by inorganic phosphate additives in processed foods (139, 150). Indeed, a recent analysis found that phosphorus intake estimated from 4-day food records was significantly lower than 24-hour urinary phosphorus excretion (151). Twenty-four-hour urinary phosphorus excretion is often used as surrogate indicator of phosphorus intake. In a healthy adult, urinary phosphorus excretion is nearly equal to dietary phosphorus intake (139). However, this does not seem to be true in patients with CKD. A recent balance study in patients with stage 3-4 CKD found that 24-hour urine phosphorus did not match intake, and varied widely between subjects and from day-to-day within subjects (152). In addition, this study found that 24-hour urine phosphorus was not correlated with net phosphorus absorption, though it was negatively correlated with whole-body phosphorus retention (152). These findings suggest 24-hour urine phosphorus is not reflective of intake but is reflective of retention. In addition, the authors note that 24-hour urine phosphorus could still be used as an indicator of absorption in the context of an intervention but should be interpreted with caution in individuals and in observational studies.

Though 24-hour urinary phosphorus may be a useful tool for studying phosphorus homeostasis, 24-hour urine collections are burdensome. Therefore, studies have explored the use of a single spot urine sample as a surrogate indicator of 24-hour urinary phosphorus excretion for assessing phosphorus intake and overall phosphorus homeostasis. A study in healthy participants found a significant correlation between the spot urine phosphate-to-creatinine ratio in random

spot urine samples to 24-hour phosphate excretion (153). However, this relationship did not hold up in similar subsequent studies, including one in urinary stone formers (75) and one that included both healthy and CKD participants for a wide range of kidney functions (154). These findings suggest that normalizing spot urine phosphorus excretion to creatinine excretion does not provide an adequate indicator of 24-hour urinary phosphorus excretion in patients with altered kidney function. As with both sodium and potassium, an equation has been developed for calculating estimated 24-hour phosphorus excretion from a spot urine sample (155). This equation was developed for use in CKD stages 3-4, and validation of the equation found that estimated 24-hour urinary phosphorus excretion was significantly correlated with measured 24-hour urinary phosphorus excretion, and provided a much more accurate prediction than spot urine phosphate-to-creatinine ratio (155). However, before widespread use of this method as a substitute for 24-hour urine collections can be recommended, more research is needed to determine how estimated 24-hour urinary phosphorus excretion compares to phosphorus intake, phosphorus absorption, and whole-body phosphorus retention.

Importance of Measuring Sodium, Potassium, and Phosphorus Intake

Sodium, potassium, and phosphorus are essential nutrients that all have implications for chronic disease risk and progression. Therefore, measuring their intakes is important in research, clinical practice, and public health settings. There are many available methods for measuring all three minerals, some of which are easier to administer than others, and some of which are more accurate than others. One method that is used for all three minerals is the collection of a spot urine sample and the use of a prediction equation to calculate estimated 24-hour urinary mineral excretion. Importantly, the accuracy of this method when compared to actual mineral intake has not been explored for either sodium, potassium, or phosphorus. The remainder of this dissertation will focus on exploring the accuracy of estimated 24-hour mineral excretion in the context of a controlled feeding study in both healthy participants as well as those with CKD. These findings could have important implications regarding the ideal method for estimating mineral intake.

References

1. Fordtran JS, Rector FC, Jr., Carter NW. The mechanisms of sodium absorption in the human small intestine. J Clin Invest 1968;47(4):884-900. doi: 10.1172/jci105781.
2. Sandle GI. Salt and water absorption in the human colon: a modern appraisal. Gut 1998;43(2):294-9.
3. Cogswell ME, Maalouf J, Elliott P, Loria CM, Patel S, Bowman BA. Use of Urine Biomarkers to Assess Sodium Intake: Challenges and Opportunities. Annu Rev Nutr 2015;35:349-87. doi: 10.1146/annurev-nutr-071714-034322.
4. Palacios C, Wigertz K, Martin BR, Jackman L, Pratt JH, Peacock M, McCabe G, Weaver CM. Sodium retention in black and white female adolescents in response to salt intake. J Clin Endocrinol Metab 2004;89(4):1858-63. doi: 10.1210/jc.2003-031446.
5. Simpson FO. Sodium intake, body sodium, and sodium excretion. Lancet 1988;2(8601):25-9.
6. Stanhewicz AE, Kenney WL. Determinants of water and sodium intake and output. Nutr Rev 2015;73 Suppl 2:73-82. doi: 10.1093/nutrit/nuv033.
7. Schafer JA. Interaction of modeling and experimental approaches to understanding renal salt and water balance. Ann Biomed Eng 2000;28(8):1002-9.
8. Titze J. Sodium balance is not just a renal affair. Curr Opin Nephrol Hypertens 2014;23(2):101-5. doi: 10.1097/01.mnh.0000441151.55320.c3.
9. Alexy U, Cheng G, Libuda L, Hilbig A, Kersting M. 24 h-Sodium excretion and hydration status in children and adolescents--results of the DONALD Study. Clin Nutr 2012;31(1):78-84. doi: 10.1016/j.clnu.2011.08.014.
10. Kanbay M, Bayram Y, Solak Y, Sanders PW. Dietary potassium: a key mediator of the cardiovascular response to dietary sodium chloride. J Am Soc Hypertens 2013;7(5):395-400. doi: 10.1016/j.jash.2013.04.009.
11. Houston MC. The importance of potassium in managing hypertension. Curr Hypertens Rep 2011;13(4):309-17. doi: 10.1007/s11906-011-0197-8.
12. Liu F, Zheng S, Mu J, Chu C, Wang L, Wang Y, Xiao H, Wang D, Cao Y, Ren K, et al. Common variation in with no-lysine kinase 1 (WNK1) and blood pressure responses to dietary sodium or potassium interventions- family-based association study. Circ J 2013;77(1):169-74.

13. Brown WJ, Jr., Brown FK, Krishan I. Exchangeable sodium and blood volume in normotensive and hypertensive humans on high and low sodium intake. Circulation 1971;43(4):508-19.
14. Beretta-Piccoli C, Davies DL, Brown JJ, Ferriss B, Fraser R, Lasaridis A, Lever AF, Morton JJ, Robertson JI, Semple PF, et al. Relation of blood pressure with body and plasma electrolytes in Conn's syndrome. J Hypertens 1983;1(2):197-205.
15. Holbrook JT, Patterson KY, Bodner JE, Douglas LW, Veillon C, Kelsay JL, Mertz W, Smith JC, Jr. Sodium and potassium intake and balance in adults consuming self-selected diets. Am J Clin Nutr 1984;40(4):786-93. doi: 10.1093/ajcn/40.4.786.
16. Schachter J, Harper PH, Radin ME, Caggiula AW, McDonald RH, Diven WF. Comparison of sodium and potassium intake with excretion. Hypertension 1980;2(5):695-9.
17. Lerchl K, Rakova N, Dahlmann A, Rauh M, Goller U, Basner M, Dinges DF, Beck L, Agureev A, Larina I, et al. Agreement between 24-hour salt ingestion and sodium excretion in a controlled environment. Hypertension 2015;66(4):850-7. doi: 10.1161/hypertensionaha.115.05851.
18. Rakova N, Juttner K, Dahlmann A, Schroder A, Linz P, Kopp C, Rauh M, Goller U, Beck L, Agureev A, et al. Long-term space flight simulation reveals infradian rhythmicity in human Na(+) balance. Cell Metab 2013;17(1):125-31. doi: 10.1016/j.cmet.2012.11.013.
19. Benjamin EJ, Blaha MJ, Chiuve SE, Cushman M, Das SR, Deo R, de Ferranti SD, Floyd J, Fornage M, Gillespie C, et al. Heart Disease and Stroke Statistics-2017 Update: A Report From the American Heart Association. Circulation 2017;135(10):e146-e603. doi: 10.1161/cir.0000000000000485.
20. de Wardener HE, He FJ, MacGregor GA. Plasma sodium and hypertension. Kidney Int 2004;66(6):2454-66. doi: 10.1111/j.1523-1755.2004.66018.x.
21. Suckling RJ, He FJ, Markandu ND, MacGregor GA. Dietary salt influences postprandial plasma sodium concentration and systolic blood pressure. Kidney Int 2012;81(4):407-11. doi: 10.1038/ki.2011.369.

22. 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):319-28.
23. Elliott P, Marmot M, Dyer A, Joossens J, Kesteloot H, Stamler R, Stamler J, Rose G. The INTERSALT study: main results, conclusions and some implications. Clin Exp Hypertens A 1989;11(5-6):1025-34.
24. He FJ, MacGregor GA. Effect of modest salt reduction on blood pressure: a meta-analysis of randomized trials. Implications for public health. J Hum Hypertens 2002;16(11):761-70. doi: 10.1038/sj.jhh.1001459.
25. Svetkey LP, Sacks FM, Obarzanek E, Vollmer WM, Appel LJ, Lin PH, Karanja NM, Harsha DW, Bray GA, Aickin M, et al. The DASH Diet, Sodium Intake and Blood Pressure Trial (DASH-sodium): rationale and design. DASH-Sodium Collaborative Research Group. J Am Diet Assoc 1999;99(8 Suppl):S96-104.
26. Sacks FM, Svetkey LP, Vollmer WM, Appel LJ, Bray GA, Harsha D, Obarzanek E, Conlin PR, Miller ER, 3rd, Simons-Morton DG, 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):3-10. doi: 10.1056/nejm200101043440101.
27. Dietary Reference Intakes for Water, Potassium, Sodium, Chloride, and Sulfate. In: Food and Nutrition Board IoM, ed. Washington, D.C.: The National Academies Press, 2005.
28. National Academies of Sciences, Engineering, and Medicine. Dietary Reference Intakes for Sodium and Potassium. Washington, DC: The National Academies Press, 2019. https://doi.org/10.17226/25353.
29. National Academies of Sciences, Engineering, and Medicine 2017. Guiding Principles for Developing Dietary Reference Intakes Based on Chronic Disease.Washington, DC: The National Academies Press. https://doi.org/10.17226/24828.
30. Dietary Guidelines for Americans 2015-2020. In: U.S. Department of Agriculture USDoHaHS, ed. 8 ed. Washington, D.C., 2015.
31. Jones DW. Dietary sodium and blood pressure. Hypertension 2004;43(5):932-5. doi: 10.1161/01.HYP.0000126610.89002.c6.

32. Benjamin EJ, Muntner P, Alonso A, Bittencourt MS, Callaway CW, Carson AP, Chamberlain AM, Chang AR, Cheng S, Das SR, et al. Heart Disease and Stroke Statistics-2019 Update: A Report From the American Heart Association. Circulation 2019;139(10):e56-e66. doi: 10.1161/cir.0000000000000659.
33. Mennuni S, Rubattu S, Pierelli G, Tocci G, Fofi C, Volpe M. Hypertension and kidneys: unraveling complex molecular mechanisms underlying hypertensive renal damage. Journal of Human Hypertension 2014;28(2):74-9. doi: 10.1038/jhh.2013.55.
34. Liu N, Sun W, Xing Z, Ma F, Sun T, Wu H, Dong Y, Xu Z, Fu Y, Yuan H. Association between sodium intakes with the risk of chronic kidney disease: evidence from a meta-analysis. Int J Clin Exp Med 2015;8(11):20939-45.
35. Mills KT, Chen J, Yang W, Appel LJ, Kusek JW, Alper A, Delafontaine P, Keane MG, Mohler E, Ojo A, et al. Sodium Excretion and the Risk of Cardiovascular Disease in Patients With Chronic Kidney Disease. Jama 2016;315(20):2200-10. doi: 10.1001/jama.2016.4447.
36. He J, Mills KT, Appel LJ, Yang W, Chen J, Lee BT, Rosas SE, Porter A, Makos G, Weir MR, et al. Urinary Sodium and Potassium Excretion and CKD Progression. J Am Soc Nephrol 2016;27(4):1202-12. doi: 10.1681/asn.2015010022.
37. McMahon EJ, Bauer JD, Hawley CM, Isbel NM, Stowasser M, Johnson DW, Campbell KL. A randomized trial of dietary sodium restriction in CKD. J Am Soc Nephrol 2013;24(12):2096-103. doi: 10.1681/ASN.2013030285.
38. Garofalo C, Borrelli S, Provenzano M, De Stefano T, Vita C, Chiodini P, Minutolo R, De Nicola L, Conte G. Dietary Salt Restriction in Chronic Kidney Disease: A Meta-Analysis of Randomized Clinical Trials. Nutrients 2018;10(6). doi: 10.3390/nu10060732.
39. Jardine MJ, Li N, Ninomiya T, Feng X, Zhang J, Shi J, Zhang Y, Zhang R, Hao J, Perkovic V, et al. Dietary Sodium Reduction Reduces Albuminuria: A Cluster Randomized Trial. J Ren Nutr 2019;29(4):276-84. doi: 10.1053/j.jrn.2018.10.009.
40. Kidney Disease: Improving Global Outcomes (KDIGO) CKD Work Group. KDIGO clinical practice guideline for the evaluation and management of chronic kidney disease.Kidney Int Suppl2013;3:1-150.

41. Graudal N, Jurgens G, Baslund B, Alderman MH. Compared with usual sodium intake, low- and excessive-sodium diets are associated with increased mortality: a meta-analysis. Am J Hypertens 2014;27(9):1129-37. doi: 10.1093/ajh/hpu028.
42. Mente A, O'Donnell M, Rangarajan S, Dagenais G, Lear S, McQueen M, Diaz R, Avezum A, Lopez-Jaramillo P, Lanas F, et al. Associations of urinary sodium excretion with cardiovascular events in individuals with and without hypertension: a pooled analysis of data from four studies. Lancet 2016;388(10043):465-75. doi: 10.1016/s0140-6736(16)30467-6.
43. O'Donnell MJ, Yusuf S, Mente A, Gao P, Mann JF, Teo K, McQueen M, Sleight P, Sharma AM, Dans A, et al. Urinary sodium and potassium excretion and risk of cardiovascular events. Jama 2011;306(20):2229-38. doi: 10.1001/jama.2011.1729.
44. O'Donnell M, Mente A, Rangarajan S, McQueen MJ, Wang X, Liu L, Yan H, Lee SF, Mony P, Devanath A, et al. Urinary sodium and potassium excretion, mortality, and cardiovascular events. N Engl J Med 2014;371(7):612-23. doi: 10.1056/NEJMoa1311889.
45. Thomas MC, Moran J, Forsblom C, Harjutsalo V, Thorn L, Ahola A, Waden J, Tolonen N, Saraheimo M, Gordin D, et al. The association between dietary sodium intake, ESRD, and all-cause mortality in patients with type 1 diabetes. Diabetes Care 2011;34(4):861-6. doi: 10.2337/dc10-1722.
46. He FJ, Campbell NRC, Ma Y, MacGregor GA, Cogswell ME, Cook NR. Errors in estimating usual sodium intake by the Kawasaki formula alter its relationship with mortality: implications for public health. Int J Epidemiol 2018;47(6):1784-95. doi: 10.1093/ije/dyy114.
47. He FJ, Ma Y, Campbell NRC, MacGregor GA, Cogswell ME, Cook NR. Formulas to Estimate Dietary Sodium Intake From Spot Urine Alter Sodium-Mortality Relationship. Hypertension 2019;74(3):572-80. doi: 10.1161/hypertensionaha.119.13117.
48. Campbell NRC, He FJ, Tan M, Cappuccio FP, Neal B, Woodward M, Cogswell ME, McLean R, Arcand J, MacGregor G, et al. The International Consortium for Quality Research on Dietary Sodium/Salt (TRUE) position statement on the use of 24-hour, spot, and short duration (<24 hours) timed urine collections to assess dietary sodium intake. J Clin Hypertens (Greenwich) 2019. doi: 10.1111/jch.13551.

49. McLean RM. Measuring population sodium intake: a review of methods. Nutrients 2014;6(11):4651-62. doi: 10.3390/nu6114651.
50. Rhodes DG, Murayi T, Clemens JC, Baer DJ, Sebastian RS, Moshfegh AJ. The USDA Automated Multiple-Pass Method accurately assesses population sodium intakes. Am J Clin Nutr 2013;97(5):958-64. doi: 10.3945/ajcn.112.044982.
51. Conkle J, van der Haar F. The Use and Interpretation of Sodium Concentrations in Casual (Spot) Urine Collections for Population Surveillance and Partitioning of Dietary Iodine Intake Sources. Nutrients 2016;9(1). doi: 10.3390/nu9010007.
52. Terry AL, Cogswell ME, Wang CY, Chen TC, Loria CM, Wright JD, Zhang X, Lacher DA, Merritt RK, Bowman BA. Feasibility of collecting 24-h urine to monitor sodium intake in the National Health and Nutrition Examination Survey. Am J Clin Nutr 2016;104(2):480-8. doi: 10.3945/ajcn.115.121954.
53. Campino C, Hill C, Baudrand R, Martinez-Aguayo A, Aglony M, Carrasco CA, Ferrada C, Loureiro C, Vecchiola A, Bancalari R, et al. Usefulness and Pitfalls in Sodium Intake Estimation: Comparison of Dietary Assessment and Urinary Excretion in Chilean Children and Adults. Am J Hypertens 2016;29(10):1212-7. doi: 10.1093/ajh/hpw056.
54. Sasaki S, Yanagibori R, Amano K. Validity of a self-administered diet history questionnaire for assessment of sodium and potassium: comparison with single 24-hour urinary excretion. Jpn Circ J 1998;62(6):431-5.
55. Murakami K, Livingstone MB, Sasaki S, Uenishi K. Ability of self-reported estimates of dietary sodium, potassium and protein to detect an association with general and abdominal obesity: comparison with the estimates derived from 24 h urinary excretion. Br J Nutr 2015;113(8):1308-18. doi: 10.1017/s0007114515000495.
56. Micheli ET, Rosa AA. Estimation of sodium intake by urinary excretion and dietary records in children and adolescents from Porto Alegre, Brazil: a comparision of two methods. Nutrition Research 2003;23(11):1477-87. doi: https://doi.org/10.1016/S0271-5317(03)00157-X.
57. Moore LB, Liu SV, Halliday TM, Neilson AP, Hedrick VE, Davy BM. Urinary Excretion of Sodium, Nitrogen, and Sugar Amounts Are Valid Biomarkers of Dietary Sodium, Protein, and High Sugar Intake in Nonobese Adolescents. J Nutr 2017. doi: 10.3945/jn.117.256875.

58. Mente A, O'Donnell MJ, Rangarajan S, McQueen MJ, Poirier P, Wielgosz A, Morrison H, Li W, Wang X, Di C, et al. Association of urinary sodium and potassium excretion with blood pressure. N Engl J Med 2014;371(7):601-11. doi: 10.1056/NEJMoa1311989.
59. Cook NR, Kumanyika SK, Cutler JA. Effect of change in sodium excretion on change in blood pressure corrected for measurement error. The Trials of Hypertension Prevention, Phase I. Am J Epidemiol 1998;148(5):431-44.
60. Cogswell ME, Elliott P, Wang CY, Rhodes DG, Pfeiffer CM, Loria CM. Assessing U.S. sodium intake through dietary data and urine biomarkers. Adv Nutr 2013;4(5):560-2. doi: 10.3945/an.113.004309.
61. Ji C, Sykes L, Paul C, Dary O, Legetic B, Campbell NR, Cappuccio FP. Systematic review of studies comparing 24-hour and spot urine collections for estimating population salt intake. Rev Panam Salud Publica 2012;32(4):307-15.
62. Brown IJ, Dyer AR, Chan Q, Cogswell ME, Ueshima H, Stamler J, Elliott P. Estimating 24-hour urinary sodium excretion from casual urinary sodium concentrations in Western populations: the INTERSALT study. Am J Epidemiol 2013;177(11):1180-92. doi: 10.1093/aje/kwt066.
63. Subramanian S, Teo BW, Toh QC, Koh YY, Li J, Sethi S, Lee EJ. Spot urine tests in predicting 24-hour urine sodium excretion in Asian patients. J Ren Nutr 2013;23(6):450-5. doi: 10.1053/j.jrn.2012.12.004.
64. Ji C, Miller MA, Venezia A, Strazzullo P, Cappuccio FP. Comparisons of spot vs 24-h urine samples for estimating population salt intake: validation study in two independent samples of adults in Britain and Italy. Nutr Metab Cardiovasc Dis 2014;24(2):140-7. doi: 10.1016/j.numecd.2013.06.011.
65. Tanaka T, Okamura T, Miura K, Kadowaki T, Ueshima H, Nakagawa H, Hashimoto T. A simple method to estimate populational 24-h urinary sodium and potassium excretion using a casual urine specimen. J Hum Hypertens 2002;16(2):97-103. doi: 10.1038/sj.jhh.1001307.
66. Kawasaki T, Itoh K, Uezono K, Sasaki H. A simple method for estimating 24 h urinary sodium and potassium excretion from second morning voiding urine specimen in adults. Clin Exp Pharmacol Physiol 1993;20(1):7-14.

67. Nerbass FB, Pecoits-Filho R, McIntyre NJ, McIntyre CW, Taal MW. Development of a formula for estimation of sodium intake from spot urine in people with chronic kidney disease. Nephron Clin Pract 2014;128(1-2):61-6. doi: 10.1159/000363297.
68. Nerbass FB, Hallvass AE, Taal MW, Pecoits-Filho R. Formula to detect high sodium excretion from spot urine in chronic kidney disease patients. J Bras Nefrol 2017;39(1):23-8. doi: 10.5935/0101-2800.20170004.
69. Wang CY, Carriquiry AL, Chen TC, Loria CM, Pfeiffer CM, Liu K, Sempos CT, Perrine CG, Cogswell ME. Estimating the population distribution of usual 24-hour sodium excretion from timed urine void specimens using a statistical approach accounting for correlated measurement errors. J Nutr 2015;145(5):1017-24. doi: 10.3945/jn.114.206250.
70. Whitton C, Gay GM, Lim RB, Tan LW, Lim WY, van Dam RM. Evaluation of Equations for Predicting 24-Hour Urinary Sodium Excretion from Casual Urine Samples in Asian Adults. J Nutr 2016;146(8):1609-15. doi: 10.3945/jn.116.232108.
71. Rhee MY, Kim JH, Shin SJ, Gu N, Nah DY, Hong KS, Cho EJ, Sung KC. Estimation of 24-hour urinary sodium excretion using spot urine samples. Nutrients 2014;6(6):2360-75. doi: 10.3390/nu6062360.
72. Rhee MY, Kim JH, Shin SJ, Gu N, Nah DY, Park JH, Kim SW, Kim HJ, Oh KW, Lee SY. Estimating 24-Hour Urine Sodium From Multiple Spot Urine Samples. J Clin Hypertens (Greenwich) 2017;19(4):431-8. doi: 10.1111/jch.12922.
73. McLean R, Williams S, Mann J. Monitoring population sodium intake using spot urine samples: validation in a New Zealand population. J Hum Hypertens 2014;28(11):657-62. doi: 10.1038/jhh.2014.10.
74. Cogswell ME, Wang CY, Chen TC, Pfeiffer CM, Elliott P, Gillespie CD, Carriquiry AL, Sempos CT, Liu K, Perrine CG, et al. Validity of predictive equations for 24-h urinary sodium excretion in adults aged 18-39 y. Am J Clin Nutr 2013;98(6):1502-13. doi: 10.3945/ajcn.113.059436.
75. Hong YH, Dublin N, Razack AH, Mohd MA, Husain R. Twenty-four hour and spot urine metabolic evaluations: correlations versus agreements. Urology 2010;75(6):1294-8. doi: 10.1016/j.urology.2009.08.061.
76. Luft FC, Fineberg NS, Sloan RS. Overnight urine collections to estimate sodium intake. Hypertension 1982;4(4):494-8.

77. Luft FC, Fineberg NS, Sloan RS. Estimating dietary sodium intake in individuals receiving a randomly fluctuating intake. Hypertension 1982;4(6):805-8.
78. Pfeiffer CM, Hughes JP, Cogswell ME, Burt VL, Lacher DA, Lavoie DJ, Rabinowitz DJ, Johnson CL, Pirkle JL. Urine sodium excretion increased slightly among U.S. adults between 1988 and 2010. J Nutr 2014;144(5):698-705. doi: 10.3945/jn.113.187914.
79. Mann SJ, Gerber LM. Estimation of 24-hour sodium excretion from spot urine samples. J Clin Hypertens (Greenwich) 2010;12(3):174-80. doi: 10.1111/j.1751-7176.2009.00241.x.
80. Wang CY, Cogswell ME, Loria CM, Chen TC, Pfeiffer CM, Swanson CA, Caldwell KL, Perrine CG, Carriquiry AL, Liu K, et al. Urinary excretion of sodium, potassium, and chloride, but not iodine, varies by timing of collection in a 24-hour calibration study. J Nutr 2013;143(8):1276-82. doi: 10.3945/jn.113.175927.
81. Mayersohn M, Conrad KA, Achari R. The influence of a cooked meat meal on creatinine plasma concentration and creatinine clearance. Br J Clin Pharmacol 1983;15(2):227-30.
82. Weaver CM, Martin BR, McCabe GP, McCabe LD, Woodward M, Anderson CA, Appel LJ. Individual variation in urinary sodium excretion among adolescent girls on a fixed intake. J Hypertens 2016;34(7):1290-7. doi: 10.1097/hjh.0000000000000960.
83. Liu K, Cooper R, McKeever J, McKeever P, Byington R, Soltero I, Stamler R, Gosch F, Stevens E, Stamler J. Assessment of the association between habitual salt intake and high blood pressure: methodological problems. Am J Epidemiol 1979;110(2):219-26.
84. Zhou L, Tian Y, Fu JJ, Jiang YY, Bai YM, Zhang ZH, Hu XH, Lian HW, Guo M, Yang ZX, et al. Validation of spot urine in predicting 24-h sodium excretion at the individual level. Am J Clin Nutr 2017;105(6):1291-6. doi: 10.3945/ajcn.116.147553.
85. Kang SS, Kang EH, Kim SO, Lee MS, Hong CD, Kim SB. Use of mean spot urine sodium concentrations to estimate daily sodium intake in patients with chronic kidney disease. Nutrition 2012;28(3):256-61. doi: 10.1016/j.nut.2011.06.006.
86. Dougher CE, Rifkin DE, Anderson CA, Smits G, Persky MS, Block GA, Ix JH. Spot urine sodium measurements do not accurately estimate dietary sodium intake in chronic kidney disease. Am J Clin Nutr 2016;104(2):298-305. doi: 10.3945/ajcn.115.127423.
87. Wedler B, Brier ME, Wiersbitzky M, Gruska S, Wolf E, Kallwellis R, Aronoff GR, Luft FC. Sodium kinetics in salt-sensitive and salt-resistant normotensive and hypertensive subjects. J Hypertens 1992;10(7):663-9.

88. Kawamura M, Kawasaki T. Clinical application of the second morning urine method for estimating salt intake in patients with hypertension. Clin Exp Hypertens 2015;37(2):89-96. doi: 10.3109/10641963.2014.913601.
89. Arakawa K, Sakaki M, Sakata S, Oniki H, Tominaga M, Tsuchihashi T. Variability of urinary salt excretion estimated by spot urine in treated hypertensive patients. Clin Exp Hypertens 2015;37(6):445-8. doi: 10.3109/10641963.2015.1057831.
90. Allen NB, Zhao L, Loria CM, Van Horn L, Wang CY, Pfeiffer CM, Cogswell ME, Wright J, Liu K. The Validity of Predictive Equations to Estimate 24-Hour Sodium Excretion: The MESA and CARDIA Urinary Sodium Study. Am J Epidemiol 2017;186(2):149-59. doi: 10.1093/aje/kwx056.
91. Falkner B. Hypertension in children and adolescents: epidemiology and natural history. Pediatr Nephrol 2010;25(7):1219-24. doi: 10.1007/s00467-009-1200-3.
92. Chen X, Wang Y. Tracking of blood pressure from childhood to adulthood: a systematic review and meta-regression analysis. Circulation 2008;117(25):3171-80. doi: 10.1161/circulationaha.107.730366.
93. Liu K, Cooper R, Soltero I, Stamler J. Variability in 24-hour urine sodium excretion in children. Hypertension 1979;1(6):631-6.
94. Savoca MR, Domel Baxter S, Ludwig DA, Evans CD, Mackey ML, Wilson ME, Hanevold C, Harshfield GA. A 4-day sodium-controlled diet reduces variability of overnight sodium excretion in free-living normotensive adolescents. J Am Diet Assoc 2007;107(3):490-4. doi: 10.1016/j.jada.2006.12.005.
95. Brier ME, Luft FC. Sodium kinetics in white and black normotensive subjects: possible relevance to salt-sensitive hypertension. Am J Med Sci 1994;307 Suppl 1:S38-42.
96. Mizehoun-Adissoda C, Houehanou C, Chianea T, Dalmay F, Bigot A, Preux PM, Bovet P, Houinato D, Desport JC. Estimation of Daily Sodium and Potassium Excretion Using Spot Urine and 24-Hour Urine Samples in a Black Population (Benin). J Clin Hypertens (Greenwich) 2016;18(7):634-40. doi: 10.1111/jch.12722.
97. Sheng HP. Sodium, chloride, and potassium. 3rd ed. In: Stipanuk MH and Caudill MA ed. Biochemical, physiological, and molecular aspects of human nutrition. St. Louis, MO: Elsevier, 2013:759-780.

98. Bia MJ, DeFronzo RA. Extrarenal potassium homeostasis. Am J Physiol 1981;240(4):F257-68. doi: 10.1152/ajprenal.1981.240.4.F257.
99. Palmer BF. Regulation of Potassium Homeostasis. Clin J Am Soc Nephrol 2015;10(6):1050-60. doi: 10.2215/cjn.08580813.
100. Binia A, Jaeger J, Hu Y, Singh A, Zimmermann D. 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):1509-20. doi: 10.1097/hjh.0000000000000611.
101. Geleijnse JM, Kok FJ, Grobbee DE. Blood pressure response to changes in sodium and potassium intake: a metaregression analysis of randomised trials. J Hum Hypertens 2003;17(7):471-80. doi: 10.1038/sj.jhh.1001575.
102. Haddy FJ, Vanhoutte PM, Feletou M. Role of potassium in regulating blood flow and blood pressure. Am J Physiol Regul Integr Comp Physiol 2006;290(3):R546-52. doi: 10.1152/ajpregu.00491.2005.
103. Penton D, Czogalla J, Loffing J. Dietary potassium and the renal control of salt balance and blood pressure. Pflugers Arch 2015;467(3):513-30. doi: 10.1007/s00424-014-1673-1.
104. Terker AS, Zhang C, McCormick JA, Lazelle RA, Meermeier NP, Siler DA, Park HJ, Fu Y, Cohen DM, Weinstein AM, et al. Potassium modulates electrolyte balance and blood pressure through effects on distal cell voltage and chloride. Cell Metab 2015;21(1):39-50. doi: 10.1016/j.cmet.2014.12.006.
105. Stamler J, Chan Q, Daviglus ML, Dyer AR, Van Horn L, Garside DB, Miura K, Wu Y, Ueshima H, Zhao L, 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):631-7. doi: 10.1161/hypertensionaha.117.09928.
106. Mirmiran P, Bahadoran Z, Nazeri P, Azizi F. Dietary sodium to potassium ratio and the incidence of hypertension and cardiovascular disease: A population-based longitudinal study. Clin Exp Hypertens 2018;40(8):772-9. doi: 10.1080/10641963.2018.1431261.
107. Cook NR, Obarzanek E, Cutler JA, Buring JE, Rexrode KM, Kumanyika SK, Appel LJ, Whelton PK. Joint effects of sodium and potassium intake on subsequent cardiovascular disease: the Trials of Hypertension Prevention follow-up study. Arch Intern Med 2009;169(1):32-40. doi: 10.1001/archinternmed.2008.523.

108. Okayama A, Okuda N, Miura K, Okamura T, Hayakawa T, Akasaka H, Ohnishi H, Saitoh S, Arai Y, Kiyohara Y, et al. Dietary sodium-to-potassium ratio as a risk factor for stroke, cardiovascular disease and all-cause mortality in Japan: the NIPPON DATA80 cohort study. BMJ Open 2016;6(7):e011632. doi: 10.1136/bmjopen-2016-011632.
109. Xing X, Liu F, Yang X, Huang C, Zhang D, Chen S, Chen J, Li J, Liu Z, Lu F, et al. Central Blood Pressure Responses to Dietary Sodium and Potassium Interventions. Am J Hypertens 2018;31(5):582-9. doi: 10.1093/ajh/hpx209.
110. Chalmers J, Morgan T, Doyle A, Dickson B, Hopper J, Mathews J, Matthews G, Moulds R, Myers J, Nowson C, et al. Australian National Health and Medical Research Council dietary salt study in mild hypertension. J Hypertens Suppl 1986;4(6):S629-37.
111. Morris RC, Jr., Sebastian A, Forman A, Tanaka M, Schmidlin O. Normotensive salt sensitivity: effects of race and dietary potassium. Hypertension 1999;33(1):18-23.
112. Weaver CM, Stone MS, Lobene AJ, Cladis DP, Hodges JK. What Is the Evidence Base for a Potassium Requirement? Nutr Today 2018;53(5):184-95. doi: 10.1097/nt.0000000000000298.
113. World Health Organization. Guideline: Potassium Intake for Adults and Children. Geneva, Switzerland: World Health Organization; 2012.
114. Fulgoni VL, 3rd, Keast DR, Bailey RL, Dwyer J. Foods, fortificants, and supplements: Where do Americans get their nutrients? J Nutr 2011;141(10):1847-54. doi: 10.3945/jn.111.142257.
115. O'Neil CE, Keast DR, Fulgoni VL, Nicklas TA. Food sources of energy and nutrients among adults in the US: NHANES 2003-2006. Nutrients 2012;4(12):2097-120. doi: 10.3390/nu4122097.
116. Department of Health and Human Services, Food and Drug Administration. Food Labeling: Revision of the Nutrition and Supplement Facts Labels. 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 Rules. Federal Register 27 May, 2016.

117. Sharma S, McFann K, Chonchol M, de Boer IH, Kendrick J. Association between dietary sodium and potassium intake with chronic kidney disease in US adults: a cross-sectional study. Am J Nephrol 2013;37(6):526-33. doi: 10.1159/000351178.
118. Kieneker LM, Bakker SJ, de Boer RA, Navis GJ, Gansevoort RT, Joosten MM. Low potassium excretion but not high sodium excretion is associated with increased risk of developing chronic kidney disease. Kidney Int 2016;90(4):888-96. doi: 10.1016/j.kint.2016.07.012.
119. Morris A, Krishnan N, Kimani PK, Lycett D. Effect of Dietary Potassium Restriction on Serum Potassium, Disease Progression, and Mortality in Chronic Kidney Disease: A Systematic Review and Meta-Analysis. J Ren Nutr 2019. doi: 10.1053/j.jrn.2019.09.009.
120. DuBose TD, Jr. Regulation of Potassium Homeostasis in CKD. Adv Chronic Kidney Dis 2017;24(5):305-14. doi: 10.1053/j.ackd.2017.06.002.
121. Gilligan S, Raphael KL. Hyperkalemia and Hypokalemia in CKD: Prevalence, Risk Factors, and Clinical Outcomes. Adv Chronic Kidney Dis 2017;24(5):315-8. doi: 10.1053/j.ackd.2017.06.004.
122. Betts KA, Woolley JM, Mu F, McDonald E, Tang W, Wu EQ. The prevalence of hyperkalemia in the United States. Curr Med Res Opin 2018;34(6):971-8. doi: 10.1080/03007995.2018.1433141.
123. Academy of Nutrition and Dietetics Evidence Analysis Library. Chronic Kidney Disease (CKD) Guideline (2010).
Internet: https://www.andeal.org/topic.cfm?menu=5303&cat=3927 (accessed 20 February 2020).
124. Leonberg-Yoo AK, Tighiouart H, Levey AS, Beck GJ, Sarnak MJ. Urine Potassium Excretion, Kidney Failure, and Mortality in CKD. Am J Kidney Dis 2017;69(3):341-9. doi: 10.1053/j.ajkd.2016.03.431.
125. De Nicola L, Di Lullo L, Paoletti E, Cupisti A, Bianchi S. Chronic hyperkalemia in non-dialysis CKD: controversial issues in nephrology practice. J Nephrol 2018;31(5):653-64. doi: 10.1007/s40620-018-0502-6.
126. Cases A, Cigarran-Guldris S, Mas S, Gonzalez-Parra E. Vegetable-Based Diets for Chronic Kidney Disease? It Is Time to Reconsider. Nutrients 2019;11(6). doi: 10.3390/nu11061263.

127. Gritter M, Vogt L, Yeung SMH, Wouda RD, Ramakers CRB, de Borst MH, Rotmans JI, Hoorn EJ. Rationale and Design of a Randomized Placebo-Controlled Clinical Trial Assessing the Renoprotective Effects of Potassium Supplementation in Chronic Kidney Disease. Nephron 2018;140(1):48-57. doi: 10.1159/000490261.
128. Freedman LS, Commins JM, Moler JE, Willett W, Tinker LF, Subar AF, Spiegelman D, Rhodes D, Potischman N, Neuhouser ML, et al. Pooled results from 5 validation studies of dietary self-report instruments using recovery biomarkers for potassium and sodium intake. Am J Epidemiol 2015;181(7):473-87. doi: 10.1093/aje/kwu325.
129. Park Y, Dodd KW, Kipnis V, Thompson FE, Potischman N, Schoeller DA, Baer DJ, Midthune D, Troiano RP, Bowles H, et al. Comparison of self-reported dietary intakes from the Automated Self-Administered 24-h recall, 4-d food records, and food-frequency questionnaires against recovery biomarkers. Am J Clin Nutr 2018;107(1):80-93. doi: 10.1093/ajcn/nqx002.
130. Va P, Dodd KW, Zhao L, Thompson-Paul AM, Mercado CI, Terry AL, Jackson SL, Wang CY, Loria CM, Moshfegh AJ, et al. Evaluation of measurement error in 24-hour dietary recall for assessing sodium and potassium intake among US adults - National Health and Nutrition Examination Survey (NHANES), 2014. Am J Clin Nutr 2019;109(6):1672-82. doi: 10.1093/ajcn/nqz044.
131. Huang Y, Van Horn L, Tinker LF, Neuhouser ML, Carbone L, Mossavar-Rahmani Y, Thomas F, Prentice RL. Measurement error corrected sodium and potassium intake estimation using 24-hour urinary excretion. Hypertension 2014;63(2):238-44. doi: 10.1161/hypertensionaha.113.02218.
132. Subar AF, Freedman LS, Tooze JA, Kirkpatrick SI, Boushey C, Neuhouser ML, Thompson FE, Potischman N, Guenther PM, Tarasuk V, et al. Addressing Current Criticism Regarding the Value of Self-Report Dietary Data. J Nutr 2015;145(12):2639-45. doi: 10.3945/jn.115.219634.
133. Tasevska N, Runswick SA, Bingham SA. Urinary potassium is as reliable as urinary nitrogen for use as a recovery biomarker in dietary studies of free living individuals. J Nutr 2006;136(5):1334-40. doi: 10.1093/jn/136.5.1334.

134. Hooft van Huysduynen EJ, Hulshof PJ, van Lee L, Geelen A, Feskens EJ, van 't Veer P, van Woerkum CJ, de Vries JH. Evaluation of using spot urine to replace 24 h urine sodium and potassium excretions. Public Health Nutr 2014;17(11):2505-11. doi: 10.1017/s1368980014001177.
135. Polonia J, Lobo MF, Martins L, Pinto F, Nazare J. Estimation of populational 24-h urinary sodium and potassium excretion from spot urine samples: evaluation of four formulas in a large national representative population. J Hypertens 2017;35(3):477-86. doi: 10.1097/hjh.0000000000001180.
136. Mercado CI, Cogswell ME, Loria CM, Liu K, Allen N, Gillespie C, Wang CY, de Boer IH, Wright J. Validity of predictive equations for 24-h urinary potassium excretion based on timing of spot urine collection among adults: the MESA and CARDIA Urinary Sodium Study and NHANES Urinary Sodium Calibration Study. Am J Clin Nutr 2018;108(3):532-47. doi: 10.1093/ajcn/nqy138.
137. Shapses SA. Calcium and phosphorus. 3rd. ed. In: Stipanuk MH and Caudill MA, eds. Biochemical, physiological, and molecular aspects of human nutrition. St. Louis, MO: Elsevier, 2013:721-746.
138. DiMeglio LA, Imel EA. Calcium and phosphate: hormonal regulation and metabolism. 1st ed. In: Burr DB and Allen MR, eds. Basic and applied bone biology. London, UK, Waltham, MA, and San Diego, CA: Elsevier, 2014:261-282.
139. Institute of Medicine 1997. Dietary Reference Intakes for Calcium, Phosphorus, Magnesium, Vitamin D, and Fluoride. Washington, DC: The National Academies Press. https://doi.org/10.17226/5776.
140. Yoon CY, Park JT, Jhee JH, Noh J, Kee YK, Seo C, Lee M, Cha MU, Kim H, Park S, et al. High dietary phosphorus density is a risk factor for incident chronic kidney disease development in diabetic subjects: a community-based prospective cohort study. Am J Clin Nutr 2017;106(1):311-21. doi: 10.3945/ajcn.116.151654.
141. Da J, Xie X, Wolf M, Disthabanchong S, Wang J, Zha Y, Lv J, Zhang L, Wang H. Serum Phosphorus and Progression of CKD and Mortality: A Meta-analysis of Cohort Studies. Am J Kidney Dis 2015;66(2):258-65. doi: 10.1053/j.ajkd.2015.01.009.

142. Isakova T, Wahl P, Vargas GS, Gutierrez OM, Scialla J, Xie H, Appleby D, Nessel L, Bellovich K, Chen J, et al. Fibroblast growth factor 23 is elevated before parathyroid hormone and phosphate in chronic kidney disease. Kidney Int 2011;79(12):1370-8. doi: 10.1038/ki.2011.47.
143. Wolf M. Mineral (Mal)Adaptation to Kidney Disease--Young Investigator Award Address: American Society of Nephrology Kidney Week 2014. Clin J Am Soc Nephrol 2015;10(10):1875-85. doi: 10.2215/cjn.04430415.
144. Moe S, Drueke T, Cunningham J, Goodman W, Martin K, Olgaard K, Ott S, Sprague S, Lameire N, Eknoyan G. Definition, evaluation, and classification of renal osteodystrophy: a position statement from Kidney Disease: Improving Global Outcomes (KDIGO). Kidney Int 2006;69(11):1945-53. doi: 10.1038/sj.ki.5000414.
145. K/DOQI clinical practice guidelines for bone metabolism and disease in chronic kidney disease. Am J Kidney Dis 2003;42(4 Suppl 3):S1-201.
146. Floege J. Phosphate binders in chronic kidney disease: a systematic review of recent data. J Nephrol 2016;29(3):329-40. doi: 10.1007/s40620-016-0266-9.
147. Block GA, Wheeler DC, Persky MS, Kestenbaum B, Ketteler M, Spiegel DM, Allison MA, Asplin J, Smits G, Hoofnagle AN, et al. Effects of phosphate binders in moderate CKD. J Am Soc Nephrol 2012;23(8):1407-15. doi: 10.1681/asn.2012030223.
148. Farrington K, Mohammed MN, Newman SP, Varghese Z, Moorhead JF. Comparison of radioisotope methods for the measurement of phosphate absorption in normal subjects and in patients with chronic renal failure. Clin Sci (Lond) 1981;60(1):55-63. doi: 10.1042/cs0600055.
149. Loghman-Adham M. Renal and intestinal Pi transport adaptation to low phosphorus diet in uremic rats. J Am Soc Nephrol 1993;3(12):1930-7.
150. Sullivan CM, Leon JB, Sehgal AR. Phosphorus-containing food additives and the accuracy of nutrient databases: implications for renal patients. J Ren Nutr 2007;17(5):350-4. doi: 10.1053/j.jrn.2007.05.008.
151. Shinozaki N, Murakami K, Asakura K, Uechi K, Kobayashi S, Masayasu S, Sasaki S. Dietary phosphorus intake estimated by 4-day dietary records and two 24-hour urine collections and their associated factors in Japanese adults. European Journal of Clinical Nutrition 2018;72(4):517-25. doi: 10.1038/s41430-018-0114-1.

152. Stremke ER, McCabe LD, McCabe GP, Martin BR, Moe SM, Weaver CM, Peacock M, Hill Gallant KM. Twenty-Four-Hour Urine Phosphorus as a Biomarker of Dietary Phosphorus Intake and Absorption in CKD: A Secondary Analysis from a Controlled Diet Balance Study. Clin J Am Soc Nephrol 2018;13(7):1002-12. doi: 10.2215/cjn.00390118.
153. Gokce C, Gokce O, Baydinc C, Ilhan N, Alasehirli E, Ozkucuk F, Tasci M, Atilkeler MK, Celebi H, Arslan N. Use of random urine samples to estimate total urinary calcium and phosphate excretion. Arch Intern Med 1991;151(8):1587-8. doi: 10.1001/archinte.1991.00400080083015.
154. Tan SJ, Smith ER, Cai MM, Holt SG, Hewitson TD, Toussaint ND. Relationship between timed and spot urine collections for measuring phosphate excretion. Int Urol Nephrol 2016;48(1):115-24. doi: 10.1007/s11255-015-1149-z.
155. Robinson-Cohen C, Ix JH, Smits G, Persky M, Chertow GM, Block GA, Kestenbaum BR. Estimation of 24-hour urine phosphate excretion from spot urine collection: development of a predictive equation. J Ren Nutr 2014;24(3):194-9. doi: 10.1053/j.jrn.2014.02.001.

CHAPTER 2: COMPARING ESTIMATED 24-HOUR URINARY SODIUM EXCRETION AGAINST ACTUAL SODIUM INTAKE USING A CONTROLLED FEEDING STUDY IN NORMOTENSIVE ADULTS

Abstract

Background: Accurate estimates of sodium intake are critical for understanding means to reduce sodium intake and developing strategies for reducing chronic disease risk. A common method for estimating sodium intake is to collect a spot urine sample and estimate 24-hour urinary sodium excretion (e24hUNa) using an equation. However, it remains unknown how well e24hUNa reflects actual sodium intake. In addition, no study has determined the ideal time to collect a spot urine sample to provide the most accurate estimate of 24hUNa.

Objective: Our objectives were to 1) compare e24hUNa calculated from multiple timed urine collections with three published equations to known sodium intake, and 2) determine the ideal time of day, relative to meal consumption, to collect a spot urine sample.

Design: This study was a secondary analysis of a multi-phase controlled feeding study in healthy, normotensive adults. Data from the control phase were used. Participants consumed their study diet with known sodium content for three days, and on day 4, ate their meals and collected their urine in timed intervals in a clinic setting. Spot urine sodium excretion was measured in each sample and used to calculate estimated 24hUNa with the INTERSALT, Tanaka, and Kawasaki equations.

Results: Regardless of timing of spot urine collection and equation used, e24hUNa was significantly lower than sodium intake on day 4 (all $p<0.05$). While some e24hUNa calculations were significantly correlated with measured 24hUNa, all correlation coefficients were weak.

Conclusions: Regardless of timing of spot urine collection, and regardless of equation used, e24hUNa does not provide a reliable estimate of sodium intake in normotensive adults. Future studies should confirm these findings in larger populations and in populations with chronic disease.

Introduction

Cardiovascular disease (CVD) is the number one cause of death in the US (1). Hypertension (HTN), a major risk factor for CVD, is highly prevalent, affecting 46% of US adults (2). Many factors contribute to the development of HTN and CVD, but one important modifiable risk factor is sodium intake. Intervention studies have shown that a modest reduction in sodium intake can significantly lower blood pressure (BP) (3-5). Indeed, the high strength of evidence from randomized-controlled trials linking sodium intake and BP provided the rationale for the recent establishment of a new Dietary Reference Intake (DRI) for Na: the Chronic Disease Risk Reduction Intake (CDRR) (6). Together, this demonstrates that reducing sodium intake is not only a viable, but a recommend strategy for reducing BP and CVD risk.

To continue studying the relationship between sodium intake and chronic disease risk in order to design effective dietary interventions and to inform policies on dietary sodium reduction, methods for estimating sodium intake are needed that are both accurate and place minimal burden on participants. A common strategy for estimating sodium intake is self-report, which includes 24-hour dietary recalls, diet records, and food frequency questionnaires. While these methods are relatively low-burden, they are often inaccurate due to systematic underreporting (7), especially in obese individuals (8), as well as difficulties quantifying discretionary salt use and sodium content in prepared foods (7). The gold standard for estimating sodium intake is 24-hour urinary sodium excretion (24hUNa) because ~90% of ingested sodium is recovered in the urine under normal conditions (9, 10). However, collecting urine for an entire day is burdensome for participants, which can affect the response rate and thus the accuracy of this method (11).

An alternative method for estimating sodium intake is calculating a predicted or estimated 24hUNa (e24hUNa) using a spot urine sample. With this method, a single urine sample is collected from participants and analyzed for spot urine sodium excretion, and this spot sodium excretion is then extrapolated to a 24-hour context. This may be done by using one of several published equations to calculate e24hUNa. The advantage of this method is that it is low-burden on participants, and the response rate is shown to be higher than for 24-hour urine collections (12). However, much remains unexplored regarding the accuracy of this method. To date, no study has determined how closely predicted 24hUNa from spot urine reflects true sodium intake. Additionally, no study has identified the ideal time of day, relative to meal intake,

to collect a spot urine sample to obtain the most accurate estimate of sodium intake. The purpose of the current study is to fill these gaps in the literature by utilizing data from a controlled feeding study in which actual sodium intake was known and timed spot urine samples were collected. Specifically, our aim was to determine how closely e24hUNa using available equations reflects known sodium intake, and to determine which spot urine sample provides the most accurate estimate.

Methods

Study Design and Participants

This was a secondary analysis of a previously completed randomized, crossover, controlled feeding study. The aim of the parent study was to assess the effect of different doses and sources of dietary potassium (K) on K kinetics, BP, and other cardiovascular outcomes. The parent study was a full-feeding intervention trial conducted in nine phases separated by a minimum seven-day washout period. Details on recruitment, randomization, enrollment, and compliance have been published previously (13). Briefly, all participants were required to be healthy, normotensive men and women (n = 39) aged 20-60 y with BMI between 17-35 kg/m^2. Exclusion criteria included having HTN or hypotension, kidney or malabsorption disorders, and taking medication that affects electrolyte metabolism. The parent study was registered on clinicaltrials.gov (NCT01881295), all procedures and protocols for the parent study were approved by the Institutional Review Board at Purdue University (IRB #1301013174), and this secondary analysis was approved by the Purdue University Institutional Review Board (IRB Protocol #2020302).

Data used for this secondary analysis were de-identified and came from the first control phase, during which K intake was not manipulated with additional supplements or food sources of K, thus eliminating the potential confounding effect of increased K intake on urinary sodium excretion. A summary of the study timeline is shown in **Figure 2.1**. Each phase consisted of a five-day controlled diet intervention. For the first three days of the phase, subjects received their meals daily at the Purdue Clinical and Translational Sciences Institute (CTSI) Clinical Research Center (CRC) and consumed them in a free-living environment. On day 4, subjects consumed their meals at the clinic during a 24-hour clinical bioavailability study. On day 5, subjects

continued the controlled diet in a free-living environment, and on day 6 subjects returned to the clinic for BP measurements and pulse wave analyses. Additional data used for the current analyses, such as demographics, anthropometry, and blood pressure were collected during screening prior to the start of the study.

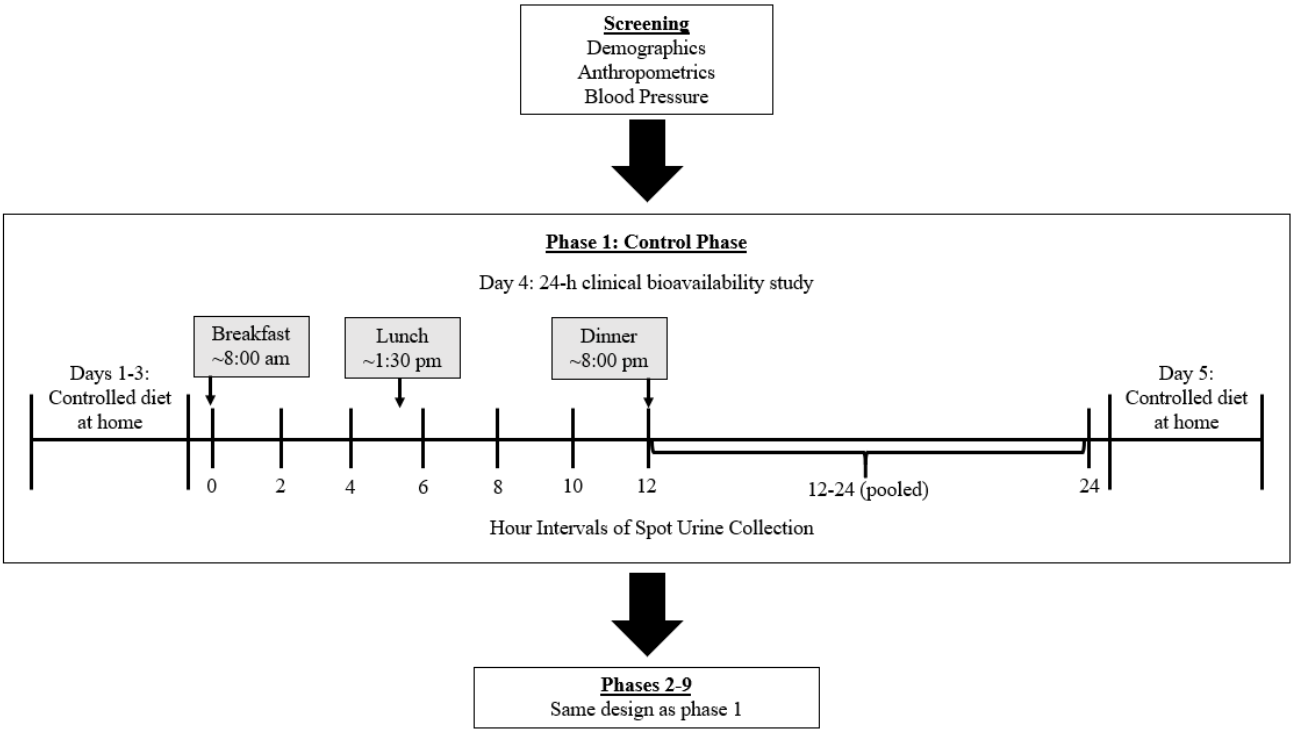


Figure 2.1 Summary of study timeline. Participants were screened, and baseline data were collected. All participants completed the control phase as phase 1, which consisted of a 3-day run in period and a 1-day clinic study day

Controlled Diets

All foods and drinks were provided to participants during the study, and the study diets were carefully designed and analyzed by a registered dietitian through the CRC. Participants were assigned to one of four energy levels (6700, 8400, 10,000, and 11,700 KJ/d, [or 1600, 2000, 2400, and 2800 kcal/d, respectively]) based on their energy requirements estimated using the Harris-Benedict equation (14). Energy and nutrient content of study diets were analyzed using ProNutra software (Viocare, Inc; Version 3.4.0). On days 1, 2, 3, and 5, three meals and two snacks were packed out for participants to pick up from the CRC. Participants were asked to return any uneaten food the next day to assess compliance. Sodium intake on days 1-3 and 5

varied depending on assigned kcal level. On day 4 at the CRC, two identical test meals were provided at breakfast (0 h, ~8:00 am) and lunch (5.5 h, ~1:30 pm). A third meal that was different from the breakfast and lunch meal was provided for dinner (12 h, ~8:00 pm), and an evening snack was provided to take home after dinner. All participants consumed the same level of dietary sodium on day 4 (~4700 mg). For all study days, the amount of dietary sodium provided by assigned kcal level is provided in **Table 2.1.** Mineral-free water was provided *ad libitum* on all days.

Table 2.1 Sodium content in controlled diet by kcal level and study day

	Total dietary Na content (mg)[1]				
Energy level (kcal)	**Day 1**	**Day 2**	**Day 3**	**Day 4[2]**	**Day 5**
1600	2114	2849	2251	4744	2855
2000	3027	3286	3395	4744	3364
2400	4169	4034	4438	4744	3682
2800	4972	4513	4925	4744	4128

[1]Na content determined using ProNutra software (Viocare, Inc; Version 3.4.0)
[2]On day 4, all participants consumed the same diet while in the clinic

Urine Collection and Biochemical Analyses

As part of the parent study 24-hour clinical bioavailability study on day 4, participants collected their urine for 24 hours starting with a fasting collection (second void) immediately before breakfast on day 4 and ending with their first void on day 5. Urine samples were pooled at baseline (fasting collection, 0 h) and at 2, 4, 6, 8, 10, and 12 hours, between 12-24 hours (evening and overnight), and at 24 hours (first void). Samples were frozen at -20˚C until further analysis. Thawed samples were diluted in 2% nitric acid by a factor of 11x (i.e. 1 mL urine and 10 mL 2% nitric acid) in duplicate and analyzed for Na, K, and other mineral content by inductively coupled plasma optical emission spectroscopy (ICP-OES) (Optima 4300; PerkinElmer). Urine samples were additionally analyzed for creatinine using a COBAS INTEGRA® 400 Plus Analyzer (Roche).

Three published equations were used to calculate e24hUNa: Kawasaki (15), Tanaka (16), and INTERSALT (17). Because the Tanaka and INTERSALT equations were originally developed using casual spot urine samples (i.e. a spot urine sample collected at any time), e24hUNa was calculated from all spot urine samples collected using these equations. However,

the Kawasaki equation was originally developed using a fasting collection (second morning void), therefore only the fasting collection (i.e. baseline, 0 h) was used to calculate e24hUNa with this equation.

Statistical Analysis

One outlier was removed because the amount of sodium excreted in the urine was ~2000 mg higher than the sodium in the provided study diet, making it physiologically improbable. The distributions of e24hUNa from the INTERSALT equations were non-normally distributed, and were log transformed prior to analysis. A repeated-measures ANOVA was used to compare spot urine sodium excretion at each time point in order to explore the pattern of urinary sodium excretion throughout the day. Additional repeated-measures ANOVA analyses were conducted to compare actual sodium intake to observed 24hUNa and e24hUNa, with separate analyses for each equation used to calculate e24hUNa (INTERSALT, Tanaka, and Kawasaki equations). *Post hoc* tests with a Bonferroni correction were used to explore the differences between measurements. Pearson bivariate correlations were calculated between e24hUNa, observed 24hUNa, and intake. All analyses were performed using SPSS statistical software (IBM; version 24.0).

Results

Baseline participant characteristics are reported in **Table 2.2**. Approximately half of the participants were women, approximately two-thirds were white, and one-third were Asian, the average BMI was on the higher end of the normal range, and all participants were normotensive. The pattern of urinary sodium excretion throughout the day for all individuals, as well as the pattern of the mean urinary sodium excretion, is shown in **Figure 2.2**. The pattern of urinary sodium excretion was highly variable between individuals, despite intake and timing of meal consumption being controlled. As shown in the inset, urinary sodium excretion increased up until hour 4 and leveled off and remained relatively stable throughout the rest of the day.

Table 2. 2 Baseline Descriptive Characteristics

Characteristic	Mean (SD)
Age, y	29.15 (10.7)
Height, cm	170.06 (9.4)
Weight, kg	70.20 (14.5)
BMI, kg/m^2	24.19 (4.2)
SBP/DBP, mmHg	107/71
Sex	
Women	21
Men	18
Race	
White	25
Asian	11
Other	3

Figure 2.3 shows e24hUNa calculated from all three equations and all spot urine samples as it compares to measured 24hUNa and actual sodium intake. Regardless of timing of spot urine sample collection, and regardless of the equation used, e24hUNa was significantly lower than sodium intake, with underestimates ranging from ~500-2200 mg. In addition, measured 24hUNa was significantly lower than sodium intake by >1300 mg ($p<0.001$). When using the INTERSALT equation, e24hUNa calculated from spot urine samples collected 3, 5, and 7 hours after lunch as well as with the first void the following morning were not significantly different from measured 24hUNa. When using the Tanaka equation, e24hUNa calculated from the fasting second collection, the sample collected 2 hours after breakfast, and the first void the following morning were not significantly different from measured 24hUNa. e24hUNa calculated from the Kawasaki equation was not significantly different from measured 24hUNa. Notably, e24hUNa from the Tanaka and Kawasaki equations are consistently higher than e24hUNa calculated from the INTERSALT equation.

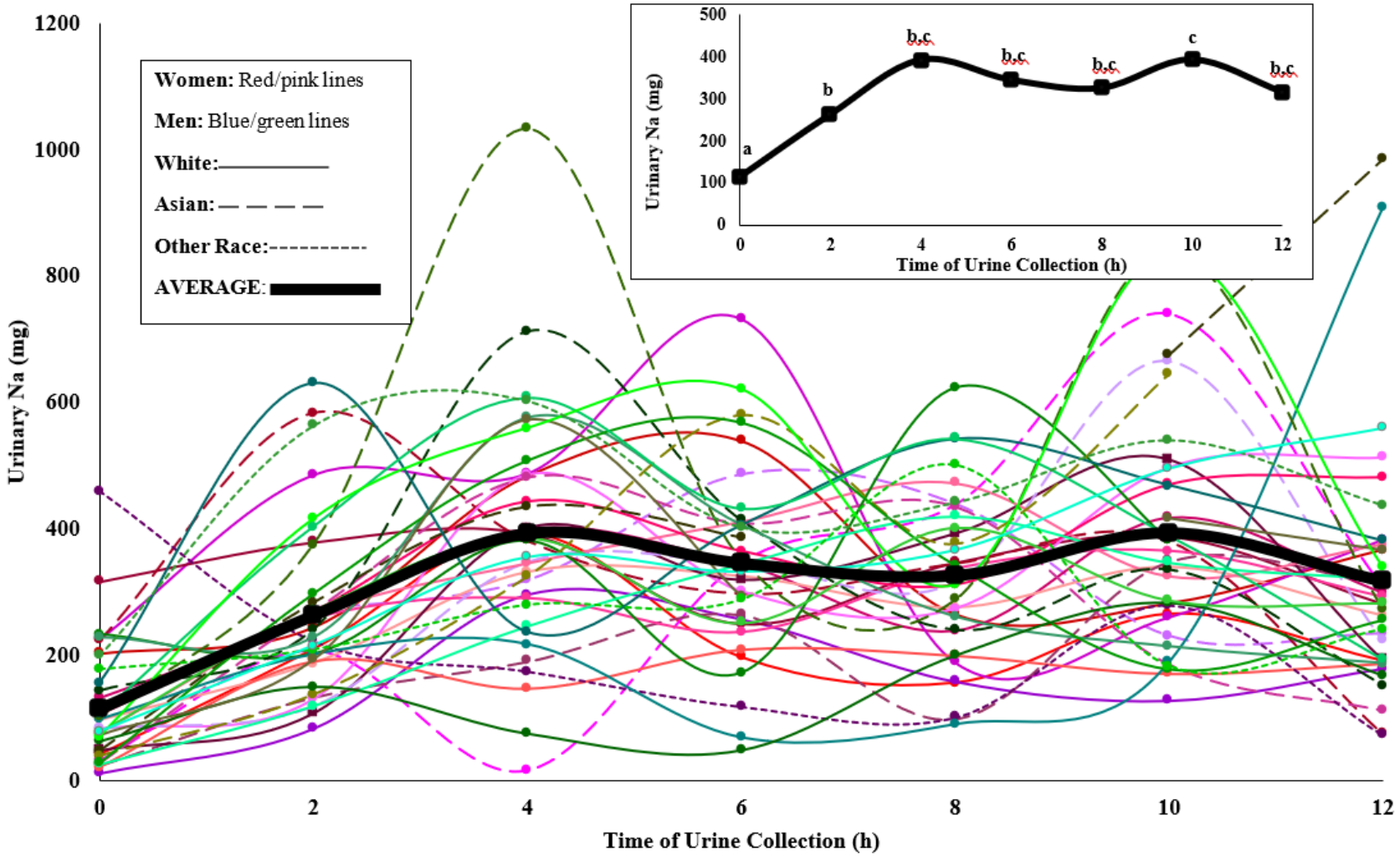


Figure 2.2 Pattern of urinary sodium excretion throughout the day in all participants. Hour 0 on the x-axis represents the fasting second urine collection (i.e. baseline), and the hour intervals represent the number of hours since baseline. Each line represents the pattern of an individual participant and the thick black line represents the mean. Inset displays the statistical differences in urinary sodium excretion at all time points. Different letters indicate statistically different levels of urinary sodium excretion.

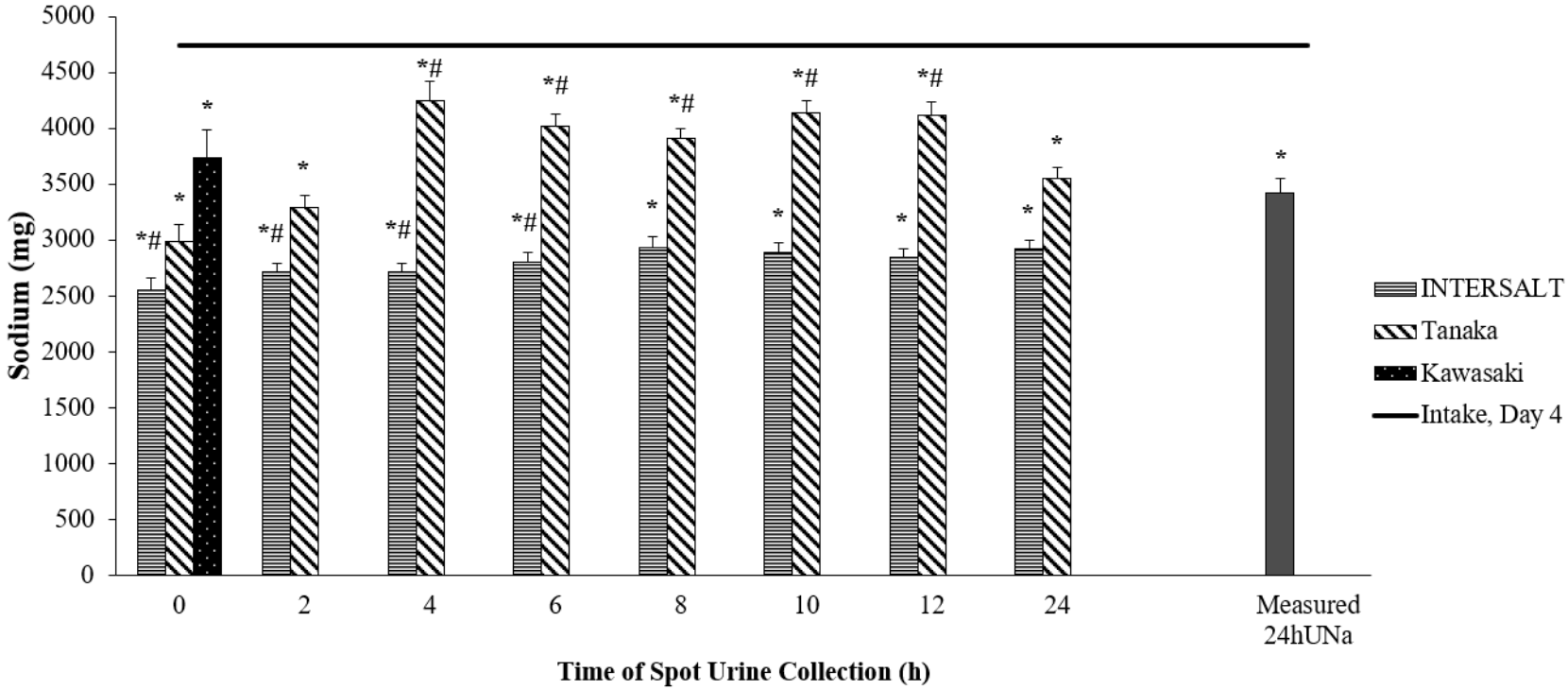


Figure 2.3 Comparison of e24hUNa, measured 24hUNa, and sodium intake on day 4. Bars represent mean ± SEM.
*significantly different from sodium intake, #significantly different from measured 24hUNa

Correlations between e24hUNa and sodium intake on day 4 could not be conducted because sodium intake was fixed for all participants on this day. **Table 2.3** shows the correlations between e24hUNa and measured 24hUNa. All correlations were weak, with correlation coefficients ranging from r=0.24 to r=0.55. For all three equations, e24hUNa calculated from the fasting second collection (hour 0) were significantly correlated with measured 24hUNa (all $p<0.01$). In addition, for both the INTERSALT and the Tanaka equations, e24hUNa calculated from the first void (hour 24) were both significantly correlated with measured 24hUNa (both $p<0.05$), and e24hUNa calculated from spot urine samples collected at least 3 hours after consuming a meal (hours 4, 8, and 10) were significantly correlated with measured 24hUNa (all $p<0.05$).

Table 2.3 Correlations between measured 24hUNa and e24hUNa from the INTERSALT, Tanaka, and Kawasaki equations[1]

	Correlations with Measured 24hUNa					
	INTERSALT		**Tanaka**		**Kawasaki**	
Time of urine collection, h	**r**	**p**	**r**	**p**	**r**	**p**
0	0.50	0.002	0.47	0.004	0.51	0.001
2	0.24	0.14	0.27	0.11		
4	0.32	0.048	0.41	0.01		
6	0.32	0.053	0.36	0.03		
8	0.40	0.03	0.55	0.001		
10	0.31	0.06	0.41	0.01		
12	0.27	0.11	0.34	0.045		
24	0.42	0.009	0.38	0.02		

[1]All values are Pearson bivariate correlations. All $p<0.05$ is considered statistically significant.

Evidence from controlled feeding studies suggests that 24hUNa may not always match sodium intake on the same day (18, 19). Therefore, we conducted additional secondary analyses to compare e24hUNa and measured 24hUNa to sodium intake on day 3. Results from these analyses are shown in **Supplemental Figure S2.1** and **Supplemental Table S2.1**. Measured 24hUNa matched day 3 sodium intake. However, regardless of equation used, nearly all e24hUNa were significantly different from day 3 sodium intake. All e24hUNa from the INTERSALT equation were significantly lower than sodium intake by ~500-900 mg (all $p < 0.05$). e24hUNa calculated from the Tanaka equation was significantly higher than actual sodium

intake by ~450-800 mg when spot urine samples collected at hours 4, 6, 8, 10, and 12 were used (all $p < 0.05$). e24hUNa from the Kawasaki equation was not significantly different from day 3 sodium intake ($p = 0.72$). Though the correlation coefficients were weak, all e24hUNa from the INTERSALT equation were significantly correlated with day 3 sodium intake. All e24hUNa calculated from the Tanaka and Kawasaki equations were not significantly correlated with day 3 sodium intake. In addition, measured 24hUNa was not significantly correlated with day 3 sodium intake ($r=0.12$, $p=0.46$).

Discussion

Our study is the first to compare e24hUNa, calculated using multiple timed spot urine collections and three published prediction equations, to both measured 24hUNa as well as actual known sodium intake. Our results suggest that, regardless of time of spot urine sample collection and regardless of equation used, e24hUNa does not provide a reliable indicator of sodium intake in normotensive adults. The INTERSALT equation consistently underestimated sodium intake, whereas the Tanaka equation either under- or overestimated intake depending on which day's intake was used. Interestingly, e24hUNa from the INTERSALT equation with all spot urine samples from day 4 was significantly correlated to sodium intake on day 3, which may indicate that applying a correction factor to this equation could improve the accuracy for estimating sodium intake. Future studies should explore strategies to improve the accuracy of these equations for estimating sodium intake.

Previous studies have sought to explore the relationship between spot urine sodium and measured 24hUNa and/or sodium intake, and to our knowledge only two have been controlled feeding studies. These controlled feeding studies showed that on both a fixed sodium intake (20) and variable sodium intake (21), urinary sodium excretion from a nocturnal, or overnight, sample is significantly correlated with measured 24hUNa, but not sodium intake (21). An important limitation of these studies was that spot urine (e.g. nocturnal) sodium excretion was not put into a 24-hour context, as with a prediction equation. Additional cross-sectional studies have aimed to compare e24hUNa calculated from the INTERSALT, Tanaka, Kawasaki, and other equations to measured 24hUNa in adult populations. Results from these studies have been mixed, with no consensus on which equation produces the least biased estimate of 24hUNa (22-27). However, e24hUNa from the INTERSALT equations seems to be consistently significantly lower than

measured 24hUNa, regardless of timing of spot urine collection (22, 24, 25, 27, 28). The Tanaka equation tends to overestimate measured 24hUNa with morning, afternoon, and evening urine samples (22, 23), and underestimate measured 24hUNa with overnight urines samples (25). The Kawasaki equation seems to consistently overestimate measured 24hUNa (22, 24, 25, 27). Based on these findings, it seems that regardless of the timing of spot urine collection, calculated e24hUNa does not provide an accurate, unbiased estimate of measured 24hUNa. Importantly, none of these studies compared e24hUNa to actual sodium intake.

Dietary sodium intake is a critical risk factor for CVD and other chronic diseases. Indeed, a recent report that reanalyzed data from the Global Burden of Disease, Injuries, and Risk Factors Study (GBD) 2017 found that high sodium intake was one of the leading dietary risk factors for morbidity and mortality (29). These findings highlight the importance of continuing to research dietary interventions that will effectively reduce disease risk. In order to further these research efforts on sodium intake, and to ultimately influence nutrition policies, we need an accurate and low-burden method for estimating sodium intake. Self-report often underestimates sodium intake, and 24-hour urine collections are considered the gold standard but are burdensome for participants. Thus, spot urine samples have been explored as a possible alternative and is widely used in population-based research. However, based on the current findings, e24hUNa calculated from spot urine samples using available equations does not accurately reflect actual sodium intake. This is problematic because many studies that use this method may be drawing inaccurate conclusions about the true relationship between sodium intake and disease risk. Indeed, a recent paper that analyzed data from the Trials of Hypertension Prevention (TOHP) follow-up study found that when sodium intake is estimated using spot urine samples and the Kawasaki equation, the relationship between sodium intake and all-cause mortality was characterized as J-shaped. (30). However, when sodium intake is measured using the gold standard of multiple 24-hour urine collections, the relationship between sodium intake and mortality was shown to be linear. Recently The International Consortium for Quality Research on Dietary Sodium/Salt (TRUE) published a position paper based on multiple systematic reviews and meta-analyses which concluded that the use of spot urine samples for estimating sodium intake is questionable at the population level and is not recommended at the individual level, especially in studies examining sodium intake related to health outcomes (31).

There are many reasons why e24hUNa from spot urine samples does not accurately reflect intake. Perhaps the most important reason is that the INTERSALT, the Tanaka, and the Kawasaki equations were not designed to estimate sodium intake: all three were designed to estimate 24hUNa (15-17). Several factors can affect the relationship between 24hUNa and sodium intake, which would not be accounted for in available prediction equations. Recent long-term balance studies have revealed that even with a fixed sodium intake, measured 24hUNa exhibits high day-to-day variability, resulting in a weekly aldosterone-dependent infradian rhythm (10, 19). Because this rhythmicity in sodium excretion is shown to occur without corresponding fluctuations in body water or blood pressure, it seems likely that sodium can be stored in body tissues without commensurate water retention (19). Indeed, findings from both animal and human studies show that sodium can be stored in skin and muscle (32), suggesting that these tissue sodium stores can significantly affect overall sodium balance. These factors could explain why we obtained disparate findings when comparing e24hUNa to sodium intake on days 3 and 4. If e24UNa will continue to be used as an indicator of sodium intake in research, new equations should be developed to better estimate sodium intake from spot urine samples that account for these and other factors that influence the relationship between sodium intake and excretion.

A strength of our study is the controlled feeding design, which allowed us to know actual sodium intake. Given the drawbacks of self-reported sodium intake, a controlled feeding study is the best way to determine how well e24hUNa reflects actual sodium intake. Another strength is the timing of spot urine collections relative to meal intake. This allowed us to consider not only the time of day, but also the timing of meals in determining the ideal time to collect a spot urine sample. One weakness of our study is the relatively small sample size compared to previous studies. However, even with the small sample size we were able to detect significant differences between e24hUNa and sodium intake. Another weakness of our study is that we only included healthy, normotensive adults. Therefore, we are unable to extrapolate our findings to adults with HTN, chronic kidney disease, diabetes, or any other chronic diseases or conditions that may affect sodium handling.

In conclusion, we found that regardless of timing of spot urine collection and prediction equation used, e24hUNa does not accurately reflect actual sodium intake in healthy, normotensive adults. Spot urine collections are advantageous over 24-hour urine collections

because they are less burdensome for participants. However, more work needs to be done to optimize this method before it should be used. Future studies should aim to develop equations using spot urine samples to predict sodium intake validated against actual intake data, establish ideal time of day for collections, and should account for additional factors that could affect the relationship between sodium intake and urinary sodium excretion. In addition, future studies should investigate the relationship between e24hUNa and sodium intake in those with HTN, chronic kidney disease, and diabetes, especially because these populations would benefit greatly from effective sodium intake reduction interventions and accurate clinical guidelines.

References

1. Heron M. Deaths: Leading causes for 2016. National Vital Statistics Reports; vol 67 no 6. Hyattsville, MD: National Center for Health Statistics. 2018.
2. Benjamin EJ, Muntner P, Alonso A, Bittencourt MS, Callaway CW, Carson AP, Chamberlain AM, Chang AR, Cheng S, Das SR, et al. Heart Disease and Stroke Statistics-2019 Update: A Report From the American Heart Association. Circulation 2019;139(10):e56-e66. doi: 10.1161/cir.0000000000000659.
3. Sacks FM, Svetkey LP, Vollmer WM, Appel LJ, Bray GA, Harsha D, Obarzanek E, Conlin PR, Miller ER, 3rd, Simons-Morton DG, 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):3-10. doi: 10.1056/nejm200101043440101.
4. He FJ, MacGregor GA. Effect of modest salt reduction on blood pressure: a meta-analysis of randomized trials. Implications for public health. J Hum Hypertens 2002;16(11):761-70. doi: 10.1038/sj.jhh.1001459.
5. Newberry SJ, Chung M, Anderson CAM, Chen C, Fu Z, Tang A, Zhao N, Booth M, Marks J, Hollands S, et al. AHRQ Comparative Effectiveness Reviews. Edtion ed. Sodium and Potassium Intake: Effects on Chronic Disease Outcomes and Risks. Rockville (MD): Agency for Healthcare Research and Quality (US), 2018.
6. National Academies of Sciences, Engineering, and Medicine. Dietary Reference Intakes for Sodium and Potassium. Washington, DC: The National Academies Press, 2019. https://doi.org/10.17226/25353.

7. McLean RM. Measuring population sodium intake: a review of methods. Nutrients 2014;6(11):4651-62. doi: 10.3390/nu6114651.
8. Rhodes DG, Murayi T, Clemens JC, Baer DJ, Sebastian RS, Moshfegh AJ. The USDA Automated Multiple-Pass Method accurately assesses population sodium intakes. Am J Clin Nutr 2013;97(5):958-64. doi: 10.3945/ajcn.112.044982.
9. Holbrook JT, Patterson KY, Bodner JE, Douglas LW, Veillon C, Kelsay JL, Mertz W, Smith JC, Jr. Sodium and potassium intake and balance in adults consuming self-selected diets. Am J Clin Nutr 1984;40(4):786-93.
10. Lerchl K, Rakova N, Dahlmann A, Rauh M, Goller U, Basner M, Dinges DF, Beck L, Agureev A, Larina I, et al. Agreement between 24-hour salt ingestion and sodium excretion in a controlled environment. Hypertension 2015;66(4):850-7. doi: 10.1161/hypertensionaha.115.05851.
11. Conkle J, van der Haar F. The Use and Interpretation of Sodium Concentrations in Casual (Spot) Urine Collections for Population Surveillance and Partitioning of Dietary Iodine Intake Sources. Nutrients 2016;9(1). doi: 10.3390/nu9010007.
12. Cogswell ME, Maalouf J, Elliott P, Loria CM, Patel S, Bowman BA. Use of Urine Biomarkers to Assess Sodium Intake: Challenges and Opportunities. Annu Rev Nutr 2015;35:349-87. doi: 10.1146/annurev-nutr-071714-034322.
13. Macdonald-Clarke CJ, Martin BR, McCabe LD, McCabe GP, Lachcik PJ, Wastney M, Weaver CM. Bioavailability of potassium from potatoes and potassium gluconate: a randomized dose response trial. Am J Clin Nutr 2016;104(2):346-53. doi: 10.3945/ajcn.115.127225.
14. Harris JA, Benedict FG. A Biometric Study of Human Basal Metabolism. Proc Natl Acad Sci U S A 1918;4(12):370-3.
15. Kawasaki T, Itoh K, Uezono K, Sasaki H. A simple method for estimating 24 h urinary sodium and potassium excretion from second morning voiding urine specimen in adults. Clin Exp Pharmacol Physiol 1993;20(1):7-14.
16. Tanaka T, Okamura T, Miura K, Kadowaki T, Ueshima H, Nakagawa H, Hashimoto T. A simple method to estimate populational 24-h urinary sodium and potassium excretion using a casual urine specimen. J Hum Hypertens 2002;16(2):97-103. doi: 10.1038/sj.jhh.1001307.

17. Brown IJ, Dyer AR, Chan Q, Cogswell ME, Ueshima H, Stamler J, Elliott P. Estimating 24-hour urinary sodium excretion from casual urinary sodium concentrations in Western populations: the INTERSALT study. Am J Epidemiol 2013;177(11):1180-92. doi: 10.1093/aje/kwt066.
18. Weaver CM, Martin BR, McCabe GP, McCabe LD, Woodward M, Anderson CA, Appel LJ. Individual variation in urinary sodium excretion among adolescent girls on a fixed intake. J Hypertens 2016;34(7):1290-7. doi: 10.1097/hjh.0000000000000960.
19. Rakova N, Juttner K, Dahlmann A, Schroder A, Linz P, Kopp C, Rauh M, Goller U, Beck L, Agureev A, et al. Long-term space flight simulation reveals infradian rhythmicity in human Na(+) balance. Cell Metab 2013;17(1):125-31. doi: 10.1016/j.cmet.2012.11.013.
20. Luft FC, Fineberg NS, Sloan RS. Overnight urine collections to estimate sodium intake. Hypertension 1982;4(4):494-8.
21. Luft FC, Fineberg NS, Sloan RS. Estimating dietary sodium intake in individuals receiving a randomly fluctuating intake. Hypertension 1982;4(6):805-8.
22. Cogswell ME, Wang CY, Chen TC, Pfeiffer CM, Elliott P, Gillespie CD, Carriquiry AL, Sempos CT, Liu K, Perrine CG, et al. Validity of predictive equations for 24-h urinary sodium excretion in adults aged 18-39 y. Am J Clin Nutr 2013;98(6):1502-13. doi: 10.3945/ajcn.113.059436.
23. Whitton C, Gay GM, Lim RB, Tan LW, Lim WY, van Dam RM. Evaluation of Equations for Predicting 24-Hour Urinary Sodium Excretion from Casual Urine Samples in Asian Adults. J Nutr 2016;146(8):1609-15. doi: 10.3945/jn.116.232108.
24. Zhou L, Tian Y, Fu JJ, Jiang YY, Bai YM, Zhang ZH, Hu XH, Lian HW, Guo M, Yang ZX, et al. Validation of spot urine in predicting 24-h sodium excretion at the individual level. Am J Clin Nutr 2017;105(6):1291-6. doi: 10.3945/ajcn.116.147553.
25. Zhang Y, Peng Y, Li K, Peng X. Assessing whether a spot urine specimen can predict 24-h urinary sodium excretion accurately: a validation study. J Hypertens 2019;37(1):99-108. doi: 10.1097/hjh.0000000000001879.

26. Mente A, O'Donnell MJ, Dagenais G, Wielgosz A, Lear SA, McQueen MJ, Jiang Y, Xingyu W, Jian B, Calik KB, et al. Validation and comparison of three formulae to estimate sodium and potassium excretion from a single morning fasting urine compared to 24-h measures in 11 countries. J Hypertens 2014;32(5):1005-14; discussion 15. doi: 10.1097/hjh.0000000000000122.
27. Charlton K, Ware LJ, Chidumwa G, Cockeran M, Schutte AE, Naidoo N, Kowal P. Prediction of 24-hour sodium excretion from spot urine samples in South African adults: a comparison of four equations. J Hum Hypertens 2019. doi: 10.1038/s41371-019-0210-2.
28. Emeville E, Lassale C, Castetbon K, Deschamps V, Salanave B, Vernay M, Guergour D, Faure P, Hercberg S, Galan P, et al. Estimating sodium intake from spot urine samples at population level: a validation and application study in French adults. Br J Nutr 2019:1-24. doi: 10.1017/s0007114519000886.
29. Health effects of dietary risks in 195 countries, 1990-2017: a systematic analysis for the Global Burden of Disease Study 2017. Lancet 2019;393(10184):1958-72. doi: 10.1016/s0140-6736(19)30041-8.
30. He FJ, Campbell NRC, Ma Y, MacGregor GA, Cogswell ME, Cook NR. Errors in estimating usual sodium intake by the Kawasaki formula alter its relationship with mortality: implications for public health†. International Journal of Epidemiology 2018:dyy114-dyy. doi: 10.1093/ije/dyy114.
31. Campbell NRC, He FJ, Tan M, Cappuccio FP, Neal B, Woodward M, Cogswell ME, McLean R, Arcand J, MacGregor G, et al. The International Consortium for Quality Research on Dietary Sodium/Salt (TRUE) position statement on the use of 24-hour, spot, and short duration (<24 hours) timed urine collections to assess dietary sodium intake. J Clin Hypertens (Greenwich) 2019. doi: 10.1111/jch.13551.
32. Titze J. Sodium balance is not just a renal affair. Curr Opin Nephrol Hypertens 2014;23(2):101-5. doi: 10.1097/01.mnh.0000441151.55320.c3.

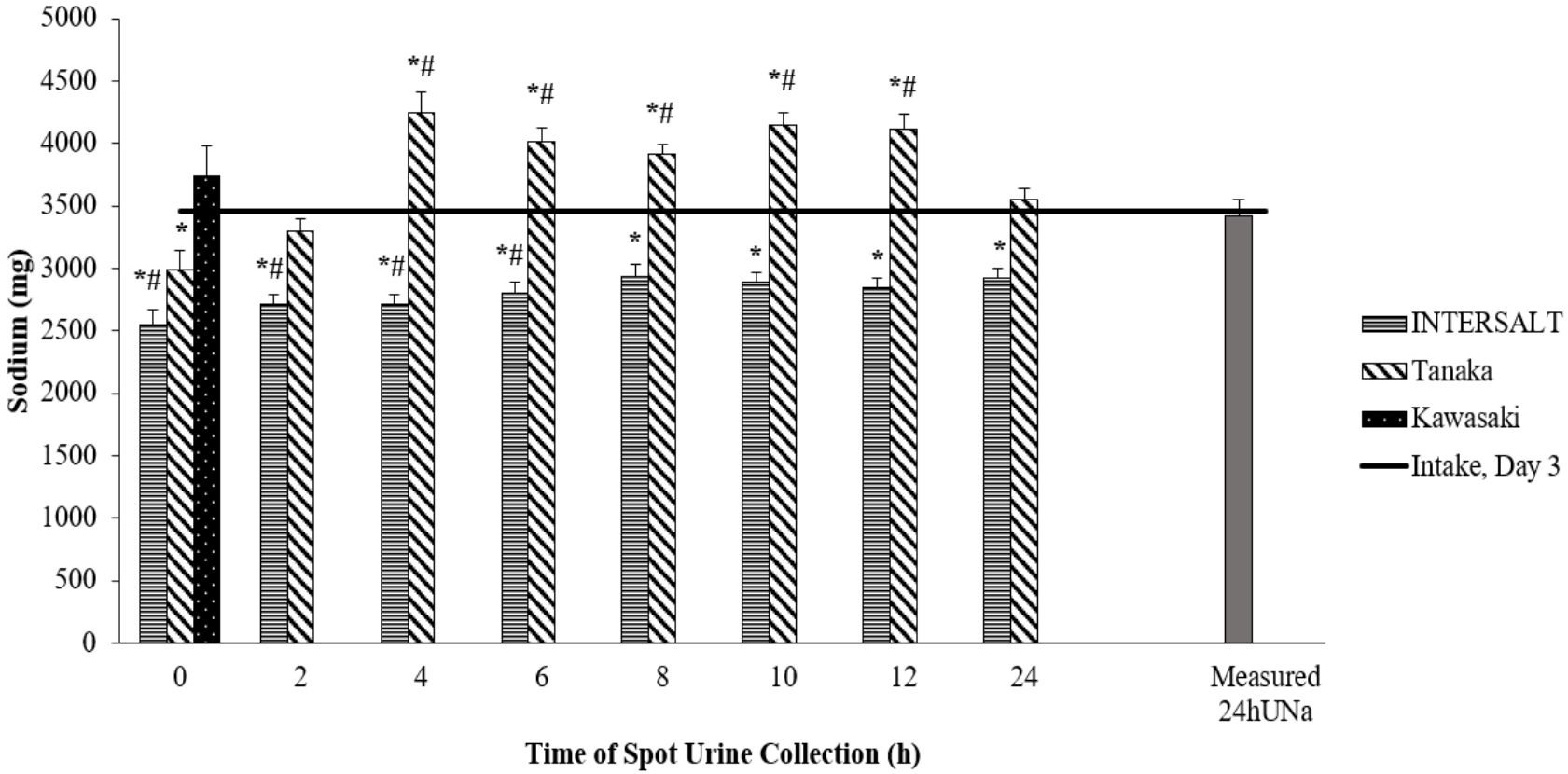


Figure S2.1 Comparison of e24hUNa, measured 24hUNa, and sodium intake on day 3. Bars represent mean ± SEM.
*significantly different from sodium intake, #significantly different from measured 24hUNa

Table S2.1 Correlations between e24hUNa, calculated using spot urine samples collected on day 4, and sodium intake on day 3[1]

	Correlations with Sodium Intake on Day 3					
	INTERSALT		Tanaka		Kawasaki	
Time of urine collection, h	**r**	**p**	**r**	**p**	**r**	**p**
0	0.36	0.03	0.19	0.26	0.12	0.46
2	0.54	0.001	0.12	0.46		
4	0.46	0.003	0.24	0.14		
6	0.60	<0.001	-0.017	0.92		
8	0.49	0.002	0.21	0.21		
10	0.52	0.001	0.25	0.13		
12	0.55	0.001	0.22	0.21		
24	0.51	0.001	-0.045	0.79		

[1]All values are Pearson bivariate correlations. All $p<0.05$ is considered statistically significant.

CHAPTER 3: SPOT URINE SAMPLES TO ESTIMATE SODIUM AND POTASSIUM INTAKE IN PATIENTS WITH CKD AND HEALTHY ADULTS: A SECONDARY ANALYSIS FROM A CONTROLLED FEEDING STUDY

Abstract

Objective: The objective of our study was to assess the agreement between estimated 24-hour urinary sodium excretion (e24hUNa) and estimated 24-hour urinary potassium excretion (e24hUK), calculated from a spot urine sample using several available equations, and actual sodium and potassium intake from a controlled diet in both healthy participants and those with chronic kidney disease (CKD).

Design and Methods: This study is a secondary analysis of a controlled feeding study in CKD patients matched to healthy controls. Participants (n=16) consumed the controlled diet, which provided ~2400 mg Na/d and ~3000 mg K/d, for 8 days. On days 7 and 8, participants consumed all meals and collected all urine in an inpatient clinic setting, and patients were discharged on day 9. The day 7 morning spot urine sample was used to calculate e24hUNa and e24hUK, which was compared to known sodium and potassium intake, respectively.

Results: Average e24hUNa from the INTERSALT and Tanaka-Na equations were higher than actual sodium intake by ~400-500 mg, though the differences were not significant. e24hUNa from the Nerbass-SALTED equation in CKD participants was significantly higher than actual sodium intake by ~2000 mg ($p<0.001$), though e24hUNa from the Nerbass-RRID equation was not different from intake. e24hUK from the Tanaka-K equation was significantly lower than actual potassium intake ($p<0.001$). For both e24hUNa and e24hUK for all participants, agreement with actual intake was poor, and e24hUNa and e24hUK were not correlated with actual sodium or potassium intake, respectively.

Conclusion: e24hUNa and e24hUK are poor indicators of true sodium and potassium intake, respectively, in both healthy and CKD participants. Findings should be confirmed in larger sample sizes with varying levels of dietary sodium and potassium.

Introduction

Cardiovascular disease (CVD) is the leading cause of death in those with chronic kidney disease (CKD), and death from CVD is more common than progression to end-stage kidney failure (ESKD) (1). Hypertension is a major risk factor for CKD (1), and kidney damage, in turn, exacerbates hypertension (2), leading to worsening kidney damage and increased CVD risk. Reducing sodium intake is shown to be effective at reducing blood pressure in both hypertensive individuals (3, 4) as well as CKD patients (5). Reducing sodium intake is also effective at reducing proteinuria and albuminuria in CKD (6). Potassium is another important mineral to consider in CKD prevention and management. Potassium supplementation is effective at lowering blood pressure, particularly in those with hypertension (7), and higher potassium intake is associated with a decreased risk of developing CKD (8, 9). However, potassium intake is often closely monitored once a patient develops CKD due to the increased risk of cardiac arrest and mortality with hyperkalemia (10, 11).

In order to 1) continue studying the relationship between sodium and potassium intake and CVD and CKD, 2) monitor the intake of these minerals in affected populations, and 3) design effective dietary interventions, we need a method for estimating intake that is both accurate as well as convenient. Twenty-four-hour urine collections are regarded as the gold standard method for estimating sodium and potassium intake because most of the sodium and potassium that we consume is excreted in our urine (12-14). However, 24-hour urine collections are burdensome on participants, which can lead to missed urine collections and ultimately affect their accuracy (15). An alternative method often used is a spot urine sample collection. This method involves collecting a spot urine sample, measuring the sodium and/or potassium content, and using one of many published prediction equations to calculate estimated 24hUNa (e24hUNa) or estimated 24hUK (e24hUK). Equations for e24hUNa and e24hUK have been developed for general populations (16-18), and additional equations for 24hUNa have been developed for CKD populations (19-21).

While spot urine samples are more convenient than a 24-hour urine collection, much remains unknown about the accuracy of this method for predicting dietary intake. Most importantly, e24hUNa and e24hUK have never been validated against known sodium and potassium intake, respectively. Given that sodium and potassium intake are the true variables of interest, this is an important gap in the literature. Therefore, the aim of our study was to assess

the agreement between e24hUNa and e24hUK, estimated using multiple available equations, against known sodium and potassium intake, respectively, in healthy and CKD participants consuming a controlled study diet.

Methods

Study Design and Participants

This study was a secondary analysis of a controlled feeding study aiming to examine differences in phosphorus absorption in healthy participants versus those with CKD. Details on participant recruitment and study design have been published previously. Briefly, men and post-menopausal women aged 45-65 years were recruited to participate in this study. Patients with moderate (stage 3) CKD were enrolled and healthy individuals were matched to CKD patients based on age, sex, and race. Exclusion criteria for healthy participants included evidence of CKD; medical conditions including diabetes, uncontrolled hypertension, or other conditions that could affect mineral absorption or metabolism; abnormal serum electrolytes; and taking any medications that could affect mineral metabolism. CKD participants were required to be in stages G3a or G3b (eGFR 30-59 mL/min) and stabilized on their medications. Exclusion criteria for CKD participants included serious underlying disease unless well-controlled; plans to initiate dialysis within the next 6 months; abnormal serum electrolytes; and medical conditions or taking any medications that could affect mineral absorption or metabolism. The parent study was registered on clinicaltrials.gov (NCT03108222) and all study procedures were approved by the Indiana University Institutional Review Board (IRB # 1612460566).

After consenting to study procedures, interested participants were screened for eligibility, at which time vital signs and a baseline blood draw and other baseline measurements were collected. After confirming eligibility, participants began the 9-day study. Three-day cycle menus were created using ProNutra software (Viocare, Inc; Version 3.4.0) and were designed to provide ~2400 mg Na/d and ~3000 mg K/d. All food and beverages were provided to participants, who came to the clinical research center (CRC) to pick up their pre-prepared and packed meals on days 1-6. Participants were provided checklists to record all foods eaten. For the first 6 days, participants consumed the controlled diet at home via meal pack-outs. Days 7-9 were inpatient study days, during which time participants completed phosphorus absorption

testing for the primary study. On the morning of day 7, participants arrived fasting to the CRC at Indiana University School of Medicine. Upon admission, a fasting urine sample was collected, and the phosphorus absorption testing began shortly thereafter. Participants collected all urine on days 7 and 8, and collection times were recorded by CRC staff. In addition, all food was provided to participants on days 7 and 8, and any uneaten food was recorded by CRC staff. On the morning on day 9, after an overnight fast, the final urine sample was collected, thus ending the study protocol.

Diet Analyses

Mineral content of the study diets (as served to participants) was confirmed by chemical analysis. Diet composites for each of the three meals of all three cycle menu days were made in duplicate according to the same procedures that would be followed if the meal were to be served to a participant. All foods and beverages from a meal were then combined and homogenized in a foodservice grade blender (Hamilton Beach). After homogenizing, composites were frozen at -20˚C, then freeze-dried (VirTis Genesis Pilot Lyophilizer, SP Scientific) for four days. After freeze drying, small amounts of the freeze dried composites were placed crucibles and ashed in a muffle furnace (Thermolyne, Thermo Scientific) at 600˚F for two days. The ash was dissolved in 1 mL of trace metal grade nitric acid, then diluted using 2% nitric acid. The dilutions were analyzed for sodium and potassium using inductively coupled plasma optical emission spectroscopy (ICP-OES, Optima 4300; PerkinElmer).

On inpatient study days 7 and 8, CRC staff provided all study food to participants and recorded the amount of food consumed for each meal. Actual food intake was then used to calculate actual mineral intake based on the chemical analysis data. If a participant ate all of their meal, their intake was assumed to be 100% of the chemically determined amount of each mineral in that meal.

Urine Sample Analyses

All urine samples were diluted in 2% nitric acid by a factor of 11x (i.e. 1 mL urine and 10 mL 2% nitric acid) in duplicate and analyzed for sodium and potassium content using ICP-OES. The spot urine sample collected in the morning on day 7 was used to calculate e24hUNa using

multiple available prediction equations. For both healthy and CKD participants, e24hUNa was calculated using the INTERSALT (16) and the Tanaka-Na (17) equations, both of which are commonly used equations that were developed for use in a general population. For CKD participants, e24hUNa was additionally calculated using two equations developed specifically for use in CKD, which will be referred to as Nerbass-RRID (19) and Nerbass-SALTED (20). For both healthy and CKD participants, e24hUK was calculated using the Tanaka-K equation (17). To our knowledge, there are no equations designed to estimate 24hUK in a CKD-specific population.

Statistical Analyses

Differences in baseline characteristics between the CKD and healthy participants were evaluated using paired-samples t-tests. Data that were non-normally distributed and were log-transformed prior to further analyses. Differences between actual sodium intake, 24hUNa, and e24hUNa from the INTERSALT and Tanaka-Na equations for all participants was determined by general linear mixed model (GLM), with CKD status and pair number included as between-subject factors. If the main GLM analysis was significant, specific differences were determined using planned contrasts. Similar analyses were conducted to compare potassium intake, 24hUK, and e24hUK from the Tanaka-K equation. A separate general linear mixed model was conducted for CKD participants only, which compared sodium intake, 24hUNa and e24hUNa from the Nerbass-RRID and Nerbass-SALTED equations. The relationship between sodium intake, 24hUNa, and e24hUNa from the INTERSALT and Tanaka-Na equations were assessed using partial correlations, which included CKD status and pair as covariates. Similar analyses were conducted to assess the relationship between actual potassium intake, 24hUK, and e24hUK from the Tanaka-K equation. A separate bivariate correlation was conducted in CKD participants only to assess the relationship between actual sodium intake, 24hUNa, and e24hUNa from the Nerbass-RRID and Nerbass-SALTED equations. The agreement between actual sodium intake and e24hUNa from the INTERSALT and Tanaka-Na equations was assessed using Bland Altman plots in all participants, as well as healthy and CKD separately. Similar Bland Altman plots were created to compare actual potassium intake and e24hUK from the Tanaka-K equation. Separate Bland Altman plots were created to assess the agreement between actual sodium intake and e24hUNa from the Nerbass-RRID and Nerbass-SALTED equations in CKD participants

only. All statistical analyses were conducted using Statistical Analysis Software (SAS, version 9.4) and $p<0.05$ was considered significant.

Results

Baseline and Descriptive Characteristics

The baseline characteristics of the participants are displayed in **Table 3.1**. Half (n=8) of the participants were men and half (n=8) were women. Approximately one-third were black (n=6) and approximately two-thirds were white (n=10). On average, participants were aged 54.6 ± 7.3 years with a BMI in the overweight to obese range. All of the CKD participants had been previously diagnosed with hypertension; 3 participants were taking diuretics, 3 were taking ACE inhibitors, and one was taking an angiotensin receptor blocker. After accounting for foods consumed, average sodium intake for all participants on day 7 of the study was 2138.48 ± 302 mg, and average 24hUNa on day 7 was 2363 ± 1020 mg. Neither sodium intake (controlled diet) nor 24hUNa were significantly different between healthy and CKD participants (2024 ± 388 vs. 2252 ± 121, p=0.15 and 2529 ± 1334 vs. 2197 ± 623, p=0.47, respectively). Average potassium intake for all participants on day 7 was 2528 ± 254 mg, and average 24hUK was 2625 ± 581 mg. Similar to sodium, neither potassium intake (controlled diet) nor 24hUK were significantly different between healthy and CKD participants (2432 ± 330 vs. 2623 ± 94, p=0.09 and 2727 ± 646 vs. 2524 ± 503, p=0.58, respectively).

Table 3.1 Baseline characteristics of study participants

Characteristic	All (*n*=16)	CKD (*n*=8)	Healthy (*n*=8)	*p*[1]
Age, y	54.6 (13.0)	56.6 (13.8)	52.5 (12.7)	0.04
Height, cm	171.3 (10.4)	166.6 (10.3)	175.4 (9.2)	0.03
Weight, kg	87.3 (17.8)	87.6 (24.1)	87.0 (11.6)	0.97
BMI, kg/m^2	30.0 (7.3)	31.7 (9.4)	28.5 (5.0)	0.39
eGFR, mL/min	58.8 (23.8)	40.7 (7.9)	84.2 (9.2)	<0.001
BUN, mg/dL	25.6 (13.9)	33.4 (12.9)	14.6 (5.0)	0.03
Cr, mg/dL	1.4 (0.45)	1.7 (0.18)	0.88 (0.09)	<0.001
SBP, mmHg	128.2 (13.3)	130.9 (16.2)	125.5 (10.0)	0.29
DBP, mmHg	68.5 (13.5)	63.0 (15.7)	74.0 (8.6)	0.11
FPG, g/dL	122.3 (32.5)	135.4 (35.0)	103.8 (18.3)	0.02
Sex				
Women	8 (50%)	4 (50%)	4 (50%)	
Men	8 (50%)			
Race				
Black	6 (37.5%)	3 (37.5%)	3 (37.5%)	
White	10 (62.5%)	5 (62.5%)	5 (62.5%)	

Data are presented as *n* (%) or mean (SD). [1]Result of paired t-tests comparing CKD and healthy participants. BMI, body mass index; eGFR, estimated glomerular filtration rate; BUN, blood urea nitrogen; Cr, creatinine; SBP, systolic blood pressure; DBP, diastolic blood pressure, FPG, fasting plasma glucose.

Agreement Between Sodium Intake, 24hUNa, and e24hUNa

The differences between actual sodium intake, measured 24hUNa, and e24hUNa from the INTERSALT and the Tanaka-Na equations are shown in **Figure 3.1A**. The main GLM analysis was not significant (p=0.18), suggesting that all methods of measuring/estimating sodium intake did not produce significantly different average values. In addition, the interaction with CKD status was not significant (p=0.51), suggesting there were no differences in sodium intake estimates in healthy compared to CKD participants. Notably, however, average e24hUNa from the INTERSALT and Tanaka-Na equations were ~400 and ~500 mg higher than sodium intake, respectively. **Figure 3.1B** shows the differences between sodium intake, measured 24hUNa, and e24hUNa from the Nerbass-RRID and Nerbass-SALTED equations in CKD participants only. The main GLM analysis was significant (p=0.001), and planned contrasts showed that e24hUNa from the Nerbass-SALTED equation was significantly higher than both actual sodium intake (p<0.001) as well as measured 24hUNa (p=0.007). The results from our correlational analyses for sodium are shown in **Table 3.2**. Overall, e24hUNa from both the INTERSALT and Tanaka-Na equations are not correlated with either actual sodium intake or measured 24hUNa (all p>0.05). In CKD participants, e24hUNa from both the Nerbass-RRID and

Nerbass-SALTED equations were not correlated with actual sodium intake (both p>0.05); however, e24hUNa from the Nerbass-SALTED was positively correlated with measured 24hUNa (r=0.78, p=0.04).

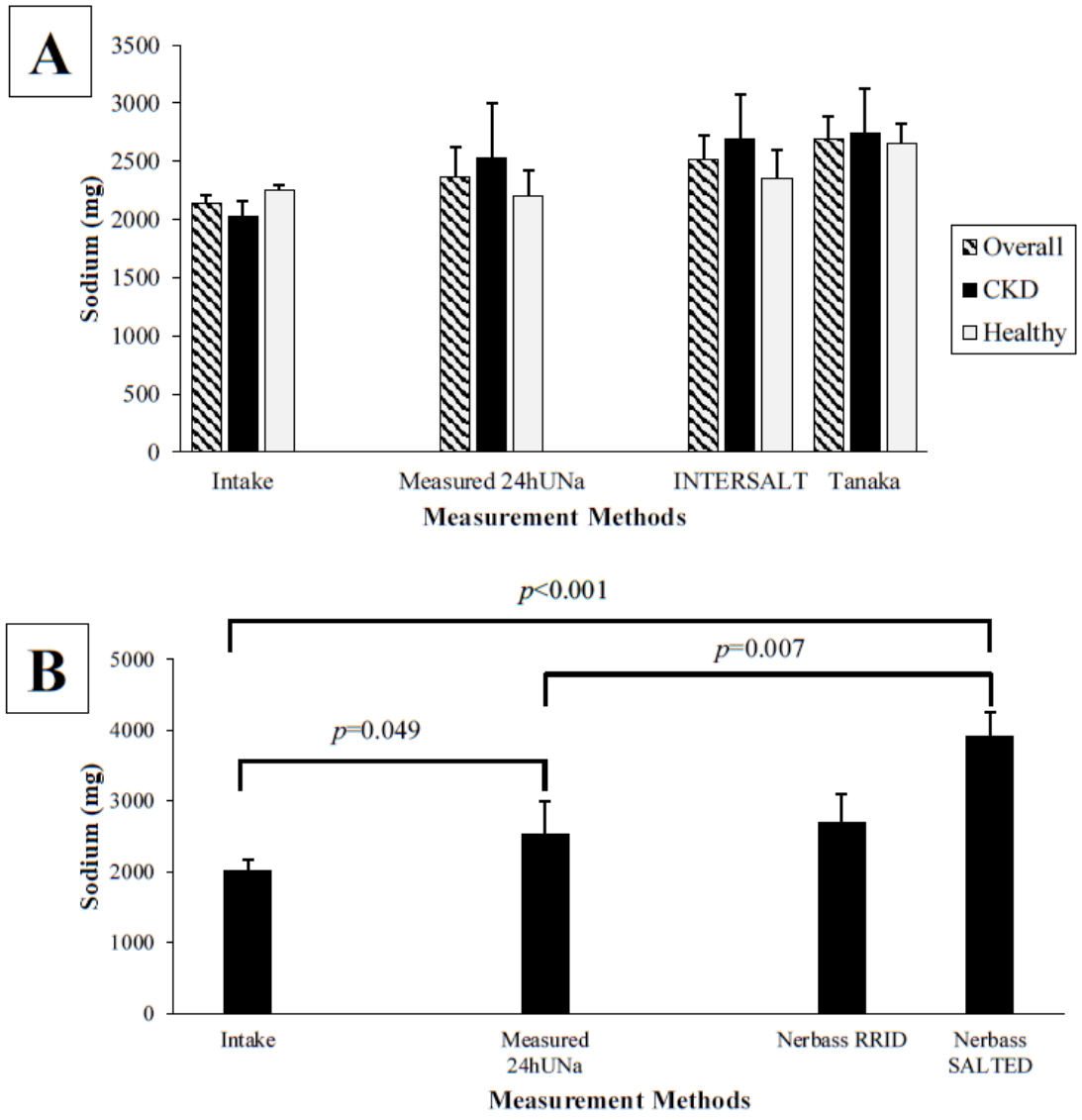


Figure 3.1 Relationship between sodium intake, measured 24hUNa, and estimated 24hUNa in A) all participants and B) CKD participants only. Bars represent mean ± SEM.

Table 3.2 Correlations between intake, measured 24h mineral excretion, and estimated 24h mineral excretion for sodium and potassium

	Actual Na Intake			Measured 24hUNa		
	r	**95% CI**	**p-value**	**r**	**95% CI**	**p-value**
Measured 24hUNa	0.19	(-0.41, 0.67)	0.53	--	--	--
Estimated 24hUNa						
INTERSALT	0.28	(-0.33, 0.72)	0.35	0.33[1]	(-0.28, 0.74)	0.27
Tanaka	0.09	(-0.48, 0.61)	0.76	0.32[2]	(-0.30, 0.73)	0.29
Nerbass—RRID*	0.18	(-0.67, 0.82)	0.70	0.67[3]	(-0.22, 0.94)	0.10
Nerbass—SALTED*	0.39	(-0.54, 0.88)	0.39	0.78[4]	(-0.01, 0.96)	0.04

	Actual K Intake			Measured 24hUK		
	r	**95% CI**	***p*-value**	***r***	**95% CI**	***p*-value**
Measured 24hUK	0.37	(-0.24, 0.75)	0.21	--	--	--
Estimated 24hUK						
Tanaka	0.55	(-0.02, 0.84)	0.051	0.32[5]	(-0.29, 0.73)	0.29

*evaluated in CKD participants only
[1]Correlation coefficient between e24hUNa and measured 24hUNa in original INTERSALT study was r=0.79 and 0.71 in men and women, respectively
[2]Correlation coefficient between e24hUNa and measured 24hUNa in original Tanaka study was r=0.54
[3]Correlation coefficient between e24hUNa and measured 24hUNa in original Nerbass-RRID study was r=0.55
[4]Correlation coefficient between e24hUNa and measured 24hUNa in original Nerbass-SALTED study was r=0.57
[5]Correlation coefficient between e24hUK and measured 24hUK in original Tanaka study was r=0.56

Agreement between e24hUNa and actual sodium intake is displayed in the Bland-Altman plots in **Figure 3.2**. On average, both the INTERSALT and Tanaka-Na equations overestimate actual sodium intake. From our observation of the pattern in the plots, both equations tend to overestimate sodium intake when the actual sodium value is low (i.e. further left on the x-axis), and tends to underestimate sodium intake to a greater extent when the actual sodium value is higher (i.e. further right on the x-axis). Similar observations can be seen when looking at the Bland-Altman plots for the Nerbass-RRID and Nerbass-SALTED equations.

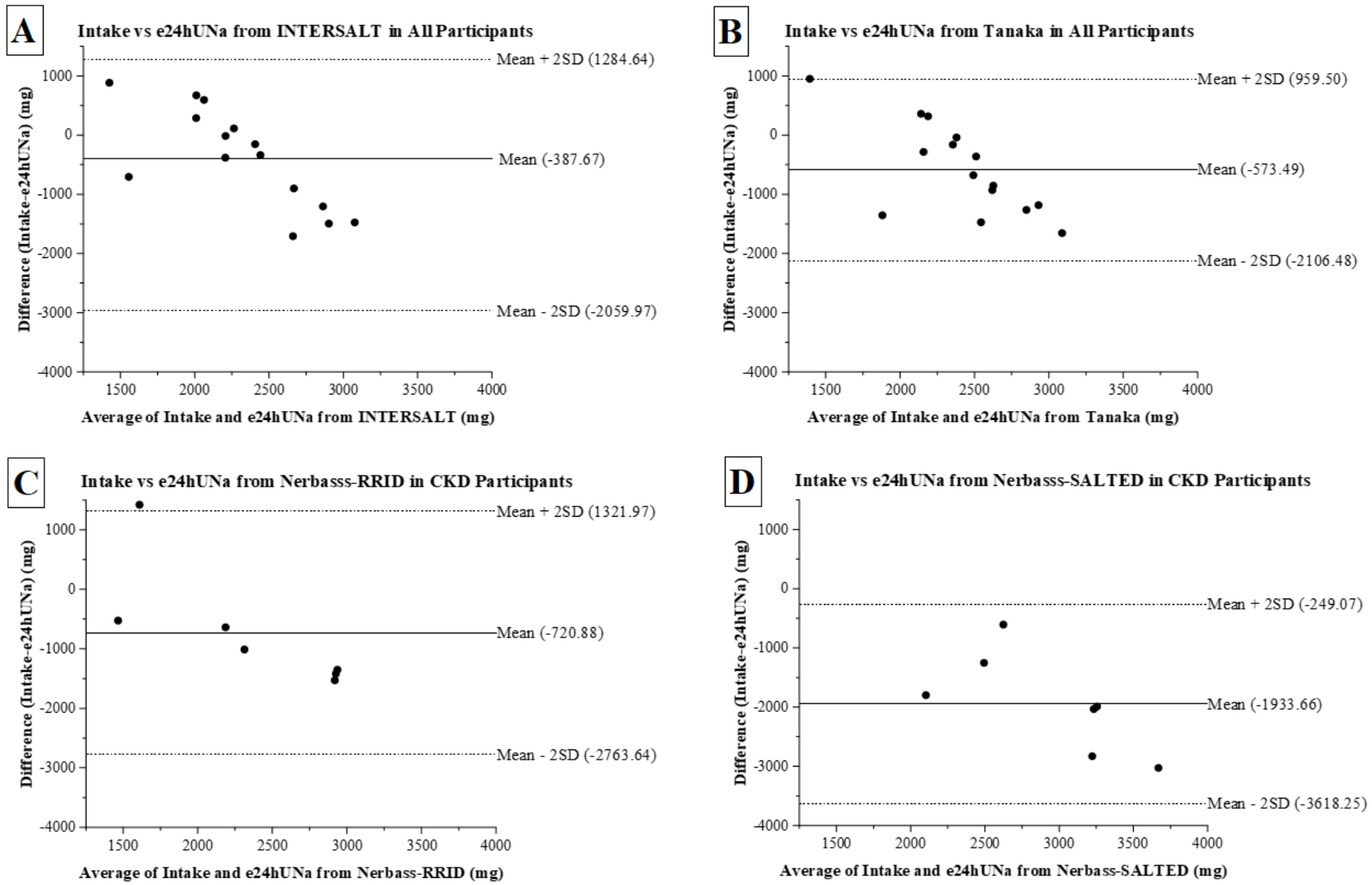


Figure 3.2 Agreement between sodium intake and e24hUNa from A) INTERSALT equation in all participants, B) Tanaka equation in all participants, C) Nerbass-RRID equation in CKD participants, and D) Nerbass-SALTED equation in CKD participants.

Agreement Between Potassium Intake, 24hUK, and e24hUK

The differences between actual potassium intake, measured 24hUK, and e24hUK from the Tanaka-K equation are shown in **Figure 3.3**. The main GLM analysis violated the assumption of sphericity, thus the Greenhouse-Geiser correction was applied. The corrected main GLM analysis was significant (p=0.02), and planned contrasts show that e24hUK from the Tanaka-K equation was significantly lower than both the actual potassium intake (p<0.0001) and measured 24hUK (p=0.02). The interaction with CKD status was not significant (p=0.82), suggesting that there were no differences in potassium intake estimates between healthy and CKD participants. The results from our correlational analyses for potassium are shown in **Table 3.2.** Overall, e24hUK was not correlated with actual potassium intake or measured 24hUK (all p>0.05). Agreement between e24hUK and actual potassium intake is shown in the Bland-Altman plot in **Figure 3.4**. On average, the Tanaka-K equation underestimates actual potassium intake. From our observation of the pattern in the plots, the Tanaka equation underestimates potassium intake to a greater extent when actual potassium intake is lower (i.e. further left on the x-axis), but better agreement between e24hUK and potassium intake is observed when the actual value is higher (i.e. further right on the x-axis).

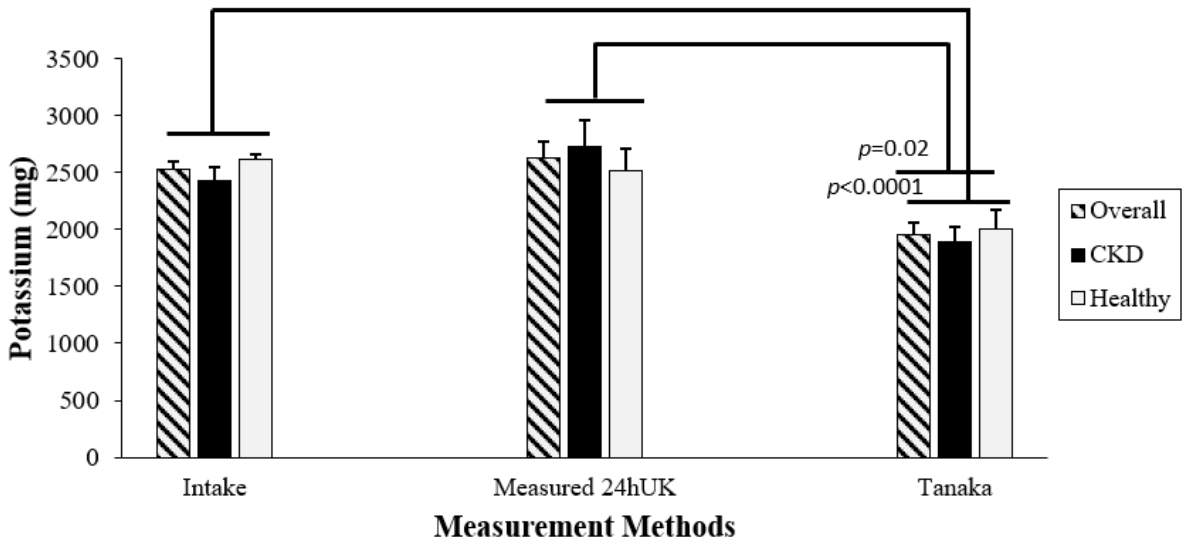


Figure 3.3 Comparison of potassium intake, measured 24hUK, and 24hUK from the Tanaka equation in all participants. Bars represent mean ± SEM.

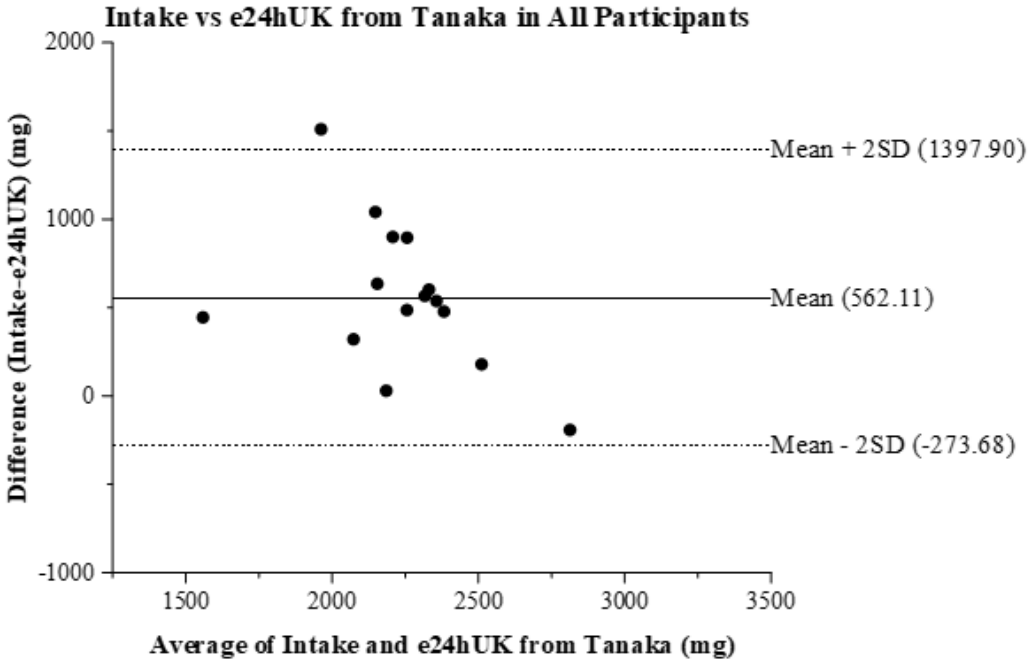


Figure 3.4 Agreement between potassium intake and e24hUK using the Tanaka equation in all participants.

Discussion

Our study was a secondary analysis of a controlled feeding study in moderate-stage CKD participants and healthy matched controls. Ours is the first study to assess the agreement between known dietary mineral intake and estimated 24-hour mineral excretion from spot urine samples for both sodium and potassium. We found that e24hUNa from the INTERSALT and Tanaka-Na equations was higher than, though not statistically different from, actual sodium intake in all participants. In CKD participants, the CKD-specific Nerbass-SALTED equation estimated an e24hUNa that was significantly higher than actual sodium intake, while the Nerbass-RRID equation estimated an e24hUNa that was closer to actual intake. Examination of Bland-Altman plots suggests that the bias in these equations becomes even more apparent at higher and lower levels of actual sodium intake. For potassium, e24hUK from the Tanaka-K equation was significantly lower than actual potassium intake, and examination of our Bland-Altman plot suggests that the bias in e24hUK becomes more apparent at low potassium intakes. Neither e24hUNa nor e24hUK were correlated with sodium or potassium intake, respectively. Overall,

our results suggest that e24hUNa and e24hUK are poor indicators of actual corresponding mineral intake in both healthy adults and CKD patients.

Several equations have been developed for calculating e24hUNa and e24hUK from spot urine samples, and these equations differ in methodology and population used to create them. Both Kawasaki (18) and Tanaka (17) developed equations for predicting e24hUNa and e24hUK from a Japanese population. Both equations use predicted 24-hour creatinine excretion as a correction factor to calculate e24hUNa and 24hUK. However, these equations are different in that the Tanaka equations were developed using a casual spot urine sample whereas the Kawasaki equations were developed using a fasting second morning void. Additionally, the Kawasaki equations were specifically developed in a clinically healthy population—having any active disease was considered exclusionary. Thus, because the intention of the current study was to calculate e24hUNa and e24hUK in a healthy as well as a CKD sample, we chose to exclude the Kawasaki equations from our current analyses. The INTERSALT equation (16) was developed as an additional equation to calculate e24hUNa. Both the INTERSALT and the Tanaka equation were developed using cohorts from the original multi-country INTERSALT study (22). However, the INTERSALT study used data from North American and Europe to develop this new equation. This equation used a casual spot urine sample and was developed using a multiple linear regression approach, with separate equations for men and women. More recently, several equations have been developed to calculate e24hUNa specifically in those with CKD: the Nerbass-RRID (19), the Nerbass-SALTED (20), and the CKDSALT (21) equations. All three equations were developed using a fasting morning spot urine sample, but the populations used were quite different. The Nerbass-RRID equation used a UK cohort with stage 3 CKD, the Nerbass-SALTED equation used a Brazilian cohort with pre-dialysis CKD of any stage, and the CKDSALT equation used a Chinese cohort with stage 1-4 CKD. The different population and CKD stage of the participants included in the development of the Nerbass-SALTED equation could explain why e24UNa was ~2,000 mg higher than sodium intake in our study sample. The CKDSALT equation was not used in our analyses because this equation includes urine urea as a factor, which was not measured in our study. To our knowledge, no equation exists to calculate e24hUK specifically in a CKD population.

Our study is the first to compare e24hUNa and e24hUK to an actual known intake in any population. However, previous studies have sought to validate e24hUNa and e24hUK against

measured 24hUNa and 24hUK, respectively, in various populations. Studies in non-CKD adults of varying racial/ethnic groups have found a large amount of bias when comparing e24hUNa to measured 24hUNa, and the relative bias varies depending on when the spot urine sample was collected, which equation was used, and what the true measured 24hUNa was (23-25). Similar results have been found when evaluating agreement between e24hUNa and measured 24hUNa in a CKD population (26). In addition, these and other studies found e24hUNa to only be weakly correlated with measured 24hUNa, if at all, with overall correlation coefficients ranging from 0.31-0.67 (23-25). These findings are similar to our results in the current study, which show a large amount of bias when comparing e24hUNa to sodium intake with insignificant correlations between the two measurements. Fewer studies have sought to validate e24hUK, but one study in non-CKD adults similarly found that e24hUK was biased when compared to measured 24hUK with similarly poor correlations, regardless of which equation was used and the timing of spot urine sample collection (27). The consistently poor validity of equations for calculating e24hUNa and e24hUK may seem surprising. However, closer examination of these shows that, even in the studies where these equations were originally developed, the correlations between estimated mineral excretion and true mineral excretion are moderate at best, with most correlation coefficients between 0.50-0.60 (16-20). These findings, combined with our results, suggest that the current method of estimating sodium and potassium intake using a spot urine sample should be further evaluated and likely revised.

Producing inaccurate estimates of mineral intake can have important implications for predicting disease risk, and the subsequent development of interventions and policies, and this implication has not gone unnoticed. Indeed, a recent analysis found that using inaccurate methods for estimating population sodium intake, specifically e24hUNa from a spot urine sample and many prediction equations, mischaracterizes the relationship between sodium intake and mortality as U- or J-shaped (28, 29). If these findings are to be believed, it would suggest that policies and interventions aimed at reducing sodium intake are misguided. However, these analyses show that when the same data are reanalyzed using the gold standard method of estimating sodium intake (i.e. multiple 24-hour urine collections), the relationship between sodium intake and mortality is shown to be linear (28, 29). Given these findings, the International Consortium for Quality Research on Dietary Sodium/Salt (TRUE) published a position statement strongly discouraging the use of spot urine samples for estimating sodium

intake (30). The implications for inaccurate estimates of potassium intake warrant further investigation.

The strength of our study is in the rigorous, highly controlled design. This study was a controlled feeding study, and all meals were chemically analyzed to confirm dietary mineral content. Therefore, we knew the actual sodium and potassium intakes of our participants. The use of a controlled feeding study is perhaps the only circumstance in which actual dietary intake could be known and compared to a urinary biomarker. In addition, participants remained in an inpatient setting for the last two days of the study, thus allowing study staff to ensure participants were properly following study protocol for the diets as well as the urine collections. This inpatient design allows for more confidence in the data collected. However, our study does have limitations. We have a small sample size (n=16) due to the fact that power and sample size calculations were originally conducted to meet the needs of the parent study. In addition, all CKD participants were in stage 3 and all participants consumed the same study menu, which limits the generalizability of our findings to other CKD stages and other levels of sodium and potassium intake. Future studies should confirm the present findings in a larger sample size, including broader stages of CKD, and with a wider range of sodium and potassium intake levels.

Practical Application

Estimated 24-hour sodium and potassium excretion calculated from spot urine samples are not reliable indicators of either sodium or potassium intake, respectively, in both CKD patients as well as those without CKD. Researchers and practitioners who use this method should do so with caution, as there is a risk of mischaracterizing a patient's disease risk.

References

1. Benjamin EJ, Muntner P, Alonso A, Bittencourt MS, Callaway CW, Carson AP, et al. Heart Disease and Stroke Statistics-2019 Update: A Report From the American Heart Association. Circulation. 2019;139(10):e56-e66. Epub 2019/02/01. doi: 10.1161/cir.0000000000000659. PubMed PMID: 30700139.

2. Mennuni S, Rubattu S, Pierelli G, Tocci G, Fofi C, Volpe M. Hypertension and kidneys: unraveling complex molecular mechanisms underlying hypertensive renal damage. Journal of Human Hypertension. 2014;28(2):74-9. doi: 10.1038/jhh.2013.55.
3. Sacks FM, Svetkey LP, Vollmer WM, Appel LJ, Bray GA, Harsha D, 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):3-10. Epub 2001/01/04. doi: 10.1056/nejm200101043440101. PubMed PMID: 11136953.
4. He FJ, MacGregor GA. Effect of modest salt reduction on blood pressure: a meta-analysis of randomized trials. Implications for public health. J Hum Hypertens. 2002;16(11):761-70. Epub 2002/11/22. doi: 10.1038/sj.jhh.1001459. PubMed PMID: 12444537.
5. McMahon EJ, Bauer JD, Hawley CM, Isbel NM, Stowasser M, Johnson DW, et al. A randomized trial of dietary sodium restriction in CKD. J Am Soc Nephrol. 2013;24(12):2096-103. Epub 2013/11/07. doi: 10.1681/ASN.2013030285. PubMed PMID: 24204003; PubMed Central PMCID: PMCPMC3839553.
6. Garofalo C, Borrelli S, Provenzano M, De Stefano T, Vita C, Chiodini P, et al. Dietary Salt Restriction in Chronic Kidney Disease: A Meta-Analysis of Randomized Clinical Trials. Nutrients. 2018;10(6). Epub 2018/06/09. doi: 10.3390/nu10060732. PubMed PMID: 29882800; PubMed Central PMCID: PMCPMC6024651.
7. Filippini T, Violi F, D'Amico R, Vinceti M. The effect of potassium supplementation on blood pressure in hypertensive subjects: A systematic review and meta-analysis. Int J Cardiol. 2017;230:127-35. Epub 2016/12/28. doi: 10.1016/j.ijcard.2016.12.048. PubMed PMID: 28024910.
8. Kieneker LM, Bakker SJ, de Boer RA, Navis GJ, Gansevoort RT, Joosten MM. Low potassium excretion but not high sodium excretion is associated with increased risk of developing chronic kidney disease. Kidney Int. 2016;90(4):888-96. Epub 2016/08/31. doi: 10.1016/j.kint.2016.07.012. PubMed PMID: 27575557.

9. Sharma S, McFann K, Chonchol M, de Boer IH, Kendrick J. Association between dietary sodium and potassium intake with chronic kidney disease in US adults: a cross-sectional study. Am J Nephrol. 2013;37(6):526-33. Epub 2013/05/15. doi: 10.1159/000351178. PubMed PMID: 23689685; PubMed Central PMCID: PMCPMC3919025.
10. Einhorn LM, Zhan M, Hsu VD, Walker LD, Moen MF, Seliger SL, et al. The Frequency of Hyperkalemia and Its Significance in Chronic Kidney Disease. Archives of Internal Medicine. 2009;169(12):1156-62. doi: 10.1001/archinternmed.2009.132.
11. An JN, Lee JP, Jeon HJ, Kim DH, Oh YK, Kim YS, et al. Severe hyperkalemia requiring hospitalization: predictors of mortality. Critical Care. 2012;16(6):R225. doi: 10.1186/cc11872.
12. Holbrook JT, Patterson KY, Bodner JE, Douglas LW, Veillon C, Kelsay JL, et al. Sodium and potassium intake and balance in adults consuming self-selected diets. Am J Clin Nutr. 1984;40(4):786-93. Epub 1984/10/01. PubMed PMID: 6486085.
13. Tasevska N, Runswick SA, Bingham SA. Urinary potassium is as reliable as urinary nitrogen for use as a recovery biomarker in dietary studies of free living individuals. J Nutr. 2006;136(5):1334-40. Epub 2006/04/15. doi: 10.1093/jn/136.5.1334. PubMed PMID: 16614426.
14. Cogswell ME, Maalouf J, Elliott P, Loria CM, Patel S, Bowman BA. Use of Urine Biomarkers to Assess Sodium Intake: Challenges and Opportunities. Annu Rev Nutr. 2015;35:349-87. Epub 2015/05/15. doi: 10.1146/annurev-nutr-071714-034322. PubMed PMID: 25974702; PubMed Central PMCID: PMCPMC5497310.
15. McLean RM. Measuring population sodium intake: a review of methods. Nutrients. 2014;6(11):4651-62. Epub 2014/10/30. doi: 10.3390/nu6114651. PubMed PMID: 25353661; PubMed Central PMCID: PMCPMC4245554.
16. Brown IJ, Dyer AR, Chan Q, Cogswell ME, Ueshima H, Stamler J, et al. Estimating 24-hour urinary sodium excretion from casual urinary sodium concentrations in Western populations: the INTERSALT study. Am J Epidemiol. 2013;177(11):1180-92. Epub 2013/05/16. doi: 10.1093/aje/kwt066. PubMed PMID: 23673246; PubMed Central PMCID: PMCPMC3664342.

17. Tanaka T, Okamura T, Miura K, Kadowaki T, Ueshima H, Nakagawa H, et al. A simple method to estimate populational 24-h urinary sodium and potassium excretion using a casual urine specimen. J Hum Hypertens. 2002;16(2):97-103. Epub 2002/02/19. doi: 10.1038/sj.jhh.1001307. PubMed PMID: 11850766.
18. Kawasaki T, Itoh K, Uezono K, Sasaki H. A simple method for estimating 24 h urinary sodium and potassium excretion from second morning voiding urine specimen in adults. Clin Exp Pharmacol Physiol. 1993;20(1):7-14. Epub 1993/01/01. PubMed PMID: 8432042.
19. Nerbass FB, Pecoits-Filho R, McIntyre NJ, McIntyre CW, Taal MW. Development of a formula for estimation of sodium intake from spot urine in people with chronic kidney disease. Nephron Clin Pract. 2014;128(1-2):61-6. Epub 2014/10/25. doi: 10.1159/000363297. PubMed PMID: 25342580.
20. Nerbass FB, Hallvass AE, Taal MW, Pecoits-Filho R. Formula to detect high sodium excretion from spot urine in chronic kidney disease patients. J Bras Nefrol. 2017;39(1):23-8. Epub 2017/03/30. doi: 10.5935/0101-2800.20170004. PubMed PMID: 28355396.
21. Hu J, Wang Y, Song N, Zhang X, Teng J, Zou J, et al. Estimating 24-Hour Urinary Sodium Excretion From Spot Urine Samples in Chronic Kidney Disease Patients. J Ren Nutr. 2020;30(1):11-21. Epub 2019/04/09. doi: 10.1053/j.jrn.2019.02.002. PubMed PMID: 30956089.
22. 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):319-28. Epub 1988/07/30. PubMed PMID: 3416162; PubMed Central PMCID: PMCPMC1834069.
23. Cogswell ME, Wang CY, Chen TC, Pfeiffer CM, Elliott P, Gillespie CD, et al. Validity of predictive equations for 24-h urinary sodium excretion in adults aged 18-39 y. Am J Clin Nutr. 2013;98(6):1502-13. Epub 2013/09/21. doi: 10.3945/ajcn.113.059436. PubMed PMID: 24047921; PubMed Central PMCID: PMCPMC3831536.

24. Allen NB, Zhao L, Loria CM, Van Horn L, Wang CY, Pfeiffer CM, et al. The Validity of Predictive Equations to Estimate 24-Hour Sodium Excretion: The MESA and CARDIA Urinary Sodium Study. Am J Epidemiol. 2017;186(2):149-59. Epub 2017/08/26. doi: 10.1093/aje/kwx056. PubMed PMID: 28838062.
25. Charlton K, Ware LJ, Chidumwa G, Cockeran M, Schutte AE, Naidoo N, et al. Prediction of 24-hour sodium excretion from spot urine samples in South African adults: a comparison of four equations. J Hum Hypertens. 2019. Epub 2019/05/12. doi: 10.1038/s41371-019-0210-2. PubMed PMID: 31076654.
26. Dougher CE, Rifkin DE, Anderson CA, Smits G, Persky MS, Block GA, et al. Spot urine sodium measurements do not accurately estimate dietary sodium intake in chronic kidney disease. Am J Clin Nutr. 2016;104(2):298-305. Epub 2016/07/01. doi: 10.3945/ajcn.115.127423. PubMed PMID: 27357090; PubMed Central PMCID: PMCPMC4962156.
27. Mercado CI, Cogswell ME, Loria CM, Liu K, Allen N, Gillespie C, et al. Validity of predictive equations for 24-h urinary potassium excretion based on timing of spot urine collection among adults: the MESA and CARDIA Urinary Sodium Study and NHANES Urinary Sodium Calibration Study. Am J Clin Nutr. 2018;108(3):532-47. Epub 2018/12/12. doi: 10.1093/ajcn/nqy138. PubMed PMID: 30535091; PubMed Central PMCID: PMCPMC6454816.
28. He FJ, Campbell NRC, Ma Y, MacGregor GA, Cogswell ME, Cook NR. Errors in estimating usual sodium intake by the Kawasaki formula alter its relationship with mortality: implications for public health. Int J Epidemiol. 2018;47(6):1784-95. Epub 2018/12/06. doi: 10.1093/ije/dyy114. PubMed PMID: 30517688; PubMed Central PMCID: PMCPMC6280933.
29. He FJ, Ma Y, Campbell NRC, MacGregor GA, Cogswell ME, Cook NR. Formulas to Estimate Dietary Sodium Intake From Spot Urine Alter Sodium-Mortality Relationship. Hypertension. 2019;74(3):572-80. Epub 2019/07/30. doi: 10.1161/hypertensionaha.119.13117. PubMed PMID: 31352828.

30. Campbell NRC, He FJ, Tan M, Cappuccio FP, Neal B, Woodward M, et al. The International Consortium for Quality Research on Dietary Sodium/Salt (TRUE) position statement on the use of 24-hour, spot, and short duration (<24 hours) timed urine collections to assess dietary sodium intake. J Clin Hypertens (Greenwich). 2019. Epub 2019/05/16. doi: 10.1111/jch.13551. PubMed PMID: 31087778.

CHAPTER 4: SPOT URINE SAMPLES TO ESTIMATE PHOSPHORUS INTAKE IN CKD PATIENTS: A SECONDARY ANALYSIS FROM A CONTROLLED FEEDING STUDY

Abstract

Objective: The objective of our study was to determine if estimated 24-hour urinary phosphorus excretion (e24hUP), calculated from a spot urine sample using a published equation, provides a reliable estimate of actual phosphorus intake in those with moderate stage chronic kidney disease (CKD) consuming a controlled diet.

Design and Methods: This was a secondary analysis of a controlled feeding study aimed at assessing differences in phosphorus absorption between healthy and CKD participants. Only the participants with CKD were included in the present analyses. Participants (n=8) consumed a controlled diet providing ~1800 mg/d P for 8 days. Days 7 and 8 were inpatient study days, during which time participants consumed all meals and collected all urine in a clinic setting. e24UP was calculated using the spot urine sample collected in the morning on day 7, and e24hUP was compared to known phosphorus intake.

Results: Actual phosphorus intake was significantly higher than both measured 24hUP (difference of 731 mg, $p<0.001$) and e24hUP (difference of 960 mg, $p<0.001$). In addition, e24hUP was not correlated with actual phosphorus intake ($r=0.56$, $p=0.19$), though e24hUP was correlated with measured 24hUP ($r=0.76$, $p=0.046$). The Bland-Altman plot shows consistently poor agreement between e24hUP and phosphorus intake, regardless of the level of actual phosphorus intake.

Conclusion: e24hUP calculated using a spot urine sample does not provide a reliable indicator of actual phosphorus intake in moderate stage CKD. These findings should be confirmed in a larger sample with varying stages of CKD and a wider range of phosphorus intake.

Introduction

Chronic kidney disease (CKD) is a major public health problem affecting >14% of the U.S. population (1). Those with CKD experience a decreased quality of life (2) as well as an increased risk for comorbidities and a two-times greater mortality rate compared to those without CKD (3). Disturbances in phosphorus homeostasis begin early in CKD and can ultimately lead to cardiovascular complications, bone fragility, and CKD progression (4). Thus, managing abnormal phosphorus is an important focus for treating those with CKD (5). Reducing absorption of dietary phosphorus, often by reducing phosphorus intake or taking phosphate binders, is a fundamental approach for managing serum phosphate and parathyroid hormone levels with the goal of preventing bone and mineral disorders (6-8).

Accurately and easily estimating phosphorus intake in both research and clinical practice is critical for furthering our understanding of the effect of phosphorus on CKD, designing effective interventions, and creating appropriate clinical guidelines and public health policies. Estimating dietary phosphorus intake can be challenging, largely due to the widespread presence of naturally-occurring phosphorus and use of phosphorus-containing additives in foods combined with inaccurate nutrient databases (9). Given that dietary phosphorus is highly absorbed (10) and that the kidneys are important regulators of phosphorus homeostasis (11), 24-hour urinary phosphorus excretion (24hUP) is often used as a surrogate indicator of dietary phosphorus intake. However, we have previously shown that 24hUP did not correlate to a known dietary intake nor intestinal phosphorus absorption from metabolic balance in eight moderate-stage CKD patients who participated in a controlled feeding study (12). We concluded that caution should be used in interpreting 24hUP values as reflecting dietary P intake or absorption in non-interventional contexts. However, we argue that our results did not undermine the use of 24hUP as a surrogate measure of intestinal P absorption in the context of randomized controlled trials where the intervention has a known or assumed mechanism affecting intestinal P absorption (e.g. dietary P restriction or P binder trials). Thus, 24hUP remains a valuable measure in certain settings. However, this method is burdensome for participants. An alternative method that has been developed is to collect a spot urine sample and estimate 24hUP (e24hUP) using a prediction equation developed specifically for CKD (13). While this method is easier to administer, it remains unknown how closely e24hUP reflects actual phosphorus intake. Thus, the aim of our study was to utilize data from a controlled feeding study to compare e24hUP, calculated from a

spot urine sample using a published equation (13), to known phosphorus intake in CKD participants.

Methods

This study was a secondary analysis of a controlled feeding study aimed at assessing phosphorus absorption in stage 3 CKD patients and healthy matched controls. More detailed study methods have been published previously. Only the CKD participants were included in the present analyses. Briefly, descriptive data and baseline characteristics were collected at the screening visit. After enrolling in the study, participants began the 9-day study. The three-day cycle menu provided ~1800 mg P/d. All prepared foods and beverages were provided to the participants. For the first 6 days, participants consumed their controlled diet outside the study clinic. For days 7-9, participants were admitted to the clinical research center (CRC) at Indiana University School of Medicine for inpatient data collection. During this time, all food was provided to participants and any uneaten food was recorded by CRC staff. Participants collected all urine in specified intervals.

To confirm dietary mineral content, study meal composites were prepared as if they would be served to a participant. These composites were homogenized, freeze-dried, and ashed to remove all organic material. The mineral ash was then diluted and analyzed using inductively coupled plasma optical emission spectroscopy (ICP-OES, Optima 4300; PerkinElmer). Actual phosphorus intake was determined using chemical analysis data combined with data from food records collected in the CRC. Urine samples were analyzed for mineral content by ICP. The spot urine sample collected in the morning on day 7 was used to calculated e24hUP using an equation developed by Robinson-Cohen et al. (13) to predict 24hUP.

Data that were non-normally distributed and were log-transformed prior to analyses. Differences between actual phosphorus intake, 24hUP, and e24hUP were determined by general linear model (GLM), and the relationship between all three measurements were assessed using Pearson bivariate correlations. Agreement between phosphorus intake and e24hUP were assessed using a Bland-Altman plot. All statistical analyses were conducted using SAS (version 9.4) and $p<0.05$ was considered significant.

Results

Baseline characteristics of study participants are shown in **Table 4.1**. On average, participants (n=8) were 56.6 ± 13.8 years old with BMI ranging from 19.5-45.6 kg/m^2 . Half of the participants were women; 3 participants were black and 5 were white. All participants had moderate stage CKD, as reflected in the average eGFR of 40.7 ± 7.9 mL/min and the elevated BUN and Cr levels of 33.4 ± 12.9 and 1.7 ± 0.18, respectively. Average phosphorus intake on day 7 was 1627.96 ± 236.79 mg, as calculated using chemical analyses of the menu as served and the food intake records collected in the CRC. **Figure 4.1** compares average phosphorus intake to measured 24hUP and e24hUP calculated using the Robinson-Cohen equation. The main GLM analysis was significant ($p<0.001$). Planned contrasts showed that phosphorus intake was significantly higher than measured 24hUNa (difference of 731 mg, $p<0.001$) and e24hUP (difference of 960 mg, $p<0.001$). Measured 24hUNa was not significantly different from e24hUP (difference of 229 mg, $p=0.41$). Correlational analyses showed that phosphorus intake is significantly correlated with measured 24hUNa ($r=0.84$, $p=0.009$), and measured 24hUP is significantly correlated with e24hUP ($r=0.76$, $p=0.046$), however phosphorus intake is not correlated with e24hUP ($r=0.56$, $p=0.19$). The agreement between phosphorus intake and e24hUP is shown in the Bland-Altman plot in **Figure 4.2**. The e24hUP underestimated phosphorus intake in all participants, and the level of underestimation remained relatively consistent regardless of the level of phosphorus intake.

Table 4.1 Baseline characteristics of participants

Characteristic	CKD Participants (*n*=8)
Age, y	56.6 (13.8)
Height, cm	166.6 (10.3)
Weight, kg	87.6 (24.1)
BMI, kg/m^2	31.7 (9.4)
eGFR, mL/min	40.7 (7.9)
BUN, mg/dL	33.4 (12.9)
Cr, mg/dL	1.7 (0.18)
SBP, mmHg	130.9 (16.2)
DBP, mmHg	63.0 (15.7)
FPG, g/dL	135.4 (35.0)
Sex	
Women	4 (50%)
Men	
Race	
Black	3 (37.5%)
White	5 (62.5%)

Data are presented as *n* (%) or mean (SD). BMI, body mass index; eGFR, estimated glomerular filtration rate; BUN, blood urea nitrogen; Cr, creatinine; SBP, systolic blood pressure; DBP, diastolic blood pressure, FPG, fasting plasma glucose.

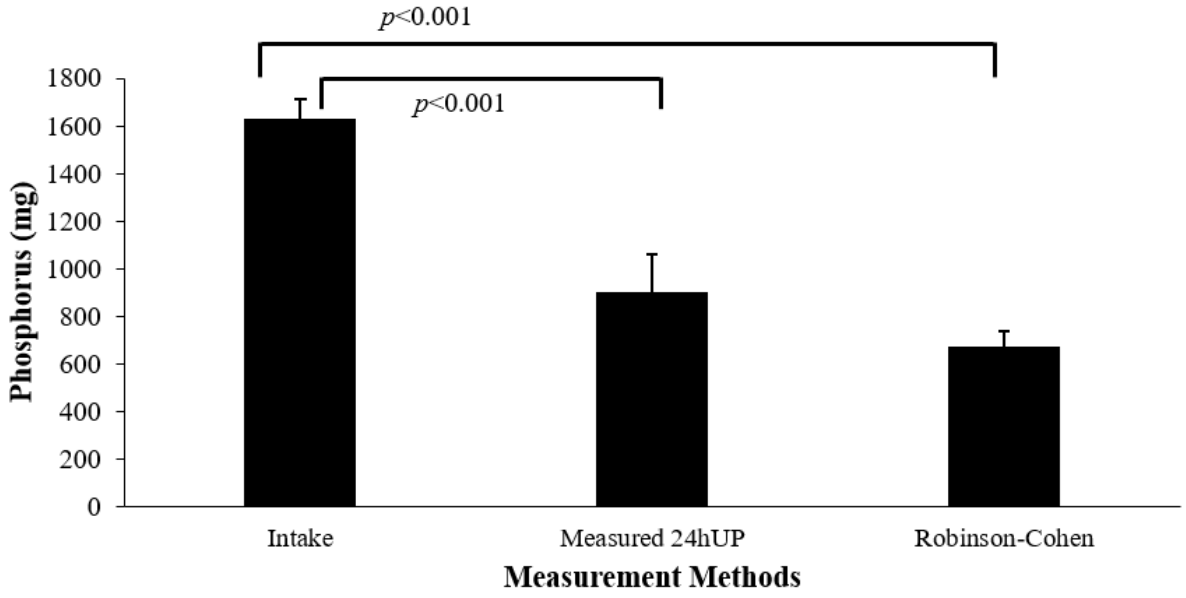


Figure 4.1 Relationship between phosphorus intake, measured 24hUP, and estimated 24hUP from the Robinson-Cohen equation. Bars represent mean ± SEM.

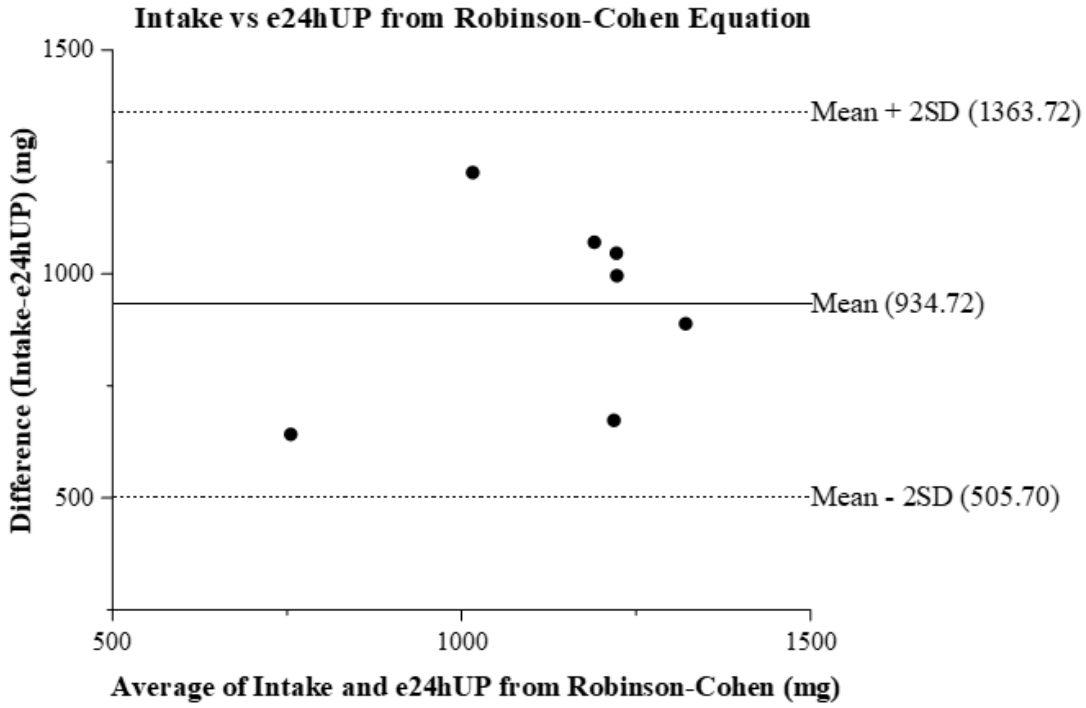


Figure 4.2 Agreement between phosphorus intake and e24hUP using the Robinson-Cohen equation.

Discussion

Our study is the first to compare e24hUP to an actual known phosphorus intake in participants with CKD. We utilized data from a controlled feeding study, thus ensuring that actual phosphorus intake was known. Our results show that e24hUP underestimates phosphorus intake by nearly 1000 mg, though e24hUP is not significantly different from measured 24hUP. In addition, though e24hUP is significantly correlated with measured 24hUP, it is not correlated with phosphorus intake, and Bland-Altman plots show consistently poor agreement between e24hUP and phosphorus intake at all levels of phosphorus intake. Overall, these findings suggest that e24hUP is a poor indicator of phosphorus intake. Our findings are important because phosphorus intake is the true exposure of interest when using a spot urine sample to calculate e24hUP. Indeed, producing inaccurate estimates of phosphorus intake could have implications for research as well as patient care in clinical settings.

Previous studies have sought to validate the use of spot urine samples for estimating 24hUP. The study by Robinson-Cohen et al (13), in which the equation for calculating e24hUP in CKD was developed, compared e24hUP against measured 24hUP in a separate validation cohort. They found that e24hUP was significantly correlated with measured 24hUP with a moderately strong regression coefficient (r^2=0.43) but e24hUP underestimated measured 24hUP in 49% of participants. A more recent cross-sectional study in both healthy and CKD participants examined whether spot urine phosphorus-to-creatinine ratio (uP/uCr) could be used as a reliable indicator of measured 24hUP (14). This study found that spot uP/uCr was not correlated with measured 24hUP, and spot urine phosphorus itself was significantly but weakly correlated with measured 24hUP (r=0.39). In addition, Bland-Altman plots displayed bias when comparing spot uP/uCr to measured 24hUP. The findings from these two studies suggest that e24hUP is superior to uP/uCr for estimating measured 24hUP; however, our findings suggest that e24hUP should be used with caution, as it may not provide a reliable indicator of phosphorus intake at a single point in time. The use of e24hUP as an indicator of change in phosphorus intake over time (e.g. in a phosphate binder or dietary intervention study) remains to be determined.

Spot urine samples are used to provide a surrogate indicator of measured 24hUP, assuming that 24hUP itself is a reliable indicator of phosphorus intake. However, whether 24hUP accurately reflects phosphorus intake in those with CKD has recently been called into question. Our previously reported analysis from a controlled feeding metabolic balance study (12) investigated the relationship between 24hUP and phosphorus intake and absorption in CKD participants. We reported that, despite the fixed phosphorus intake, 24hUP varied widely among participants and day-to-day within participants. In addition, 24hUP was not a reliable indicator of phosphorus intake nor net intestinal phosphorus absorption, but rather was inversely correlated to whole-body phosphorus retention. This suggests that caution should be used in interpreting 24hUP as a reflection of phosphorus intake or absorption in those with CKD.

The strength of our study lies in the tightly controlled study setting. Participants consumed a controlled diet, which was chemically analyzed to confirm the amount of phosphorus provided. In addition, dietary intake data and urine samples were collected in an inpatient setting, which helps ensure the accuracy and completeness of the data. Weaknesses of the current study include a small sample size, providing only one level of dietary phosphorus in the prescribed menu, and only including participants with moderate stage CKD. In order to make

these results more generalizable, future studies should confirm these findings in a larger sample with participants in varying stages of CKD consuming varying levels of dietary phosphorus.

Practical Application

Collecting a spot urine sample and calculating estimated 24-hour urinary phosphorus excretion may not provide an accurate indicator of actual phosphorus intake in CKD patients. Estimating phosphorus intake is important in both research and clinical settings, and investigators and clinicians should carefully consider which method they use. Future work should investigate more reliable methods for estimating phosphorus intake in this population

References

1. Chapter 1: CKD in the General Population. American Journal of Kidney Diseases. 2019;73(3):S1-S28. doi: 10.1053/j.ajkd.2018.12.001.
2. Perlman RL, Finkelstein FO, Liu L, Roys E, Kiser M, Eisele G, et al. Quality of life in chronic kidney disease (CKD): a cross-sectional analysis in the Renal Research Institute-CKD study. American journal of kidney diseases : the official journal of the National Kidney Foundation. 2005;45(4):658-66. doi: 10.1053/j.ajkd.2004.12.021. PubMed PMID: 15806468.
3. Chapter 3: Morbidity and Mortality in Patients with CKD. American Journal of Kidney Diseases. 2019;73(3):S45-S78. doi: 10.1053/j.ajkd.2018.12.003.
4. Wolf M. Mineral (Mal)Adaptation to Kidney Disease--Young Investigator Award Address: American Society of Nephrology Kidney Week 2014. Clin J Am Soc Nephrol. 2015;10(10):1875-85. Epub 2015/09/10. doi: 10.2215/cjn.04430415. PubMed PMID: 26350436; PubMed Central PMCID: PMCPMC4594069.
5. Levin A, Stevens PE, Bilous RW, Coresh J, De Francisco ALM, De Jong PE, et al. Kidney disease: Improving global outcomes (KDIGO) CKD work group. KDIGO 2012 clinical practice guideline for the evaluation and management of chronic kidney disease. Kidney International Supplements. 2013;3(1):1-150. doi: 10.1038/kisup.2012.73.

6. Barreto FC, Barreto DV, Massy ZA, Drueke TB. Strategies for Phosphate Control in Patients With CKD. Kidney Int Rep. 2019;4(8):1043-56. Epub 2019/08/24. doi: 10.1016/j.ekir.2019.06.002. PubMed PMID: 31440695; PubMed Central PMCID: PMCPMC6698320.
7. Stremke ER, Hill Gallant KM. Intestinal Phosphorus Absorption in Chronic Kidney Disease. Nutrients. 2018;10(10). Epub 2018/09/27. doi: 10.3390/nu10101364. PubMed PMID: 30249044; PubMed Central PMCID: PMCPMC6213936.
8. KDIGO 2017 Clinical Practice Guideline Update for the Diagnosis, Evaluation, Prevention, and Treatment of Chronic Kidney Disease–Mineral and Bone Disorder (CKD-MBD). Kidney International Supplements. 2017;7(1):1-59. doi: 10.1016/j.kisu.2017.04.001.
9. Sullivan CM, Leon JB, Sehgal AR. Phosphorus-containing food additives and the accuracy of nutrient databases: implications for renal patients. J Ren Nutr. 2007;17(5):350-4. Epub 2007/08/28. doi: 10.1053/j.jrn.2007.05.008. PubMed PMID: 17720105; PubMed Central PMCID: PMCPMC2020846.
10. Institute of Medicine 1997. Dietary Reference Intakes for Calcium, Phosphorus, Magnesium, Vitamin D, and Fluoride. Washington, DC: The National Academies Press. https://doi.org/10.17226/5776.
11. Blaine J, Chonchol M, Levi M. Renal Control of Calcium, Phosphate, and Magnesium Homeostasis. Clinical Journal of the American Society of Nephrology. 2015;10(7):1257. doi: 10.2215/CJN.09750913.
12. Stremke ER, McCabe LD, McCabe GP, Martin BR, Moe SM, Weaver CM, et al. Twenty-Four-Hour Urine Phosphorus as a Biomarker of Dietary Phosphorus Intake and Absorption in CKD: A Secondary Analysis from a Controlled Diet Balance Study. Clin J Am Soc Nephrol. 2018;13(7):1002-12. Epub 2018/06/21. doi: 10.2215/cjn.00390118. PubMed PMID: 29921736; PubMed Central PMCID: PMCPMC6032595.
13. Robinson-Cohen C, Ix JH, Smits G, Persky M, Chertow GM, Block GA, et al. Estimation of 24-hour urine phosphate excretion from spot urine collection: development of a predictive equation. J Ren Nutr. 2014;24(3):194-9. Epub 2014/04/25. doi: 10.1053/j.jrn.2014.02.001. PubMed PMID: 24759300.

14. Tan SJ, Smith ER, Cai MM, Holt SG, Hewitson TD, Toussaint ND. Relationship between timed and spot urine collections for measuring phosphate excretion. Int Urol Nephrol. 2016;48(1):115-24. Epub 2015/11/17. doi: 10.1007/s11255-015-1149-z. PubMed PMID: 26568386.

CHAPTER 5: DISCUSSION

Summary and Synthesis

The overall aim of my dissertation is to examine the use of spot urine samples for estimating mineral intake in healthy participants as well as those with CKD. Sodium, potassium, and phosphorus are minerals of interest that have implications for chronic disease risk as well as disease progression. Therefore, accurately estimating their intake is important for research, clinical practice, and public health purposes.

Spot Urine Samples for Estimating Sodium Intake in Normotensive Adults

HTN is a major risk factor for CVD (1), and sodium intake is an important modifiable risk factor for elevated blood pressure (2). Accurately and easily measuring sodium intake is important for studying diet-disease relationships and designing effective interventions. The gold standard method is 24hUNa, but 24-hour urine collections are burdensome for participants. Spot urine samples have been used as convenient alternative, but how well e24hUNa from spot urine samples reflects actual sodium intake has not been determined. Our aim was to explore the relationship between actual sodium intake and e24hUNa from multiple spot urine samples and published equations in healthy, normotensive men and women (n=35). We utilized data from a controlled feeding study, in which actual sodium intake was known. On day 4 of the 4-day control phase, participants consumed all meals and collected timed urine samples in a clinic setting. All timed spot urine samples were used to calculate e24hUNa using the INTERSALT (3), Tanaka (4), and Kawasaki (5) equations. We found that, regardless of spot urine sample or equation used, e24hUNa exhibited poor agreement with sodium intake. Our results suggest that e24hUNa does not provide a reliable indicator of sodium intake in normotensive adults. These findings align with previous studies, which have demonstrated bias and only weak correlations when comparing e24hUNa to measured 24hUNa (6) and provides further justification that researchers should exercise caution when using this method to characterize diet-disease relationships (7, 8).

Spot Urine Samples for Estimating Sodium and Potassium Intake in Patients with CKD and Healthy Adults

Sodium and potassium are two minerals with important implications for CKD prevention (9, 10) and progression (11, 12). Thus, it is important to have accurate and convenient methods for estimating sodium and potassium intake in both research and clinical practice settings. Twenty-four-hour urine collections are the gold standard method for both minerals because dietary sodium and potassium are readily excreted in the urine (13). Because 24-hour urine collections are burdensome, an alternative method that has been explored is using a spot urine sample to calculate e24hUNa and e24hUK, which are used as surrogate indicators of sodium and potassium intake, respectively. Multiple equations have been developed for calculating e24hUNa (3, 4) and e24hUK (14), including CKD-specific equations for 24hUNa (15, 16). However, how closely e24hUNa and e24hUK reflect actual sodium and potassium intake, respectively, in patients with CKD has not been explored. Therefore, our aim was to examine the relationship between e24hUNa and 24hUK in patients with moderate stage CKD (n=8) and matched healthy adults (n=8). We utilized data from a controlled feeding study, in which actual sodium and potassium intake is known. Diet samples were chemically analyzed using ICP to confirm mineral content. The study lasted 9 days; on days 7-9, participants consumed all meals and collected all urine in an inpatient setting. The morning spot urine sample collected on day 7 was used to calculate e24hUNa and e24hUK. We found generally poor agreement between e24hUNa and sodium intake, even with CKD-specific equations, as well as poor agreement between e24hUK and potassium intake. Our findings align with previous studies in CKD patients, which have demonstrated bias when comparing e24hUNa to measured 24hUNa (17). Overall, this suggests that spot urine samples are not a reliable method for estimating sodium or potassium intake in either healthy adults or patients with CKD.

Spot Urine Samples for Estimating Phosphorus Intake in Patients with CKD

Phosphorus is another mineral of concern in terms of CKD morbidity and mortality (18, 19). Targeting phosphorus intake and absorption is an important therapeutic strategy for managing CKD (20). Therefore, accurately and easily measuring phosphorus intake in patients with CKD as well as for research purposes is important. Twenty-four-hour urinary phosphorus excretion is often used as a surrogate indicator of phosphorus intake, though newer evidence

suggests 24hUP may not be reflective of phosphorus intake nor absorption (21). Despite this, it is still frequently used, and given the burden of 24-hour urine collections, the use of spot urine samples and e24hUP as an alternative method in patients with CKD has been explored (22). However, how well e24hUP reflects actual phosphorus intake has not been explored. The aim of our study was to examine the relationship between e24hUP and phosphorus intake in patients with moderate stage CKD (n=8). We utilized data from a controlled feeding study, in which true phosphorus intake was known, and in which urine samples and dietary intake data were collected in an inpatient setting. We used the spot urine sample collected in the morning on day 7 to calculate e24hUP. Our results show poor agreement between e24hUP and phosphorus intake, but good agreement between e24hUP and measured 24hUP. These findings corroborate previous research that suggests 24hUP is not reflective of intake (21). However, measured 24hUP may still be useful as an indicator of whole-body phosphorus retention (21), and also as an indicator of change in phosphorus absorption, as in an intervention study, and our results do not preclude the use of e24hUP as an indicator of measured 24hUP in that context.

Strengths and Limitations

The primary strength of all three studies is the use of data from controlled feeding studies. This helps ensure the actual mineral intake of study participants. In addition, dietary intake data and urine collections were all conducted in a clinical setting, particularly in the second and third studies which involved a two-day inpatient visit. This helps ensure that dietary intake is recorded accurately and ensures that no urine samples were missed, which could affect the accuracy of the measured 24-hour mineral data. A limitation of all three studies is the relatively small sample sizes (n=39, n=16, and n=8). For similar studies, much larger sample sizes are typically used).

Each study has its own unique strengths and limitations. A strength of my first study is the collection of multiple spot urine samples, which allowed us to examine e24hUNa when spot urine samples were collected at various times of the day relative to meal intake. A unique limitation of my first study is the lack of chemical analysis data on the study diets. Therefore, though menus were analyzed using the gold standard software for controlled feeding studies, we cannot confirm the reported sodium content. An additional limitation is the lack of generalizability. The study only included normotensive white and Asian adults. Therefore, these

findings cannot be extrapolated to hypertensive adults, adults with other chronic conditions, other races, or to children. A strength of my second and third studies is the chemical analysis of the study diet, which ensured the reported mineral intake was accurate. Unique limitations of my second and third studies are the inclusion of only patients with stage 3 CKD, and the provision of only one study diet. This prevents generalizability of our findings to all stages of CKD and prevented us from exploring relationships between mineral intake and estimated 24-hour mineral excretion over a wider range of intakes. An additional limitation of my second and third studies is the collection of only one true spot urine sample. This prevented us from exploring whether e24hUNa, e24hUK, and e24hUP may better reflect intake when using a spot urine sample collected at a different time of the day.

Future Directions

The studies in my dissertation were the first to examine the relationship estimated 24-hour mineral excretion and actual mineral intake (namely sodium, potassium, and phosphorus) in both healthy populations as well as patients with CKD. Previously, studies have only explored the relationship between estimated and measured 24-hour mineral excretion. Given that sodium, potassium, and phosphorus intake are often the true exposure of interest in both research and clinical practice settings, these fill an important gap in the literature regarding the use of alternative methods for estimating mineral intake. However, more work needs to be done. Future studies should confirm these findings in larger, more diverse samples with a wider range of mineral intakes. Given that current published equations were all designed to predict 24-hour mineral excretion, which itself is a surrogate measure of mineral intake, future studies should also aim to develop new equations designed to directly predict mineral intake. This would likely need to be done in the context of a controlled feeding study in order to ensure actual mineral intake is known. In addition, because e24hUP exhibited good agreement with measured 24hUP, future studies should explore whether e24hUP could be used as a surrogate indicator of phosphorus retention or changes in phosphorus absorption in the context of an intervention.

Conclusions

Regardless of equation used, estimated 24-hour mineral excretion does not provide a reliable indicator of mineral intake in either healthy adults or patients with CKD. For sodium, potassium, and phosphorus, 24-hour urine excretion is frequently used as a surrogate indicator of intake. Given the burden of 24-hour urine collections, spot urine samples have been explored as a more convenient alternative strategy to estimate intake. This involves collecting a spot urine sample, then using one of many available prediction equations to calculate an estimate of 24-hour mineral excretion. Our findings suggest that this strategy should be used with caution, which aligns with expert recommendations advising against using e24hUNa to examine diet-disease relationships (23). For phosphorus specifically, e24hUP may be useful as a surrogate indicator of other measures of phosphorus metabolism and homeostasis, though this should be confirmed with additional studies. Having an accurate and convenient method for estimating sodium, potassium, and phosphorus intake is undeniably important for chronic disease prevention and management. Therefore, future work should focus on improving current methods or developing new methods for directly estimating sodium, potassium, and phosphorus intake.

References

1. Benjamin EJ, Muntner P, Alonso A, Bittencourt MS, Callaway CW, Carson AP, Chamberlain AM, Chang AR, Cheng S, Das SR, et al. Heart Disease and Stroke Statistics-2019 Update: A Report From the American Heart Association. Circulation 2019;139(10):e56-e66. doi: 10.1161/cir.0000000000000659.
2. He FJ, MacGregor GA. Effect of modest salt reduction on blood pressure: a meta-analysis of randomized trials. Implications for public health. J Hum Hypertens 2002;16(11):761-70. doi: 10.1038/sj.jhh.1001459.
3. Brown IJ, Dyer AR, Chan Q, Cogswell ME, Ueshima H, Stamler J, Elliott P. Estimating 24-hour urinary sodium excretion from casual urinary sodium concentrations in Western populations: the INTERSALT study. Am J Epidemiol 2013;177(11):1180-92. doi: 10.1093/aje/kwt066.

4. Tanaka T, Okamura T, Miura K, Kadowaki T, Ueshima H, Nakagawa H, Hashimoto T. A simple method to estimate populational 24-h urinary sodium and potassium excretion using a casual urine specimen. J Hum Hypertens 2002;16(2):97-103. doi: 10.1038/sj.jhh.1001307.
5. Kawasaki T, Itoh K, Uezono K, Sasaki H. A simple method for estimating 24 h urinary sodium and potassium excretion from second morning voiding urine specimen in adults. Clin Exp Pharmacol Physiol 1993;20(1):7-14.
6. Cogswell ME, Wang CY, Chen TC, Pfeiffer CM, Elliott P, Gillespie CD, Carriquiry AL, Sempos CT, Liu K, Perrine CG, et al. Validity of predictive equations for 24-h urinary sodium excretion in adults aged 18-39 y. Am J Clin Nutr 2013;98(6):1502-13. doi: 10.3945/ajcn.113.059436.
7. He FJ, Campbell NRC, Ma Y, MacGregor GA, Cogswell ME, Cook NR. Errors in estimating usual sodium intake by the Kawasaki formula alter its relationship with mortality: implications for public health†. International Journal of Epidemiology 2018;47(6):1784-95. doi: 10.1093/ije/dyy114.
8. He FJ, Ma Y, Campbell NRC, MacGregor GA, Cogswell ME, Cook NR. Formulas to Estimate Dietary Sodium Intake From Spot Urine Alter Sodium-Mortality Relationship. Hypertension 2019;74(3):572-80. doi: 10.1161/hypertensionaha.119.13117.
9. Liu N, Sun W, Xing Z, Ma F, Sun T, Wu H, Dong Y, Xu Z, Fu Y, Yuan H. Association between sodium intakes with the risk of chronic kidney disease: evidence from a meta-analysis. Int J Clin Exp Med 2015;8(11):20939-45.
10. Sharma S, McFann K, Chonchol M, de Boer IH, Kendrick J. Association between dietary sodium and potassium intake with chronic kidney disease in US adults: a cross-sectional study. Am J Nephrol 2013;37(6):526-33. doi: 10.1159/000351178.
11. Garofalo C, Borrelli S, Provenzano M, De Stefano T, Vita C, Chiodini P, Minutolo R, De Nicola L, Conte G. Dietary Salt Restriction in Chronic Kidney Disease: A Meta-Analysis of Randomized Clinical Trials. Nutrients 2018;10(6). doi: 10.3390/nu10060732.
12. Einhorn LM, Zhan M, Hsu VD, Walker LD, Moen MF, Seliger SL, Weir MR, Fink JC. The Frequency of Hyperkalemia and Its Significance in Chronic Kidney Disease. Archives of Internal Medicine 2009;169(12):1156-62. doi: 10.1001/archinternmed.2009.132.

13. Holbrook JT, Patterson KY, Bodner JE, Douglas LW, Veillon C, Kelsay JL, Mertz W, Smith JC, Jr. Sodium and potassium intake and balance in adults consuming self-selected diets. Am J Clin Nutr 1984;40(4):786-93.
14. Allen NB, Zhao L, Loria CM, Van Horn L, Wang CY, Pfeiffer CM, Cogswell ME, Wright J, Liu K. The Validity of Predictive Equations to Estimate 24-Hour Sodium Excretion: The MESA and CARDIA Urinary Sodium Study. Am J Epidemiol 2017;186(2):149-59. doi: 10.1093/aje/kwx056.
15. Nerbass FB, Pecoits-Filho R, McIntyre NJ, McIntyre CW, Taal MW. Development of a formula for estimation of sodium intake from spot urine in people with chronic kidney disease. Nephron Clin Pract 2014;128(1-2):61-6. doi: 10.1159/000363297.
16. Nerbass FB, Hallvass AE, Taal MW, Pecoits-Filho R. Formula to detect high sodium excretion from spot urine in chronic kidney disease patients. J Bras Nefrol 2017;39(1):23-8. doi: 10.5935/0101-2800.20170004.
17. Dougher CE, Rifkin DE, Anderson CA, Smits G, Persky MS, Block GA, Ix JH. Spot urine sodium measurements do not accurately estimate dietary sodium intake in chronic kidney disease. Am J Clin Nutr 2016;104(2):298-305. doi: 10.3945/ajcn.115.127423.
18. Da J, Xie X, Wolf M, Disthabanchong S, Wang J, Zha Y, Lv J, Zhang L, Wang H. Serum Phosphorus and Progression of CKD and Mortality: A Meta-analysis of Cohort Studies. Am J Kidney Dis 2015;66(2):258-65. doi: 10.1053/j.ajkd.2015.01.009.
19. Wolf M. Mineral (Mal)Adaptation to Kidney Disease--Young Investigator Award Address: American Society of Nephrology Kidney Week 2014. Clin J Am Soc Nephrol 2015;10(10):1875-85. doi: 10.2215/cjn.04430415.
20. Barreto FC, Barreto DV, Massy ZA, Drueke TB. Strategies for Phosphate Control in Patients With CKD. Kidney Int Rep 2019;4(8):1043-56. doi: 10.1016/j.ekir.2019.06.002.
21. Stremke ER, McCabe LD, McCabe GP, Martin BR, Moe SM, Weaver CM, Peacock M, Hill Gallant KM. Twenty-Four-Hour Urine Phosphorus as a Biomarker of Dietary Phosphorus Intake and Absorption in CKD: A Secondary Analysis from a Controlled Diet Balance Study. Clin J Am Soc Nephrol 2018;13(7):1002-12. doi: 10.2215/cjn.00390118.

22. Robinson-Cohen C, Ix JH, Smits G, Persky M, Chertow GM, Block GA, Kestenbaum BR. Estimation of 24-hour urine phosphate excretion from spot urine collection: development of a predictive equation. J Ren Nutr 2014;24(3):194-9. doi: 10.1053/j.jrn.2014.02.001.
23. Campbell NRC, He FJ, Tan M, Cappuccio FP, Neal B, Woodward M, Cogswell ME, McLean R, Arcand J, MacGregor G, et al. The International Consortium for Quality Research on Dietary Sodium/Salt (TRUE) position statement on the use of 24-hour, spot, and short duration (<24 hours) timed urine collections to assess dietary sodium intake. J Clin Hypertens (Greenwich) 2019. doi: 10.1111/jch.13551.

APPENDIX A: PROTOCOLS

A1: Diet Homogenizing Protocol

Note: this protocol is written assuming the "unit" is one meal. The same steps apply if you are homogenizing a single food item or even food for an entire day.

1. Make meal according to the same protocol you would use if you were making it for a participant in the study.
    a. Make sure to use the same ingredients and products you would use for the study.
    b. Portion the food and prepare it the same way you would for the study.
2. Combine all food for the meal, including non-water beverages, in a large container.
    a. Store food in a labeled container until further analyses can be done.
    b. If analyses will not be done right away, food samples can be stored in the freezer, but must be thawed before they can be processed further.
3. Make sure food samples are thawed, and begin further processing
    a. Record the container codes in your processing records—see table at the end of this protocol for how to record all processing data.
4. Take the lid off of the container and weigh the container with the food in it. Record the weight of the container + food.
5. Add all (thawed) food from the meal/sample to a large, industrial-grade blender.
    a. If necessary, use a spatula or other utensil to scrape as much food off the container into the blender as you can.
    b. Weigh the empty container and record the weight.
6. Add ~50-100g of ultrapure water to the blender.
    a. The actual amount of ultrapure water you add to the blender doesn't matter, as long as you record the exact weight of ultrapure water added (i.e. weigh the water, record it, then add it to the blender).
7. Blend the food until it is fully homogenized.
    a. It should have the texture of a smoothie.
    b. If you still see lumps or whole chunks of food, continue blending.
    c. You can always add more ultrapure water to the blender, as long as you weigh the ultrapure water and record the weight prior to adding.
8. While food is blending, prepare your sample trays. These are the trays you will pour the blended sample into, and these are the trays you will put in the freeze dryer.
    a. Weigh the empty tray and record the weight.
    b. Label the tray with the sample code for the sample you will put into it.
    c. Depending on how much food you are homogenizing, one sample will sometimes require more than one tray. You will need to weigh both trays, and make sure to label them as "samplecode.1" and "samplecode.2" (or some other coding scheme).
9. When the sample is fully homogenized, empty the sample into the prepared tray(s)
    a. Trays should only be max ¾ full, so use multiple trays if necessary
10. Add about 50-100 g of ultrapure water to the blender to rinse it and use a spatula or other tool to scrape as much of the food sample off the sides of the blender.

    a. Make sure you record the weight of the ultrapure water added to the blender.
    b. Add the ultrapure water/sample mixture on top of the filled trays with the food sample.
11. Weigh and record the weight of the tray + homogenate.
12. Cover the tray with foil and put in freezer.
    a. Sample must be fully frozen before it can be freeze dried, which is the first step in further processing the sample for chemical analysis.

Diet Homogenate Sample Record Sheet

Container Code	Food + Container Weight (g)	Empty Container Weight (g)	U.P. Water added before blending (g)	U.P. Water used to rinse blender (g)	Empty tray weight (g)	Tray + homogenate weight (g)	Homogenized date	Date in Freeze dryer	Date out of freeze dryer	Tray + dry food weight (g)

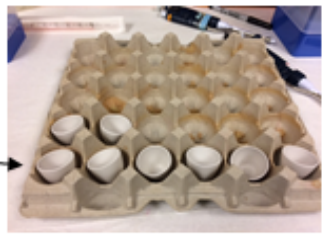
A2: Diet Preparation for ICP Analysis Protocol

- To run diet on the ICP you will need to have a dried composite that is freeze dried (or if animal diet just crushed) to eliminate the water weight.
- **The procedure will first be explained with individual steps to follow:**

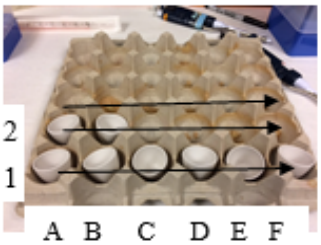
A diet is combined with all appropriate weights and measurements taken. This combined homogenate is frozen in plastic containers no more than ¾" thick. These frozen diets are placed in the freeze dryer and run until all moisture is removed. The freeze dried diets are crushed in a bag. (Multiple trays of the same diet are combined and well mixed). Crucibles are weighed empty with this weight recorded. The freeze dried diet is added to the crucibles (approximately 2 grams), and the crucibles are reweighed. Lids are added after the weighing procedure. A marker is used to add the labels to the lids for ID purposes prior to entering the muffle furnace. The diets are placed in the muffle furnace using a map that identifies where each crucible is since the label will not be visible after burning. They should be run in the furnace on Program 1 which is 300 degrees for 16 hours which will automatically increase to 600 degrees for 3 days. After 3 days, the furnace will be turned off (manually) and the crucibles are allowed to cool. After about 4 hours the crucibles can be removed from the furnace and allow to cool completely before weighing. Once cooled, the lids will be removed, but kept with their respective crucible and the crucible (without lid) will be weighed for an ash weight. Lids can be placed on crucible after weighing and a marker can be used to label lids post furnace. After weighing the crucibles will have 1ml of concentrated HNO3 added to dissolve the pellet. This will need to sit over night, but not more than 48 hours. After the pellet is dissolved the acid will be transferred to a 25mL volumetric flask using Ultrapure water. Rinse the crucible at least 3 times with a disposable transfer pipet and the ultrapure water. Once the crucible is empty and rinsed the volumetric flask can be filled to the 25mL mark with Ultrapure water. Parafilm should be placed on the top of each flask where they will be inverted and shaken to allow the solution to mix well. After mixing the solution, it can be stored in a pre-labeled scintillation vial (if you need 20ml to store) or a 15 mL centrifuge tube. Be sure lids are on tight. Any extra solution can be discarded (but make note of the total volume the crucible was diluted to). This is considered the STOCK SOLUTION. Dilutions for ICP will be made from this tube. Dilute accordingly to have the appropriate concentration for the machine. See the ICP tech for appropriate dilutions. Always check a few dilutions first to see if it is correct before doing your entire set of samples.

- **STEP BY STEP INSTRUCTIONS:**
- **Prepare the Ash the diet for dilutions:**
    - Crucibles are found in drawer

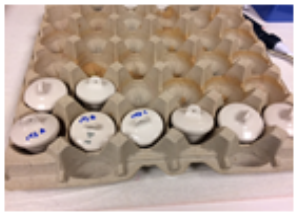
    - Place the appropriate number of crucibles in an egg carton
    - Weigh empty crucible without cover
    - Place approximately 2-3grams of crushed diet in crucible.



        - Weigh crucible with diet without cover
            - When organizing samples always go from left to right and front (closest to you) to back.
            - Ex: Picture at left: Sample 1 would be in 1A, sample 2 in 1B, 3 in 1C, Sample 7 would be in 2A, sample 8 in 2B, etc.



    - Place cover on crucible
    - Add label to lid



    - Make a map of your crucibles
        - **Top and Middle shelf has 30 spaces; 6 across, 5 deep**
        - **Bottom shelf has up to 49 spaces; 7 across, 7 deep**

Diet 1 – 1	Diet 1 -2	Diet 1-3	ETC.		

Front of egg carton

    - Put diet in muffle furnace.
        - Crucibles cannot be touching each other or covering holes in the shelf.
        - Start program
            - Should be 600°
            - Leave in for 3 days after it reaches 600. If put in on Monday, would come out on Friday. Thursday, to Monday etc.
        - Shut off muffle furnace and open door slightly to allow crucibles to cool.

- Leave at least 4-6 hours to cool in furnace
  - When crucibles are completely cool – weigh them without cover
    - Re-label lids for ID

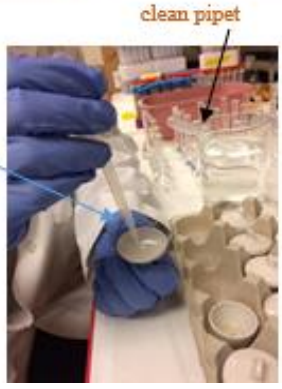
- **For ICP testing**
  - Using a repeat pipetor, place 1 ml HNO_3 acid into each crucible (under hood)
    - Be careful not to splash the acid when adding to the crucibles.
  - Put lids on crucibles
  - Leave under hood for 1 days
  - Remove from hood
    - Transfer liquid to 25ml volumetric flask (see below)
      - Excessive sit time after acid is added will cause evaporation and make it harder to transfer the solution.

- Items needed for transfer
  - Volumetric flask
  - Ultrapure water
  - Beaker with 2% HNO_3
  - Pump with 2%HNO_3
  - Clean plastic transfer pipette
  - Enough additional pipettes to provide one for each diet sample

Volumetric Flasks are found in a drawer.

*Be sure there are enough volumetric flasks prior to adding acid so you can transfer you samples.

- **Transfer procedure**
  - ***LIDS***
    - Remove lid
    - Hold upside down – using clean pipette, add one dropper full of ultrapure water into lid
      - Do not touch any part of the crucible with the clean pipette.



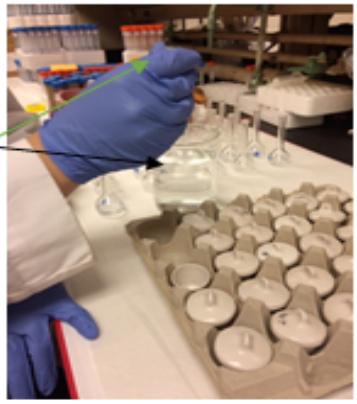
Rev. 2-2-18

- If you do touch the crucible or its contents then it would be considered a dirty pipette and should only be used for that particular crucible and then discarded afterwards.

- Pipette liquid up and down 2-3 times to dissolve particles in lid (using dirty pipette)
- Remove liquid; place in volumetric flask
- Put lid into container to be washed.

- ***CRUCIBLES***
  - Using clean pipette, add one dropper full of ultrapure water to crucible

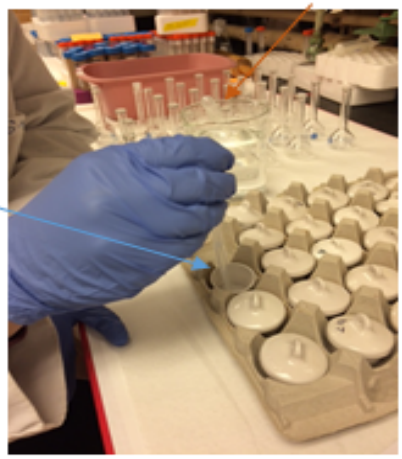
Ultrapure water
Clean pipet only in ultrapure water.
(Do not touch any part of lid or crucible with clean pipet)



  - Using dirty pipette(one used only with a single crucible and corresponding lid), pipette up and down 2-3 times
    - This dirty pipette is the same one you used for the corresponding lid.

clean pipet

Dirty pipet (only used for this crucible)



    - Remove all liquid; place in volumetric flask
    - Using clean pipette add dropper full of ultrapure water to crucible.
    - Using dirty pipette, rinse inside of crucible with liquid in crucible 3 times, using a circular pattern (around the top edge of the crucible, rinsing downward).
    - Place liquid from crucible into volumetric flask using dirty pipette
  - Repeat previous step 2 more times with ultrapure water (A total of 3 times addition of ultrapure water) more is ok, less is not.
  - Fill 25ml labeled flask with ultrapure water up to 25 ml mark, making sure the bottom of the meniscus is at the line (view at eye level).

- Add parafilm onto the top of the flask and invert and shake vigorously to mix the solution.
- Pour this solution into pre-labeled 15ml centrifuge tubes for storage (Note the total volume is 25ml). This is the stock solution.

- DILUTIONS for ICP
  - Take 0.1ml of original diet (25ml) and put in a new labeled 15ml tube.
  - Add 10ml of 2% HNO^3 (found in a bottle with 10 ml pump)
    - Be sure to write the dilution factor on the tube

Back					
Front					

Map for Crucibles: Top Shelf

Back					
Front					

Map for Crucibles: Middle Shelf

Back						
Front						

Map for Crucibles: Bottom Shelf

APPENDIX B: SAS CODE FOR CHAPTERS 3 AND 4

```
/*Imported dataset using ImportWizard, no code needed*/
/*label CKD status, sex, and race*/
PROC FORMAT;
VALUE CKD_status
      1= 'CKD'
      2= 'Healthy'
      ;
VALUE sex
      1= 'Female'
      2= 'Male'
      ;
VALUE race
      1= 'White'
      2= 'Black'
      ;
      RUN;
/*apply formats to data*/
DATA spot_u.spoturinedataformatted; SET spot_u.spoturinedata;
FORMAT CKD_Status CKD_status.;
FORMAT sex sex.;
Format race race.;
RUN;
/*descriptives overall*/
PROC MEANS DATA=spot_u.spoturinedataformatted;
run;
PROC FREQ DATA=spot_u.spoturinedataformatted;
TABLES CKD_status sex race;
run;
/*descriptives for CKD and Healthy separately*/
PROC MEANS DATA=spot_u.spoturinedataformatted;
class CKD_status;
var age_y ht_cm wt_kg BMI_kg_m2 eGFR_mL_min BUN_mg_dL Cr_mg_dL SBP_mmHg
DBP_mmHg FPG_g_dL Intake_Na_mg Intake_K_mg Intake_P_mg TwentyfourhUNa_mg
TwentyfourhUK_mg TwentyfourhUP_mg;
run;
/*descriptives for CKD and Healthy for all intake and urine variables*/
PROC MEANS DATA=spot_u.spoturinedataformatted;
class CKD_status;
run;
/*checking for normality using graphs*/
PROC UNIVARIATE DATA=spot_u.spoturinedataformatted;
var Intake_Na_mg Intake_K_mg Intake_P_mg TwentyfourhUNa_mg TwentyfourhUK_mg
TwentyfourhUP_mg INTERSALT_Na INTERSALT_woK_Na Tanaka_Na Tanaka_K Kawasaki_Na
Kawasaki_K;
HISTOGRAM Intake_Na_mg Intake_K_mg Intake_P_mg TwentyfourhUNa_mg
TwentyfourhUK_mg TwentyfourhUP_mg INTERSALT_Na INTERSALT_woK_Na Tanaka_Na
Tanaka_K Kawasaki_Na Kawasaki_K;
run;
PROC UNIVARIATE DATA=spot_u.spoturinedataformatted;
var Intake_Na_mg Intake_K_mg Intake_P_mg TwentyfourhUNa_mg TwentyfourhUK_mg
TwentyfourhUP_mg INTERSALT_Na INTERSALT_woK_Na Tanaka_Na Tanaka_K Kawasaki_Na
Kawasaki_K;
```

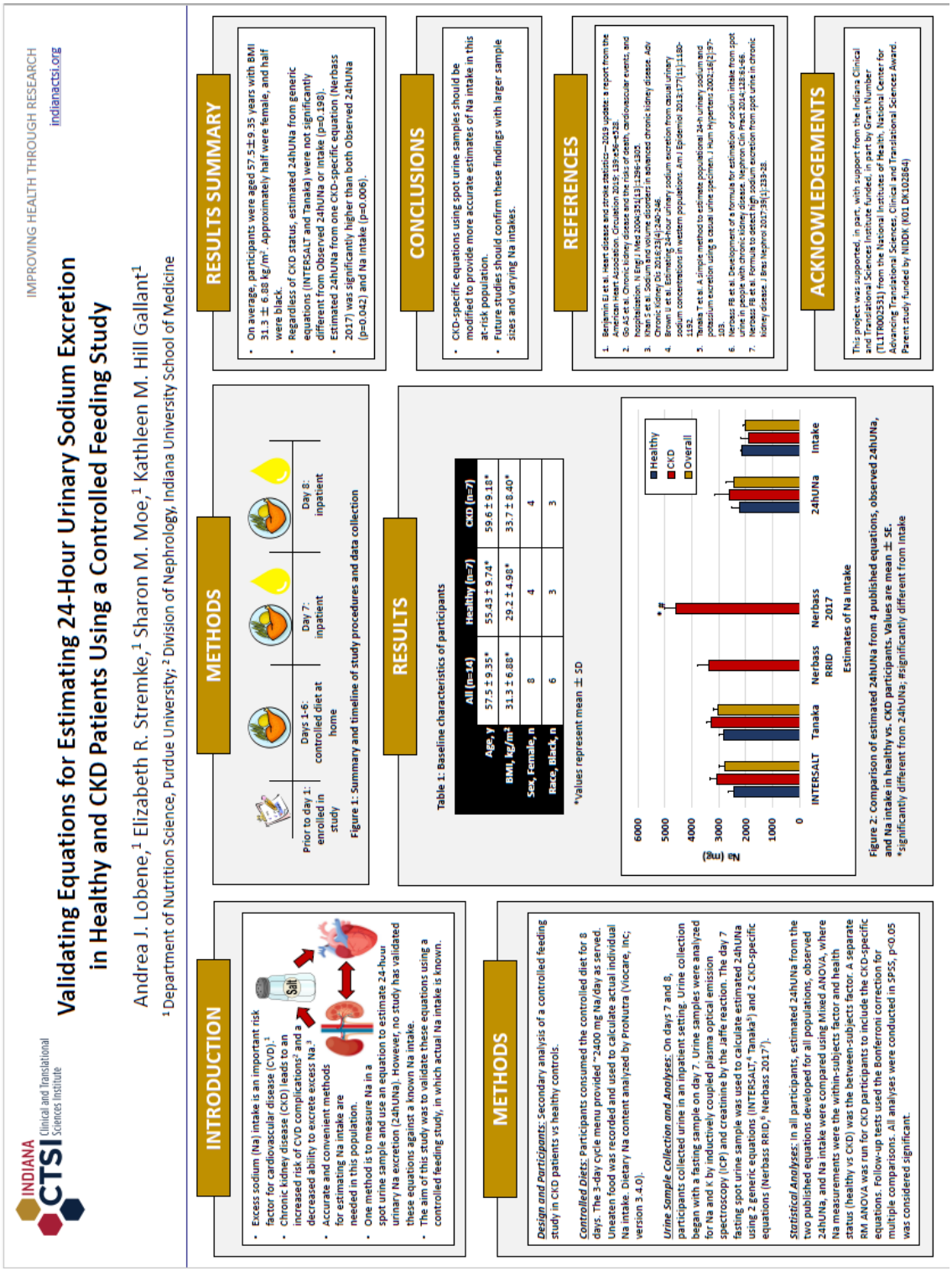
```
ppplot;
run;
PROC UNIVARIATE DATA=spot_u.spoturinedataformatted;
var Intake_Na_mg Intake_K_mg Intake_P_mg TwentyfourhUNa_mg TwentyfourhUK_mg
TwentyfourhUP_mg INTERSALT_Na INTERSALT_woK_Na Tanaka_Na Tanaka_K Kawasaki_Na
Kawasaki_K;
qqplot /NORMAL (MU=EST SIGMA=EST);
run;
/*need to transform variables because intake variables are non-normally
distributed*/
DATA spot_u.spoturinedatatransformed; SET spot_u.spoturinedata;
logIntake_Na_mg = LOG(Intake_Na_mg);
logIntake_K_mg = LOG(Intake_K_mg);
logIntake_P_mg = LOG(Intake_P_mg);
logTwentyfourhUNa_mg = LOG(TwentyfourhUNa_mg);
logTwentyfourhUK_mg = LOG(TwentyfourhUK_mg);
logTwentyfourhUP_mg = LOG(TwentyfourhUP_mg);
logINTERSALT_Na = LOG(INTERSALT_Na);
logINTERSALT_woK_Na = LOG(INTERSALT_woK_Na);
logTanaka_Na = log(Tanaka_Na);
logTanaka_K = log(Tanaka_K);
logKawasaki_Na = log(Kawasaki_Na);
logKawasaki_K = log(Kawasaki_K);
logNerbass_RRID_Na = log(Nerbass_RRID_Na);
logNerbass_SALTED_Na = log(Nerbass_SALTED_Na);
logRobCoh_P = log(RobCoh_P);
run;
/*create new dataset with only CKD participants*/
DATA spot_u.spoturinedataCKDonly; SET spot_u.spoturinedatatransformed;
IF CKD_status EQ 2 THEN DELETE;
run;
/*create new dataset with only healthy participants*/
DATA spot_u.spoturinedataHealthyonly; SET spot_u.spoturinedatatransformed;
IF CKD_status EQ 1 THEN DELETE;
run;


/*TERTIARY AIM: repeated measures ANOVA comparing P intake, 24hUP, and
estimated 24hUP in CKD only*/
PROC GLM DATA=spot_u.spoturinedataCKDonly;
MODEL logIntake_P_mg logTwentyfourhUP_mg logRobCoh_P = /NOUNI;
/*NOUNI means no univariate*/
REPEATED phosphorus 3 /PRINTE;
/*PRINTE adds a test of sphericity*/
MANOVA H=intercept M= (1 -1 0) / SUMMARY;
MANOVA H=intercept M= (1 0 -1) / SUMMARY;
MANOVA H= intercept M= (0 1 -1) / SUMMARY;
/*these are the defined contrasts; I'm comparing intake, 24hUP, and esimtated
24hUP to each other*/
RUN;


/*SECONDARY AIM: repeated measures ANOVA comparing Na intake, 24hUNa, and
estimated 24hUNa in CKD only*/
PROC GLM DATA=spot_u.spoturinedataCKDonly;
MODEL logIntake_Na_mg logTwentyfourhUNa_mg logNerbass_RRID_Na
logNerbass_SALTED_Na = /NOUNI;
```

```
REPEATED sodium 4 CONTRAST (1) / SUMMARY PRINTE;
REPEATED sodium 4 CONTRAST (2) /SUMMARY;
RUN;


/*PRIMARY AIM: mixed ANOVA comparing Na intake, 24hUNa, and estimated 24hUNa
in both healthy and CKD*/
PROC GLM DATA=spot_u.spoturinedatatransformed;
CLASS CKD_status;
CLASS Pair;
/*CLASS tells SAS these variables are categorical*/
MODEL logIntake_Na_mg logTwentyfourhUNa_mg logINTERSALT_Na logTanaka_Na =
CKD_status Pair/NOUNI;
/*NOUNI means no univariate*/
/*different measures of Na are a within-subjects factor, and CKD status is a
between-subjects factor*/
/*adding Pair as a covariate*/
/*main effect of CKD_status tells us if Na is different based on CKD status,
regardless of method of Na measurement*/
/*main effect of sodium tells us if Na measurements differ, regardless of CKD
status*/
/*interaction between Na and CKD status tells us if measurements of Na using
different measurement methods differ between CKD and healthy*/
REPEATED sodium 4 CONTRAST (1) / SUMMARY PRINTE;
REPEATED sodium 4 CONTRAST (2) /SUMMARY;
/*contrast 1 compares all measures of Na to Na intake*/
/*contrast 2 compares all measures of Na to 24hUNa*/
/*PRINTE adds a test of sphericity*/
RUN;


/*PRIMARY AIM: mixed ANOVA comparing K intake, 24hUK, and estimated 24hUK in
both healthy and CKD*/
PROC GLM DATA=spot_u.spoturinedatatransformed;
CLASS CKD_status;
CLASS Pair;
/*CLASS tells SAS these variables are categorical*/
MODEL logIntake_K_mg logTwentyfourhUK_mg logTanaka_K = CKD_status Pair/NOUNI;
/*NOUNI means no univariate*/
/*different measures of K are a within-subjects factor, and CKD status is a
between-subjects factor*/
/*adding Pair as a covariate*/
/*main effect of CKD_status tells us if K is different based on CKD status,
regardless of method of K measurement*/
/*main effect of potassium tells us if K measurements differ, regardless of
CKD status*/
/*interaction between K and CKD status tells us if measurements of K using
different measurement methods differ between CKD and healthy*/
REPEATED potassium 3 CONTRAST (1)/ SUMMARY PRINTE;
REPEATED potassium 3 CONTRAST (2)/ SUMMARY;
/*contrast 1 compares all measures of K to K intake*/
/*contrast 2 compares all measures of K to 24hUK*/
/*PRINTE adds a test of sphericity*/
RUN;


/*run Pearson bivariate correlation for tertiary aim*/
```

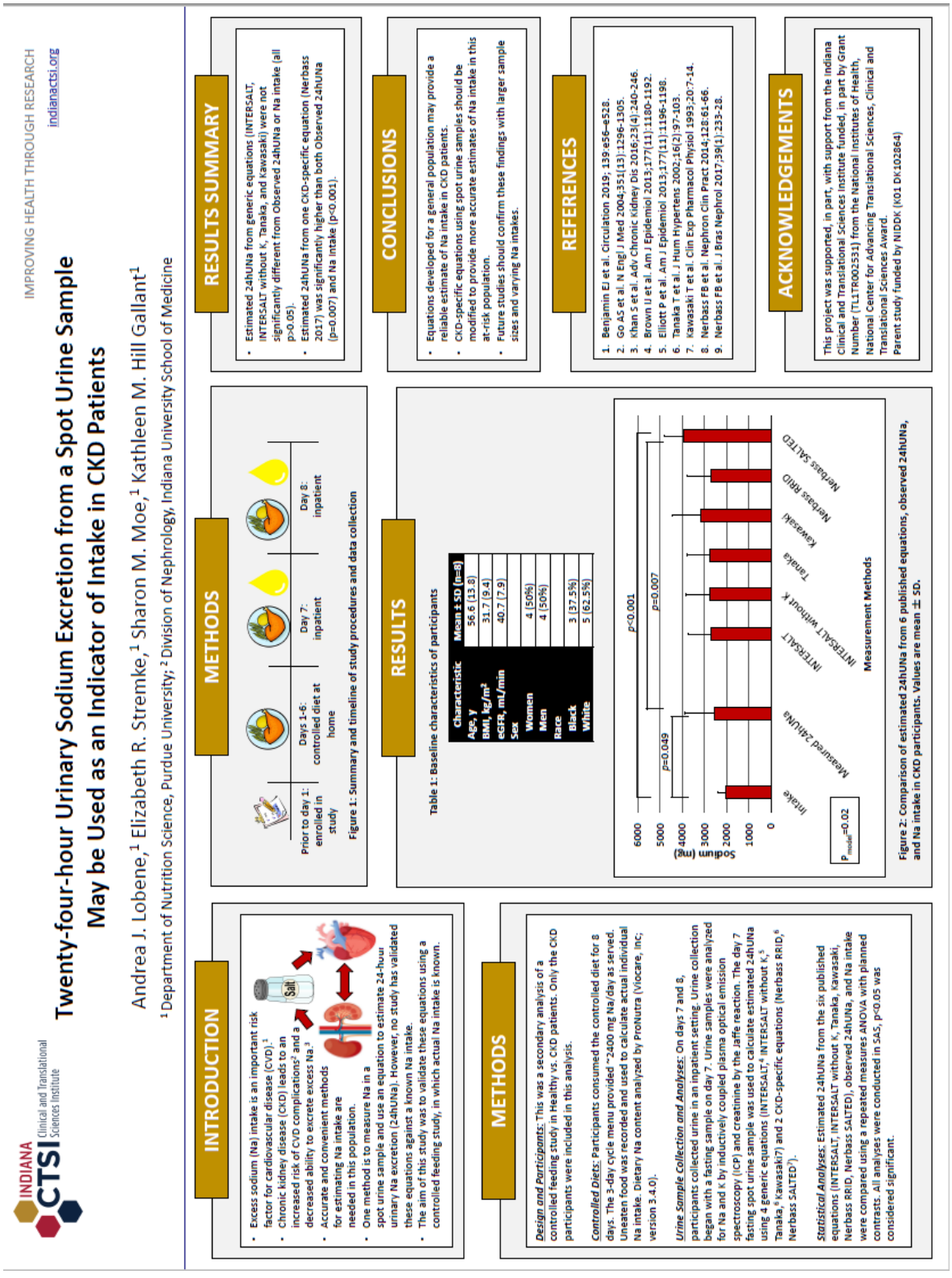
```
PROC CORR data=spot_u.spoturinedatackdonly fisher;
/*fisher generates confidence intervals*/
VAR logIntake_P_mg logTwentyfourhUP_mg logRobCoh_P;
RUN;


/*run Pearson bivariate correlation for secondary aim*/
PROC CORR data=spot_u.spoturinedatackdonly fisher;
/*fisher generates confidence intervals*/
VAR logIntake_Na_mg logTwentyfourhUNa_mg logNerbass_RRID_Na
logNerbass_SALTED_Na;
RUN;


/*run partial correlations for primary aim, controlling for CKD status and
pair*/
PROC CORR data=spot_u.spoturinedatatransformed fisher;
/*fisher generates confidence intervals*/
VAR logIntake_Na_mg logTwentyfourhUNa_mg logINTERSALT_Na logTanaka_Na;
PARTIAL CKD_status pair;
RUN;
PROC CORR data=spot_u.spoturinedatatransformed fisher;
/*fisher generates confidence intervals*/
VAR logIntake_K_mg logTwentyfourhUK_mg logTanaka_K logKawasaki_K;
PARTIAL CKD_status pair;
RUN;
```

APPENDIX C: INDIANA CLINICAL AND TRANSLATIONAL SCIENCES INSTITUTE (CTSI) POSTER PRESENTATIONS

C1: Poster for Indiana CTSI Annual Meeting, September 2019 in Indianapolis, IN



C2: Poster for Indiana CTSI Annual Retreat, January 2020 in West Lafayette, IN



VITA

Andrea Lobene CV

ANDREA J. LOBENE, MS, RD

EDUCATION and TRAINING

2016-2020 (anticipated) **Purdue University, West Lafayette, IN, USA**
Doctor of Philosophy Candidate, Nutrition Science
Advisor: Kathleen Hill Gallant, PhD, RD, FAND, Assistant Professor and Director, Didactic Program in Dietetics
Dissertation: Sodium and related mineral intake in chronic disease

2014-2016 **The University of Georgia, Athens, GA, USA**
Dietetic Internship

2014-2016 **The University of Georgia, Athens, GA, USA**
Master of Science, Foods and Nutrition
Advisor: Richard Lewis, PhD, RD, FACSM, UGA Foundation Professor in Family and Consumer Sciences
Thesis: Zinc supplementation and insulin secretion in children

2010-2014 **The University of Georgia, Athens, GA, USA**
Bachelor of Science in Family and Consumer Science, Dietetics
Spanish Minor
Advisor: Richard Lewis, PhD, RD, FACSM, UGA Foundation Professor in Family and Consumer Sciences
Thesis: The relationships between zinc and bone strength in healthy children
Summa cum laude; with highest honors

CERTIFICATIONS

- Graduate Teaching Certificate, Center for Instructional Excellence, Purdue University, 2018
- Graduate Instructional Development Certificate, Center for Instructional Excellence, Purdue University, 2017
- Registered Dietitian Nutritionist (Registration #86015133), Commission on Dietetic Registration, 2016-present

ADDITIONAL TRAINING and PROFESSIONAL DEVELOPMENT

- Scientific Writing from the Reader's Perspective, delivered by Dr. George Gopen; Purdue University, October 2019
- Causal Inference in Behavioral Obesity Research Short Course, Hosted by Dr. David Allison; Indiana University School of Public Health-Bloomington, July 2019
- Using National Dietary Data: Building Blocks to Expand Your Research Portfolio, Nutrition 2019 Preconference Workshop; Baltimore MD, June 2019

ACADEMIC POSITIONS

2019-present Indiana Clinical and Translational Sciences Institute Predoctoral Fellow, Purdue University

2017-present Graduate Teaching Assistant, Department of Nutrition Science, Purdue University

2016-2019 Graduate Research Assistant, Department of Nutrition Science, Purdue University

2014-2016 Graduate Teaching Assistant, Department of Foods and Nutrition, The University of Georgia

TEACHING EXPERIENCE

Purdue University Department of Nutrition Science, West Lafayette, IN

NUTR 481 Medical Nutrition Therapy II, ~40 students
Semester: Spring 2019, Spring 2020
Instructors of Record: Dr. Kathleen Hill Gallant, Assistant Professor and Director, Didactic Program in Dietetics; Donna Zoss, Continuing Lecturer and Assistant Director, Didactic Program in Dietetics

NUTR 480, Medical Nutrition Therapy I, ~50 students
Semester: Fall 2019
Instructors of Record: Dr. Kathleen Hill Gallant, Assistant Professor and Director, Didactic Program in Dietetics; Donna Zoss, Continuing Lecturer and Assistant Director, Didactic Program in Dietetics

NUTR 330, Diet Selection and Planning: Dietary Guidance for Human Health, 30-45 students/semester
Semesters: Summer 2019, Fall 2018, Summer 2018
Instructor of Record: Dr. Marie Allsopp, Clinical Assistant Professor (Summer 2019)
Instructor of Record: Dr. Regan Bailey, Associate Professor (Fall 2018, Summer 2018)

NUTR 424 Communication Techniques in Foods and Nutrition, ~20 students/semester
Semesters: Spring 2019, Spring 2018, Fall 2017
Instructor of Record: Dr. Heather Leidy, Associate Professor

NUTR 442 Foodservice Systems Management; ~80 students
Semester: Spring 2017
Instructor of Record: Donna Zoss, Continuing Lecturer and Assistant Director, Didactic Program in Dietetics

The University of Georgia Department of Foods and Nutrition, Athens, GA

FDNS 3610/3610L Quantity Foods; ~75 students/semester
Semesters: Spring 2016, Spring 2015
Instructor of Record: Tracey Brigman, Clinical Assistant Professor

FDNS 3600/3600L Food Principles; ~90 students/semester
Semesters: Fall 2015, Fall 2014
Instructor of Record: Tracey Brigman, Clinical Assistant Professor

The University of Georgia Peer Nutrition Educators, Athens, GA

Peer Nutrition Educator
Summer 2014-Spring 2015
Supervisors: Katherine Ingerson, UGA Foodservices Dietitian; Ben Gray, University Health Center Dietitian

PUBLICATIONS in PEER-REVIEWED JOURNALS

Bailey RL, Kiesel VA, **Lobene AJ**, Zou P. Redesigning an undergraduate nutrition course through active learning and group-based projects enhances student performance. *Accepted,* Curr Dev Nutr 2020.

Coyne M, **Lobene AJ**, Neumann C, Lachcik P, Weaver CM, Nie LH. Determination of bone sodium (Na) and Na exchange in pig leg using *in vivo* neutron activation analysis (IVNAA). Physiol Meas 2019;40(7):075009.

Kindler JM, **Lobene AJ**, Vogel KA, Martin BR, McCabe LD, Peacock M, Warden SJ, Henry CN, McCabe GP, Weaver CM. Adiposity, insulin resistance, and bone health in children and adolescents. J Clin Endocrinol Metab 2019; 104(3):892-899.

Weaver CM, Bailey RL, McCabe LD, Moshfegh AJ, **Lobene AJ**, McCabe GP. Mineral intake ratios are a weak but significant factor in blood pressure variability in U.S. adults. J Nutr 2018; 148: 1845-1451.

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

Lobene AJ, Kindler JM, Jenkins NT, Pollock NK, Laing EM, Grider A, Lewis RD. Zinc supplementation does not alter indicators of insulin secretion and sensitivity in black and white female adolescents. J Nutr 2017; 147(3):1296-1300.

MANUSCRIPTS in PROGRESS

Song Y, **Lobene AJ,** Wang Y, Hill Gallant KM. The DASH Diet and CKD: A Review of the Evidence with a Focus on China. *Submitted.*

Lobene AJ, Stremke ER, McCabe GP, Moorthi RN, Moe SM, Hill Gallant KM. Spot urine samples to estimate sodium and potassim intake in patients with CKD and healthy adults: a secondary analysis from a controlled feeding study. *In Preparation.*

Lobene AJ, Stremke ER, McCabe GP, Moorthi RN, Moe SM, Hill Gallant KM. Spot urine samples to estimate phosphorus intake in patients with CKD: a secondary analysis from a controlled feeding study. *In Preparation.*

PLANNED MANUSCRIPTS

Lobene AJ, Hill Gallant KM, Chow LS et al. Time restricted eating and bone outcomes.

Lobene AJ, Hand RK, Watowicz RP, Hill Gallant KM. Collaborative implementation of a structured evidence-based practice unit for senior level undergraduate dietetics students.

BOOK CHAPTERS

Lobene AJ, McCabe LD, Stone MS, Kindler JM, Bailey RL, Moshfegh AJ, Rhodes DG, Goldman JD, McCabe GP, Weaver CM. Dietary minerals, mineral ratios, and bone. In: Nutritional Influences on Bone Health, Proceedings of the 10th International Symposium on Nutritional Aspects of Osteoporosis.

PEER-REVIEWED ABSTRACTS

Lobene AJ, Stremke ER, McCabe GP, Moorthi RN, Moe SM, Hill Gallant KM. Using spot urine samples to estimate sodium and potassium intake in healthy vs CKD patients: results from a controlled feeding study. Nutrition 2020, Seattle, WA, June 2020 *[virtual meeting due to COVID-19].*

Lobene AJ, Stremke ER, Moorthi RN, Moe SM, Hill Gallant KM. Twenty-four-hour Urinary Sodium Excretion from a Spot Urine Sample May Be Used as an Indicator of Intake in CKD Patients. Association for Clinical and Translational Sciences Annual Meeting, Washington, DC, April 2020 *[virtual meeting due to COVID-19].*

Lobene AJ, McCabe LD, McCabe GP, Martin BR, Weaver CM. Estimated 24-hour Sodium Excretion from Available Equations is a Poor Predictor of Intake. Nutrition 2019, Baltimore, MD, June 2019.

Lobene AJ, Macdonald-Clarke CJ, Martin BR, McCabe LD, McCabe GP, Weaver CM. Estimating Sodium Intake Using Timed Urine Collections from a Controlled Feeding Study. EPI|Lifestyle Scientific Sessions, Houston, TX, March 2019.

Lobene AJ, Martin BR, McCabe LD, McCabe GP, Weaver CM. Variability in Urinary Sodium Excretion in Timed Spot Urine Samples. Nutrition 2018, Boston, MA, June 2018.

Lobene AJ, Martin BR, Weaver CM. Predicting Population Sodium Intake from a Single Timed Urine Collection. Foods and Nutrition Conference and Expo, Chicago, IL, October 2017.

Coyne M, **Lobene A**, Lachcik P, Zhang X, Hsieh MJ, Newman C, Weaver C, Nie LH. Determination of Bone Sodium (Na) and Na Exchange in Pig Leg Using *In Vivo* Neutron Activation Analysis (IVNAA). Healthy Physics Society Annual Meeting, Raleigh, NC, July 2017.

Lobene AJ, Martin BR, Macdonald-Clarke CJ, Anderson CAM, McCabe LD, McCabe GP, Weaver CM. Pattern of Urinary Sodium Excretion Following Consumption of a Known Quantity of Sodium. Experimental Biology, Chicago, IL, April 2017.

Lobene AJ, Kindler JM, Pollock NK, Laing EM, Lewis RD. Zinc supplementation, beta cell function, insulin secretion, and insulin resistance in black and white female adolescents. Experimental Biology, San Diego, CA, April 2016.

ORAL PRESENTATIONS at NATIONAL MEETINGS

Lobene AJ, McCabe LD, McCabe GP, Martin BR, Weaver CM. Estimated 24-hour Sodium Excretion from Available Equations is a Poor Predictor of Intake. Nutrition 2019, Concurrent oral session on Vitamins and Minerals, Baltimore, MD, June 2019.

POSTER PRESENTATIONS at NATIONAL and LOCAL MEETINGS

Lobene AJ, Stremke ER, Moorthi RN, Moe SM, Hill Gallant KM. Twenty-four-hour Urinary Sodium Excretion from a Spot Urine Sample May Be Used as an Indicator of Intake in CKD Patients. Indiana Clinical and Translational Sciences Institute Annual Retreat, West Lafayette, IN, January 2020.

Lobene AJ, Stremke ER, Moe SM, Hill Gallant KM. Validating Equations for Estimating 24-Hour Urinary

Sodium Excretion in Healthy vs CKD Patients Using a Controlled Feeding Study. Indiana Clinical and Translational Sciences Institute Annual Meeting, Indianapolis, IN, September 2019.

Lobene AJ, Macdonald-Clarke CJ, Martin BR, McCabe LD, McCabe GP, Weaver CM. Estimating Sodium Intake Using Timed Urine Collections from a Controlled Feeding Study. EPI|Lifestyle Scientific Sessions, Houston, TX, March 2019.

Lobene AJ, Martin BR, McCabe LD, McCabe GP, Weaver CM. Variability in Urinary Sodium Excretion in Timed Spot Urine Samples. Nutrition 2018, Boston, MA, June 2018.

Lobene AJ, Martin BR, Weaver CM. Predicting Population Sodium Intake from a Single Timed Urine Collection. Foods and Nutrition Conference and Expo, Chicago, IL, October 2017.

Lobene AJ, Martin BR, Macdonald-Clarke CJ, Anderson CAM, McCabe LD, McCabe GP, Weaver CM. Pattern of Urinary Sodium Excretion Following Consumption of a Known Quantity of Sodium. Experimental Biology, Chicago, IL, April 2017.

Lobene AJ, Kindler JM, Pollock NK, Laing EM, Grider A, Lewis RD. Zinc supplementation, beta cell function, insulin secretion, and insulin resistance in black and white female adolescents. Experimental Biology. San Diego, CA 2016

Lobene AJ, Kindler JM, Pollock NK, Laing EM, Grider A, Lewis RD. Zinc supplementation, beta cell function, insulin secretion, and insulin resistance in black and white female adolescents. Georgia Academy of Nutrition and Dietetics Annual Conference and Exhibition. Atlanta, GA 2016.

Lobene AJ, Chertin V, Kindler JM, Grider A, Laing EM, Lewis RD. The relationships between zinc and bone strength in healthy children. Center for Undergraduate Research Opportunities Symposium. Athens, GA, 2014.

FUNDED GRANTS

Indiana Clinical and Translational Sciences Institute, (TL1 Program), TL1TR002531 (T. Hurley, PI), 5/18/2018 – 4/30/2023, and UL1TR002529 (A. Shekhar, PI), 5/18/2018 – 4/30/2023 from the National Institutes of Health, National Center for Advancing Translational Sciences, Clinical and Translational Sciences Award
Role: Predoctoral Fellowship, 7/1/2019 – 6/30/2020, $24,500 annual stipend
Title: *Sodium Intake and Physiology in Healthy Adults versus CKD Patients and its Association with Chronic Disease Risk Factor*

HONORS and AWARDS

- Recipient of the 2020 Outstanding Graduate Student Teaching Award, Purdue University Department of Nutrition Science.

- Recipient of a 2020 Burroughs Wellcome Fund Trainee Travel Award to support my travel to Translational Science 2020, the annual meeting for the Association for Clinical and Translational Science. Accompanied by a $500 travel stipend. ***unable to receive funds, in-person meeting/trip cancelled due to COVID-19*

- Paula Dudley White Scholar, 2019, American Heart Association, EpiLifestyle Scientific Sessions. Award given to the primary author of the highest ranked abstract from each country.

- Recipient of a 2019 Early Investigator Travel Award to present at the EpiLifestyle Scientific Sessions, American Heart Association Council on Lifestyle and Cardiometabolic Health. Accompanied by a $1,000 cash prize.

- Recipient of a 2018-2019 Compton Graduate Research Travel Award, Purdue University College of Health and Human Sciences. Accompanied by a $500 travel award.

- Recipient of the 2017 Rickard Family Graduate Nutrition Support Fund, Purdue University Department of Nutrition Science. Award accompanied by $4,000 scholarship.

- Recipient of a 2017 Student Stipend to attend Food and Nutrition Conference and Expo, from the Academy of Nutrition and Dietetics Foundation. Award accompanied by a $100 stipend.

- Winner, 2017 Nutrition Science Graduate Student 3 Minute Thesis ® Competition, Purdue University. Accompanied by a $500 travel award.

- Recipient of a 2017 Purdue Graduate Student Government Travel Grant, Purdue University. Award accompanied by a $500 travel stipend.

- Recipient of a 2017 College of Health and Human Sciences/Graduate School Scholarship, Purdue University. Award accompanied by a $2,000 scholarship.

- Recipient of the 2016 Rickard Family Graduate Nutrition Support Fund, Purdue University Department of Nutrition Science. Award accompanied by $4,000 scholarship.

- Recipient of a 2016-2018 Fredrick N. Andrews Fellowship, Purdue University Graduate School. Award accompanied by a $17,299 stipend.

- Recipient of a 2016 Graduate Student Travel Award, University of Georgia College of Family and Consumer Sciences. Award accompanied by $300 travel stipend.

- Recipient of the 2015 Georgia Dietetic Foundation Scholarship. Award accompanied by $1,500 scholarship.

- Recipient of the 2015 Ruth Rowan Morrison Scholarship, University of Georgia College of Family and Consumer Sciences. Award accompanied by $3,000 scholarship.

- Recipient of the 2015 Northeast Georgia Dietetic Association Graduate Level Scholarship. Award accompanied by $150 scholarship.

- Recipient of the 2014 Northeast Georgia Dietetic Association Graduate Level Scholarship. Award accompanied by $150 scholarship.

- Recipient of the 2014 Marian Wang Nutrition Scholarship, University of Georgia College of Family and Consumer Sciences. Award accompanied by $1,000 scholarship.

- Recipient of the 2014 Outstanding Student in a DPD Program award, Georgia Academy of Nutrition and Dietetics.

- Recipient of the 2013 Flatt Dietetic Scholarship, University of Georgia College of Family and Consumer Sciences. Award accompanied by $1,000 scholarship.

- Recipient of the 2012 Flatt Dietetic Scholarship, University of Georgia College of Family and Consumer Sciences. Award accompanied by $1,000 scholarship.

GUEST LECTURES and SEMINAR PRESENTATIONS

Purdue University, West Lafayette, IN

Coordinated Program in Dietetics, *Preparing for and Applying to Graduate School*, May 2017, May 2018, May 2019

The University of Georgia, Athens, GA

FDNS 6530: Medical Nutrition Therapy II, *Kidney Stones, Dietary Determinants and Approaches to Treatment*, April 2015, April 2016

FDNS 8900: Seminar in Foods and Nutrition, *A Brief Review of Artificial Sweeteners and their Comparison to Fructose*, December 2014

NON-ACADEMIC PUBLICATIONS

Food & Nutrition Magazine, Stone Soup Blog. *How to Handle Casual Nutrition Conversations*. April 4, 2018. Link: https://foodandnutrition.org/blogs/stone-soup/handle-casual-nutrition-conversations/

STUDENT MENTORING AND SUPERVISORY EXPERIENCE

- Mentoring postbaccalaureate visiting scholar Sophia Song through writing a narrative review paper for publication in a peer-reviewed journal
- Supervised three undergraduate student graders for NUTR 330, Summer 2018 and Fall 2018
- Helped undergraduate student Nicolas Lisowski analyze data and create a poster for the Purdue Undergraduate Research Conference, April 2018
- Trained, scheduled, and supervised a team of 23 undergraduate laboratory staff members for a large clinical research study, Summer 2017

SERVICE ACTIVITIES

AD HOC REVIEWER of MANUSCRIPTS for SCIENTIFIC JOURNALS

2018	European Journal of Clinical Nutrition
2017	PLOS ONE

LEADERSHIP in PROFESSIONAL ORGANIZATIONS and COMMITTEES

2019-present	Accreditation Council for Education in Nutrition and Dietetics (ACEND) Liaison, Research Dietetic Practice Group of the Academy of Nutrition and Dietetics

2019-present Career Advocate, Nutrition Science Graduate Student Organization, Purdue University

2017-present Graduate Student Representative, Purdue University Nutrition Science Alumni Network
2018-2019 Student Ambassador, Purdue Institute of Inflammation, Immunology, and Infectious Disease

2017-2019 President, Nutrition Science Graduate Student Organization, Purdue University

2016-2018 Board Member, FACS Young Alumni Council, College of Family and Consumer Sciences Alumni Association, The University of Georgia

2015-2016 President, Foods and Nutrition Graduate Student Organization, The University of Georgia

2014-2016 Nominating Committee, Northeast Georgia Dietetic Association

2015-2016 Graduate Student Representative, Open House and Graduate Student Recruitment Committee, Department of Foods and Nutrition

2014-2015 Graduate Student Representative, Diversity Committee, Department of Foods and Nutrition

2013-2014 President, Student Dietetic Association, The University of Georgia

2012-2013 Secretary, Student Dietetic Association. The University of Georgia

EDUCATIONAL PRESENTATIONS in the COMMUNITY

CAMP: Clinical Applications for future Medical Professionals, College of Health and Human Sciences, Purdue University, West Lafayette, IN
Know your Nutrients: The Dietitian's Role in Recognizing and Treating Malnutrition, July 2019

Purdue Lecture Hall Series, West Lafayette, IN
My Path from "What do I want to do when I grow up?" to "I want to save the world," October 2018

Association of Nutrition & Foodservice Professionals Meeting, West Lafayette, IN
Understanding Nutrition Labels: An important tool for making informed decisions, October 2018

Baseball Umpires Meeting, Lafayette, IN
Nutrition Tips for Gamedays (and Beyond), April 2018

Osher Lifelong Learning Institute (OLLI@UGA) class
The Often Confusing and Overwhelming Principles of Nutrition, May 2016

Athens-Clarke County Wellness Program, Healthy Hour
What You Should Know about Kidney Stones, March 2016
Folate Facts, February 2016
Get the Skinny on Soft Drinks, October 2015
Fad Diets: The Truth Behind the Myths, September 2015
Understanding Nutrition Labels: An important tool for making informed decisions, March 2015
Glycemic Index: What You Need to Know, October 2014

Human Development and Family Sciences Graduate Student Organization, Self-Care Night
How to Eat Healthier in Graduate School, February 2016

Boy Scouts Cooking and Nutrition Class
The Many Methods of Cooking, November 2015
The Many Methods of Cooking, October 2014

OTHER SERVICE ACTIVITIES

2019 Helped organize and implement "A Leap from Lab," a life sciences graduate student career event

2018 Conducted grocery store tour for National Nutrition Month through the Indiana Academy of Nutrition and Dietetics at Fresh Thyme in Lafayette, IN

2017 Helped organize and implement "Tuesday Toasts," a weekly email blast to our nutrition Department at Purdue that highlights all our individual accomplishments over the past week

2017 Organized graduate student 3 Minute Thesis (3MT®) competition for the Department of Nutrition Science, Purdue University. 6 students participated, $500 award given to winner.

PROFESSIONAL ORGANIZATION MEMBERSHIPS

2019-present American Society of Nephrology
2018-present American Heart Association
2018-present Nutrition Educators of Health Professionals Dietetic Practice Group
2016-present Western Indiana Academy of Nutrition and Dietetics
2016-present Indiana Academy of Nutrition and Dietetics
2015-present American Society for Nutrition
2014-present Research Dietetic Practice Group
2011-present Academy of Nutrition and Dietetics
2018-2019 American Society for Bone and Mineral Research
2013-2016 Northeast Georgia Dietetic Association
2011-2016 Georgia Academy of Nutrition and Dietetics

ADDITIONAL WORK EXPERIENCE

Purdue University Center for Instructional Excellence, Instructional Data Processing, West Lafayette, IN
2019 Exam Proctor

University of Georgia Food Services, Athens, GA
2013-2014 Dietitian's Assistant
2012-2013 Mystery Shopper

Georgia School Nutrition Culinary Institute I, Athens, GA
2013, 2014 Culinary Assistant

University of Georgia Sports Nutrition, Athens, GA
2013 Intern