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URINE ELECTROLYTE EXCRETION IN A HYPERTENSIVE POPULATION OF EAST AFRICANS

A Thesis
Presented to
The Faculty of the Department of Biology
Western Kentucky University
Bowling Green, Kentucky

In Partial Fulfillment
Of the Requirements for the Degree
Master of Science

By
Aiste Dobrovolskaite

May 2017
URINE ELECTROLYTE EXCRETION IN A HYPERTENSIVE POPULATION OF EAST AFRICANS

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CONTENTS

INTRODUCTION ............................................................................................................................................. 1
  Global perspective of Noncommunicable Diseases ................................................................. 1
  Cardiovascular Diseases ............................................................................................................. 3
  Hypertension ............................................................................................................................... 4
  Hypertension in Kenya ............................................................................................................... 6
  Physiology and Renal Blood Pressure ....................................................................................... 9
  Salt-sensitive Hypertension and the Renin-Angiotensin System ............................................ 10
  Salt-sensitivity in a Hypertensive Cohort in Kenya .............................................................. 12

METHOD AND MATERIALS ...................................................................................................................... 15
  Ethics Review and Compliance ................................................................................................. 15
  Study Participants ..................................................................................................................... 15
  Urine Stability Tests ................................................................................................................. 15
  Urine Sampling Protocols ........................................................................................................ 16
  Anthropometric Data Collection ............................................................................................... 17
  Urine Sample Collection and Analysis .................................................................................... 18
  Statistical Analysis ...................................................................................................................... 19

RESULTS ..................................................................................................................................................... 20
  Urine Stability Tests ............................................................................................................... 20
  Demographic Results ............................................................................................................... 22
  Electrolyte Excretion Comparison by Hypertensive Category ............................................. 24
  Electrolyte Excretion Comparison by Age ............................................................................ 29
  Linear Regression Analysis – Sodium ..................................................................................... 33
  Linear Regression Analysis – Potassium and Chloride ........................................................ 37
  Electrolyte Excretion Comparison by BMI ............................................................................. 39

DISCUSSION .................................................................................................................................................. 45

REFERENCES .............................................................................................................................................. 51

APPENDIX ................................................................................................................................................... 57

ABBREVIATIONS ....................................................................................................................................... 63
LIST OF FIGURES

Figure 1. Deaths due to NCDs. ................................................................. 2
Figure 2. Prevalence of hypertension in age-standardized populations for males and females................................................................. 5
Figure 3. Map of Kenya and Kasigau ....................................................... 8
Figure 4. Renin-Angiotensin System ......................................................... 11
Figure 5. Mean electrolyte excretion for males and females in the study population ................................................................. 23
Figure 6. Electrolyte excretion levels of estimated 24HU Na⁺, K⁺ and SU Cl⁻ for different hypertensive stages. ................................................................. 26
Figure 7. Mean electrolyte excretion levels of estimated 24HU Na⁺, K⁺ and SU Cl⁻ for different hypertensive stages for females (a) and males (b) .......... 28
Figure 8. Mean systolic BP values of males and females, divided by age .......... 31
Figure 9. Mean electrolyte values of estimated 24HU Na⁺, K⁺ and SU Cl⁻ for males and females, divided by age ................................................................. 32
Figure 10. Relationship between estimated 24HU Na⁺ excretion (mmol/L) and systolic BP (mmHg) ................................................................. 34
Figure 11. Relationship between estimated 24HU Na⁺ excretion (mmol/L) and systolic BP (mmHg) for females ................................................................. 35
Figure 12. Relationship between estimated 24HU Na⁺ excretion (mmol/L) and systolic BP in (mmHg) for males ................................................................. 36
Figure 13. Relationship between electrolytes excretion (mmol/L) for estimated 24HU K⁺ and SU Cl⁻ vs systolic BP (mmHg) ................................................................. 38
Figure 14. Mean systolic BP values of males and females divided by BMI .......... 40
Figure 15. Mean electrolyte excretion levels of estimated 24HU Na⁺, K⁺ and SU Cl⁻ for different BMI categories ................................................................. 41
Figure 16. Mean electrolyte excretion levels of estimated 24HU Na⁺, K⁺ and SU Cl⁻ for different BMI categories for (a) females and (b) males ................. 44
Figure 17. Cycle of aging, inflammation, and oxidative stress in relationship to hypertension ................................................................. 46
LIST OF TABLES

Table 1. SU stability tests in different temperatures (~23 °C, 4 °C and –20 °C) and different time (0h, 24h, 48h, 72h). .................................................................21

Table 2. Multiple analyzes of one SU sample at ~23 °C and 0h after changing K+ electrode. .................................................................21

Table 3. Characteristics of study population.................................................................23

Table 4. Mean systolic BP and urine electrolyte excretion means for normotensive, pre-hypertensive and combined hypertensive stage I & II categories .................25

Table 5. Mean systolic BP and urine electrolyte excretions for overall study population, males and females, divided by age. .................................................................30

Table 6. Mean systolic BP and urine electrolyte excretion means for underweight, normal weight, overweight and obese individuals........................................42
Chronic noncommunicable diseases (NCDs) are the largest contributor to mortality rates worldwide including in low- and middle- income countries (LMICs) which already suffer from high rates of infectious disease. Among the four major NCDs that cause 38 million deaths annually, cardiovascular disease (CVD) causes 17.5 million of these annual deaths. The primary risk factor of CVD is hypertension. Kenya, a developing country in Sub-Saharan Africa, has a high rate of hypertension with low (2.6%) management rates. Prior research from our lab has identified a population of Kenyans with a high prevalence of hypertension that is not statistically correlated with typical known risk factors such as obesity, hypercholesterolemia, and behaviors of smoking and lack of exercise. This study investigated the hypothesis that high dietary salt consumption and low K⁺ dietary intake are contributing to the etiology of high blood pressure in this community. To test our hypothesis, two spot urine samples representing nocturnal excretions (evening and morning) and blood pressure measurements were collected from 135 participants. All samples were analyzed for Na⁺, K⁺ and Cl⁻ content using the Smartlyte Electrolyte Analyzer. The average of each spot urine sample was extrapolated to an estimated 24-h value by the method of Mills, et al. The overall population mean urine electrolyte excretion values for Na⁺, K⁺ and Cl⁻ were 170.6 ± 89.3 mmol/L, 82.0 ± 54.0 mmol/L, and 87.7 ± 42.1 mmol/L, respectively. While these values fall within the suggested levels for Na⁺ (40-220 mmol/L) and K⁺ (25-125 mmol/L), they
are under normal excretion levels for Cl\(^-\) (110-250 mmol/L). Overall ion excretion was higher in females than males, although only K\(^+\) values were statistically significant (p < 0.05). Analysis of Na\(^+\) and Cl\(^-\) excretion from individuals stratified by blood pressure, revealed significant differences (p < 0.05) between normotensive and hypertensive stage I individuals for both electrolytes (57.9 mmol/L vs. 88.9 mmol/L and 65.5 mmol/L vs. 96.7 mmol/L, respectively). Overall, these results suggest that our sample population consumes dietary salt within a normal range and thus, the observed prevalence of hypertension likely results from other genetic and environmental factors.
INTRODUCTION

Global perspective of Noncommunicable Diseases

Noncommunicable diseases (NCDs) are the leading cause of death worldwide, with an annual mortality rate of 38 million (World Health Organization (WHO), 2014a). Among all NCDs, cardiovascular disease (CVD), cancer, diabetes, and respiratory diseases account for 82% of all NCD deaths (WHO, 2014a). In the past it was thought that NCDs mainly occurred in high-income countries, however, currently the highest death rate from NCDs (28 million) is seen in low- and middle-income counties (LMIC) (Sommer et al., 2015; WHO, 2014a). Through many advancements in the last few centuries such as screening for diseases, clinical practice, improved technology and treatment, medicine as well as increased funding for biomedical research, human life expectancy has increased above 65 years and older by 22% in the past 50 years (Olshansky, 2015). Notwithstanding the positives of living longer, there are also negatives such as accumulating NCDs over a lifetime. The World Bank defines low-income countries as those with a Gross National Income (GNI) per capita per year of $1,025 or less and lower middle-income countries having GNI per capita between $1,026 and $4,035 per year (“The World Bank Country and Lending Groups”, 2015). Fifty one percent of all LMICs belong to the continent of Africa where poverty and NCDs collide (Figure 1) (“The World Bank Country and Lending Groups”, 2015).
As a result of the poverty in African countries, there is a lack of access to health care and shortages of medication. Because of such poor health management, people in Africa have the highest death rates of concomitant infections and NCDs (BeLeu et al. 2009). Since most government funding in these areas is used to fight infectious diseases, such as Ebola, human immunodeficiency virus (HIV), dengue and malaria, NCDs do not get sufficient resources to address the high number of deaths. The last decade provided evidence that vaccinations for most of the communicable diseases reduced mortality rate among children, however, this caused African populations to undergo an epidemiological transition (Byass et al., 2014; Bygbjerg, 2012).

Epidemiological transitions are recognized by a shift from high mortality rates of infants and children to a high mortality rates of the elderly. This transition in developing countries is mainly caused by urbanization, development, industrialization and aging of
the whole population (Bygbjerg, 2012; Mathenge et al., 2010; Van de Vijver et al., 2013). Out of 16 million NCD premature deaths that occur between ages 30 and 70, developing countries have the highest mortality rates (85%) (WHO, 2014b). To deal with this modern century problem, the WHO suggests focusing on prevention of NCDs in order to prevent chronic health problems that would be significantly more expensive to treat.

Cardiovascular Diseases

Out of four major NCDs, CVD has the highest annual mortality rate of 17.5 million, followed by cancer, respiratory diseases and diabetes with 8.2, 4 and 1.5 million respectively (WHO, 2011; WHO, 2014a). The continent most impacted by CVD is Africa, with the death rates for both males and females expected to increase more than 100% in developing countries by the year 2020 (Kayima et al., 2013; Yach et al., 2004). To address this problem, the prevalence of CVD risk factors needs to be determined and subsequently managed. CVD is considered to have two major categories of risk markers: modifiable (reversible) and non-modifiable (irreversible) (Van de Vijver et al., 2013).

Modifiable risk factors include hypertension (also known as high blood pressure (BP)), obesity, hyperlipidemia, and environmental exposure, while non-modifiable risk markers refer to age, gender, and family history (WHO, 2011). While some scientists argue that race and ethnicity are also contributing factors to CVD, there has not been sufficient evidence to support this hypothesis (Kurian & Cardarelli, 2007). However, race and ethnicity are thought to correlate to socioeconomic status, which can influence CVD as a risk factor (Kurian & Cardarelli, 2007). According to the WHO, hypertension is identified as the primary risk factor for CVD (WHO, 2013) as well as other NCDs that
lead to disability or death worldwide (Lim et al., 2012). To treat the increase in CVD over the next ten years it would cost approximately one trillion dollars if precautionary measures are not implemented today (Bloom et al., 2011; Vedanthan et al., 2014). Thus, it is necessary to control and treat high BP now in order to reduce the risk for CVD and other NCDs.

**Hypertension**

In the last four decades, the number of people with hypertension increased from 594 million to 1.3 billion worldwide with the highest increase in LMIC in both genders, (Figure 2) (Zhou et al., 2017). The number of individuals with elevated BP are undeniably rising, and by 2025, worldwide hypertension prevalence is predicted to increase by 60%, of which developing countries will have 25% of the global hypertension population (Chockalingam et al., 2006; WHO, 2014a). Adults over 25 years old are considered to have hypertension if their systolic BP ≥ 140 mmHg, their diastolic BP ≥ 90 mmHg, they are taking antihypertensive medication or any combination of the above (WHO, 2013).
Hypertension can be further categorized as primary (essential) hypertension or secondary hypertension. The majority of all hypertension cases (95%) are said to be essential with unknown etiology, while secondary hypertension has a known cause (Guyton and Hall, 2000; Staessen et al., 2003). It is thought that essential hypertension is influenced by polygenic and environmental factors or a combination of both (Staessen et
al., 2003). Despite years of research on essential hypertension, specific causes for the disease are still not fully understood (Staessen et al., 2003). Countries in Africa not only have significantly higher numbers of people suffering from essential hypertension but also show lower levels of awareness and treatment of the disease, which ultimately leads to increased risks for morbidity and mortality (Ataklte et al., 2015; Kayima et al., 2013). Thus, it is crucial to investigate the possible causes for hypertension in African countries in order to lower the escalating disease burden there.

Hypertension in Kenya

Kenya, a LMIC located in East Africa, has a population of ~47 million people. It is estimated that 45.2% of Kenyans live below the poverty line of Kenyan shillings (Ksh) 1,562 ($15.08) and Ksh 2,913 ($28.12) per rural and urban households per month respectively (Ngugi, 2013). Despite rapid urbanization, the majority (74.4%) of Kenyans live in rural areas where the availability of health care is scarce (“The World Factbook: KENYA”, 2017). According to the World Bank, Kenya is at the low-end globally for the number of physicians, with only 20 per 100,000 people (“The World Bank”, 2014). By comparison, the United States of America has 250 physicians per 100,000 people. The shortage of doctors leads to lack of access to healthcare, especially in rural areas. Specifically, elevated BP in rural areas of Kenya was reported to be managed in less than 3% of all cases (Mwita et al., 2013). This number might be even lower considering that only 18% of Kenyan people are aware of hypertension (Hendriks et al., 2012). The lack of information about hypertension is an additional risk factor, especially for older people, since age and elevated BP are positively correlated. In twenty years, the number of
individuals above age 60 is foreseen to be higher than children under 10 with almost three quarters of the world’s elderly living in developing countries like Kenya (van de Vijver et al., 2013).

This study takes place in Kasigau, located in the southeast of Kenya, in the Taita-Taveta District. As shown in Figure 3, the Kasigau region has five villages surrounding Mount Kasigau: Rukanga, Jora, Bungule, Makwasinyi, and Kiteghe. Ngambenyi and Buguta are additional villages included in the study that do not directly associate with the mountain but are within 1 and 14 km of it respectively and belong to the Kasigau region. Altogether, the seven villages have a population of ~13,000 people (Ngugi, 2013). Residents of Kasigau are subsistence farmers, although they are frequently unable to grow crops due to scarce rains and wildlife conflicts. According to Ngugi (2013), only ~18% of the Kasigau people work for pay which makes them very vulnerable to health care problems. Kenya makes a good research model country for expanding our understanding of hypertension and how to treat it in LMIC.
Since 2008, a team of U.S. physicians led by Dr. Nancy Rice and accompanied by Western Kentucky University (WKU) students have traveled to Kasigau and provided medical assistance and education for the local people through WKU’s Partners in Caring: Medicine in Kenya (PiC:MiK) program. Clinical work along with self-reported cases revealed that essential hypertension is prevalent in the region. Preliminary epidemiological research has shown that 70% of the local population (age 45 years old and above) has elevated BP with no correlation to known risk factors such as high cholesterol, lack of physical activity, tobacco use, etc. (Williams, 2012). Since no

**Figure 3.** Map of Kenya and Kasigau. (A) Overview map of Kenya. (B) Zoomed-in view of Mt. Kasigau with surrounding villages. From the very top and clockwise: Buguta, Kiteghe, Makwasinyi, Bungule, Jora, Ngambenyi and Rukanga. Source: Google Earth.
known typical risk factors have been identified, a hypothesis was made that a potential cause might be increased sensitivity to sodium (Na\textsuperscript{+}) through dietary salt consumption.

**Physiology and Renal Blood Pressure**

In order to understand hypertension, it is important to know the basic physiology of BP. With each heartbeat, blood is pumped out with a certain force (BP) in the circulation. Maximum arterial BP is referred to as systolic, whereas minimum arterial BP is referred to as diastolic. In order to keep blood flowing in the circulatory system, a difference between systolic and diastolic BP is needed. Hemodynamics, or dynamics of blood flow, is controlled by the circulatory system in order to maintain homeostasis.

Blood is a complex fluid that includes plasma (liquid portion) and formed elements, such as red and white blood cells and platelets. According to the American Red Cross, water (~92\%) is the main component of plasma while proteins make up 7\%, and other solutes like mineral salts, fats, and vitamins make up only 1\% ("Blood Components", n.d.). Thus, a change in water and salt balance can increase BP in several ways (Johns et al., 2011) including increased blood vessel resistance, increased blood volume and reduced blood flow via renal dysfunction (Drenjancevic-Peric et al., 2011; Osborn & Foss, 2016).

Renal (ultrafiltration) physiology is an important component in maintaining healthy BP via homeostasis of water and salt. Ultrafiltration occurs in the Bowman’s (glomerular) capsule in kidneys when blood flows into a dense network of capillaries. Due to BP force and a differential concentration gradient, small molecules like water, urea, glucose, amino acids and salts are filtered through arterioles into renal tubules (Koushanpour, 1986). The filtered fluid is then modified by reabsorption of useful
solutes back into the circulation of blood and secretion processes, which produces urine.

If there is a decrease in BP, a reduction of blood flow results, which then decreases the glomerular filtration rate via decreased levels of Na\(^+\) in macula densa. Ultimately, this is fixed by increasing reabsorption of Na\(^+\) which also leads to osmosis and increased volume of plasma. Ultrafiltration in the kidney is a main control mechanism for blood volume (Ehlenz, 1995). Since blood volume can directly affect cardiac output, it also is able to manipulate blood pressure.

**Salt-sensitive Hypertension and the Renin-Angiotensin System**

Salt-sensitivity is when an individual’s BP fluctuates depending on their Na\(^+\) intake (Richardson et al., 2013). It is recommended that Na\(^+\) intake per day ranges from 1.5 – 2.4 g which equals 3-5 grams of table salt per day (O’Donnell et al., 2014; WHO, 2012b) which is more than sufficient for bodily needs (Adrogue & Madias, 2007). Despite the recommended levels, the average global Na\(^+\) consumption has been increasing over time from 3.95 g/day in 2010 to 4.93 g/day in 2014 (Mozaffarian et al., 2014; O’Donnell et al., 2014). Elevated levels of consumption are a big concern because they can lead to essential hypertension and CVD (Mozaffarian et al., 2014; O’Donnell et al., 2014).

Multiple genetic factors impact BP and an individual’s sensitivity to dietary salt. The renin-angiotensin system (RAS) is the most studied and physiologically significant complex hormonal system that controls salt balance and BP homeostasis (Drenjancevic-Peric et al., 2011; Giner et al., 2000; Mendoza & Lazartigues, 2015). When there is a drop in BP, it is detected in the kidney in specialized arteriole (juxtaglomerular) cells and renin is generated.
At the same time, the liver excretes inactive angiotensinogen (AGT) protein which is then converted to active angiotensin I (Ang-I) by renin. Upon activation, Ang-I is transported via the blood stream to the lungs where it is further converted to angiotensin II (Ang-II) by angiotensin converting enzyme (ACE). Ang-II is a potent vasoconstrictor meaning it can regulate the narrowing of blood vessels via regulation of smooth muscle contraction, increasing blood flow and cardiac output. Ang-II can influence various other pathways as well such as the secretion of aldosterone in adrenal glands which increases reabsorption of Na\(^+\) and water in the nephron leading to hypertension (Figure 4). Each of the polypeptides involved in the RAS system are encoded by a separate gene. One of the ways to target hypertension is to prevent Ang-II formation by inhibiting enzymes (renin or ACE) that facilitate the pathway (Mendoza & Lazartigues, 2015). A number of people have impairments in the RAS system and are not able to excrete Na\(^+\) and water appropriately.

**Figure 4.** Renin-Angiotensin System (RAS). Arrows refer to the Angiotensin-II synthesis pathway that results in elevated blood pressure due to a change in blood [Na\(^+\)]

Data source: http://fcpspart1dentistry.com/renin-angiotensin-aldosterone-system-video-lecture/
Salt-sensitivity in a Hypertensive Cohort in Kenya

Kenyan people from the Kasigau region could possibly be genetically predisposed for salt-sensitive essential hypertension. According to Richardson et al. (2013), 75% of hypertensive salt-sensitive individuals are non-Hispanic blacks, with other studies showing a positive correlation between Na\(^+\) intake and hypertension in African populations (Tayo et al., 2012). Epidemiological studies suggest that it takes significantly less dietary salt to develop hypertension for Africans than Caucasians due to an evolutionary maladaptation in RAS (Drenjacevic-Peric et al., 2011). In addition, some studies argue that the main cause of elevated BP in aging populations is due to rising salt consumption. A study on Yanomamo Indians revealed that incredibly low salt intake does not lead to increasing BP with age, with prevalence of hypertension at only ~5%, as would be expected in African population before westernization (Drenjacevic-Peric et al., 2011). As more African societies become westernized, their consumption of processed food, which is high in salt, increases which can lead to elevated BP.

Likewise, there has been some evidence positively correlating chloride (Cl\(^-\)) to high BP, and it is important to acknowledge it because Na\(^+\) and Cl\(^-\) can come from different sources (McCallum & Lip, 2015). Conversely, studies done by Hajjar et al. (2006) have shown a negative correlation in high potassium (K\(^+\)) intake and hypertension. Thus, a Na\(^+\)/K\(^+\) ratio can also be used as an indicator for hypertension since several studies have shown that a positive correlation exists between an increase in BP and a corresponding increase in Na\(^+\)/K\(^+\) (Hedayati et al., 2012). Hence, salt sensitive hypertension does not only refer to dietary Na\(^+\) intake, it can also be influenced by other
diet components: K\(^+\), Cl\(^-\), fat, etc. (McCallum & Lip, 2015). This study will evaluate Na\(^+\), K\(^+\) and Cl\(^-\) excretions in urine in order to extrapolate salt consumption in a Kenyan cohort living in the Kasigau area. This work will provide important information regarding whether the observed hypertension in this population may be a result of salt-sensitivity.

While 24-hour urine (24HU) collection has traditionally been the standard protocol for determining salt consumption, this technique is limited by difficulty on the part of the patient and lack of compliance. Recently, multiple studies have shown that Na\(^+\) excretion can be effectively estimated from spot urine (SU) samples and SU samples correlate well with 24HU samples. Therefore, SU can be used as an estimate of Na\(^+\)/K\(^+\) ratios in urine samples (Brown et al., 2013; Kawasaki et al., 1993; Mill et al., 2015; Tanaka et al., 2002; Wang et al., 2013).

Moreover, Mill et al. (2012) showed that nocturnal 12-h urine collection correlates to 24HU collection, making 12-h urine collection useful in epidemiological studies. In the study, two collection periods were established: nocturnal (overnight: between 1900 and 0700 h) and diurnal (over day: between 0700 and 1900 h). It was found that nocturnal urine collection represents 47% (Na\(^+\)) and 39% (K\(^+\)) of 24HU collection. Averaged and divided by 0.47 or 0.39 for Na\(^+\) and K\(^+\), these values are a good representation of 24HU electrolyte excretion. Hence, evening and morning SU values represent nocturnal urine electrolyte values.

Thus, I will test the hypothesis that the high rate of hypertension in Kenyan individuals is due to a high dietary Na\(^+\) intake. To test this hypothesis, I will use a two
SU analysis (evening and overnight) based upon the method of Mill et al. (2012), since these SU samples are a good representation of 12-h (overnight) urine collection and can be correlated to 24HU collection to represent dietary salt consumption.
MATERIALS AND METHODS

Ethics Review and Compliance

This study was approved by Western Kentucky University’s Human Subject Review Board (IRB 14-387, Appendix A) and the Taita District Health Officer in Kenya, the University of Nairobi Ethics Review Board (Appendix B) as well as the village chiefs/elders. Participants were provided an informed document that they either read or was read to them. This document explained the study purpose and goals to identify salt excretion in the samples and individuals voluntarily consented to participate.

Study Participants

Study participants were recruited from the seven Kasigau villages: Buguta, Makwasinyi, Rukanga, Bungule, Ngambenyi, Kiteghe, and Jora. Participants in this study were a subset of a longitudinal study in our lab (Williams, 2012). Initially, participants were selected as a random sample of the normal population and included males and non-pregnant females, ages 45 and above.

Urine Stability Tests

Because field sampling in Kenya lacks many common sample storage abilities, prior to data collection in Kenya, urine stability tests were performed. Urine samples were tested for Na⁺, K⁺ and Cl⁻ electrolyte stability in a sample over time; 0h, 24h, 48h
and 72h. Urine samples were also tested for Na\(^+\), K\(^+\) and Cl\(^-\) electrolyte stability after 24h at room temperature (~23°C), 4°C and –20°C.

**Urine Sampling Protocols**

To increase compliance in this study, the SU sampling approach was chosen over 24HU sampling. Spot urine samples have been proven to be a good indicator of 24HU collection, therefore it is a good indicator of dietary salt intake (Kawasaki et al., 1993; Mill et al., 2015; Wang et al., 2013). Over the course of two months, seven community meetings, barazza, were held for study participants in each of the Kasigau sub-locations: Buguta, Makwasinyi, Rukanga, Bungule, Ngambenyi, Kiteghe, and Jora. Participants as a group were given a verbal explanation of the study aims along with instructions for urine sample collection. A translator assisted in this phase of the project. Each participant was instructed to collect an evening (between 1800 and 2359 h) and overnight (between 0400 and 0900 h) urine sample. Each participant was given two of each of the following items for sample collection: Dixie cup, previously labeled 15 mL falcon tube, 3 mL disposable plastic transfer pipet, and a towelette. Instructions on collection process were provided in English (Appendix C) and Swahili (Appendix D). Individuals either read these instructions or the instructions were read to them. Study participants were to transfer the void from the Dixie cups to corresponding falcon tubes for morning and evening samples.
Anthropometric Data Collection

BP measurements were taken after each participant received instructions on how to collect the samples. In order to obtain the most accurate BP results, all individuals were asked to be seated and remain seated for at least five minutes without talking and with both flat feet touching the floor prior to BP measurement (Frese et al., 2011). BP was measured using an automatic BP monitor by Omron (model: BP742N, Omron Healthcare, Inc.). Participants were classified as either: normotensive (below 120 mmHg systolic BP / 80 mmHg diastolic BP), pre-hypertensive (120-140 / 80-90 mmHg), Stage I (140-160 / 90-100 mmHg) or Stage II (above 160/100 mmHg) categories (WHO, 2014a; Richardson et al., 2013). Systolic BP is a more accurate predictor for cardiovascular problems than diastolic BP especially among older populations, so systolic value is the primary comparison tool that is used in this study (Hajjar et al., 2006). Two blood pressure measurements were taken from each individual and the average of both systolic BP measurements was used for statistical correlations. Study participants also were asked if they were currently using any antihypertensive medicine.

Other anthropometric data, such as, age, body weight (kg), height (cm), waist to hip ratio was obtained from the preliminary work done in the same community by Lindsay Williams (2012). Body mass index (BMI) was calculated as kg/m². Based on BMI, participants were divided into the following categories: underweight (< 18.5 kg/m²), normal (18.5 – 25 kg/m²), overweight (25 – 30 kg/m²), and obese (> 30 kg/m²) according to the standards set forth by the WHO.
Urine Sample Collection and Analysis

Urine samples were collected and analyzed within 6 - 48 hours. A Diamond Diagnostics Smartlyte Electrolyte Analyzer was used to perform the electrolyte analysis. Each sample was diluted to a ratio of 1 part urine sample (1 ml urine) to 2 parts urine diluent (2mL urine diluent) (AV-BP0344D, Diamond Diagnostics). Samples were then analyzed for Na\(^+\) and Cl\(^-\) concentrations. Before measuring K\(^+\), the diluted urine samples were diluted one more time with distilled water in a 1:2 ratio respectively. Potassium values were subsequently multiplied by 3 to control for the dilution factor. Samples that were below 15 mmol/L for K\(^+\) prior to the dilution were not diluted further. Subjects who failed to collect at least 3mL of void for each of the morning and evening samples were excluded from the study.

The Smartlyte Electrolyte Analyzer uses a calibration curve based on measured points of standard solutions with known ion concentrations. The calibration procedure, either 2-point or 3-point, was performed automatically every 4 hours or after 100 analytical runs, whichever came first. A 1-point calibration was automatically performed with each sample measurement. Quality control and standard cleaning and conditioning procedures were executed every time after powering on the Smartlyte Electrolyte Analyzer. These included using commercially prepared reagents from Diamond Diagnostics: Mission Control 1-2-3 standards (DD-92123) for quality control tests; ISE Cleaning Solution (AV-BP1025D) and Electrode Conditioning Solution (AV-BP0380D) for cleaning and conditioning, respectively. Each urine sample was measured as a duplicate and the average of both trials were used for statistical analysis. Prior to data
analysis, averaged crude SU sample values were divided by 0.47 and 0.39 for Na\(^+\) and K\(^+\) respectively in order to represent 24HU collection (Mill et al., 2012). These values were used in further data analysis.

**Statistical Analysis**

Statistical analysis was performed using the SigmaPlot 11.0 package (Systat Software, Inc, USA). Statistical relationships between electrolyte excretion and anthropometric data were analyzed via linear regression and overall anthropometric population data were analyzed statistically by t-test. If a data set failed the normality test, Mann-Whitney Rank Sum test was applied. P values of < 0.05 were used to infer statistical significance.
RESULTS

Urine Stability Tests

Since the Kasigau area does not have reliable electricity, samples collected in the field would not be able to be refrigerated, frozen or transported to the USA for analysis. Thus, prior to our sample collection in Kenya, random urine samples were tested for stability of electrolyte quantification at room temperature (~23 °C) at 0h, 24h, 48h and 72h. Urine samples were also tested after 24 h at 4 °C, and after one round of freezing at -20°C and thawing at room temperature. The average temperature during the data collection period in Kenya ranged from 20 °C to 28 °C, which is within the range of room temperature (~23 °C) during stability testing.

Regardless of storage temperature, after 24h, no significant differences in Na⁺ and Cl⁻ were observed in comparison with 0h; the highest differences were 6.7 mmol/L for Na⁺ and 10.3 mmol/L for Cl⁻ (Table 1). After 48h and 72h, Sample 1 indicated slightly higher values when compared to 0 h for Na⁺ and Sample 2 had slightly higher values for Cl⁻, which were likely a result of improper mixing of the stationary samples prior to analysis. K⁺ levels varied the most when compared to 0 h (Table 1). Such variation could not be explained by error coming from serial dilutions and mixing efficiency. Therefore, since K⁺ values did not appear to be stable over time, commercially prepared quality control tests were executed (Mission Control 1-2-3, Diamond Diagnostics) which led to the replacement of the K⁺ electrode. After the K⁺ electrode was replaced, another
stability test was performed and this time $K^+$ values did not fluctuate (Table 2). Thus, it was concluded that SU samples are stable at room temperature for up to 48 h.

Table 1. SU stability tests in different temperatures (~23°C, 4°C and –20°C) and different time (0h, 24h, 48h, 72h); values represent the average of three measurements, ± S.D.

<table>
<thead>
<tr>
<th>Sample 1</th>
<th>0 h (~23°C)</th>
<th>24 h (~23°C)</th>
<th>24 h (4°C)</th>
<th>24 h (~20°C)</th>
<th>48 h (~23°C)</th>
<th>72 h (~23°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na$^+$</td>
<td>198.8 ± 3.2</td>
<td>201.0 ± 0.9</td>
<td>202.7 ± 3.5</td>
<td>200.7 ± 2.5</td>
<td>207.0 ± 0.0</td>
<td>211.5 ± 4.4</td>
</tr>
<tr>
<td>K$^+$</td>
<td>41.8 ± 1.8</td>
<td>40.2 ± 1.0</td>
<td>75.3 ± 48.3</td>
<td>98.0 ± 69.1</td>
<td>80.3 ± 15.8</td>
<td>55.4 ± 11.1</td>
</tr>
<tr>
<td>Cl$^-$</td>
<td>135.8 ± 3.2</td>
<td>138.9 ± 1.5</td>
<td>141.3 ± 1.5</td>
<td>141.3 ± 2.5</td>
<td>143.3 ± 0.5</td>
<td>143.8 ± 5.6</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sample 2</th>
<th>0 h (~23°C)</th>
<th>24 h (~23°C)</th>
<th>24 h (4°C)</th>
<th>24 h (~20°C)</th>
<th>48 h (~23°C)</th>
<th>72 h (~23°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na$^+$</td>
<td>156.0 ± 1.5</td>
<td>153.0 ± 1.6</td>
<td>156.0 ± 1.0</td>
<td>157.0 ± 1.0</td>
<td>157.5 ± 1.3</td>
<td>162.3 ± 0.5</td>
</tr>
<tr>
<td>K$^+$</td>
<td>42.3 ± 0.8</td>
<td>39.4 ± 5.6</td>
<td>77.3 ± 38.0</td>
<td>94.8 ± 54.1</td>
<td>65.9 ± 7.7</td>
<td>53.0 ± 13.2</td>
</tr>
<tr>
<td>Cl$^-$</td>
<td>159.3 ± 3.8</td>
<td>162.3 ± 1.6</td>
<td>164.3 ± 2.1</td>
<td>166.3 ± 2.5</td>
<td>167.0 ± 1.2</td>
<td>173.3 ± 2.5</td>
</tr>
</tbody>
</table>

Table 2. Multiple analyzes of one SU sample at ~23°C and 0h after changing K$^+$ electrode.

<table>
<thead>
<tr>
<th>Sample 3</th>
<th>0 h (~23°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na$^+$</td>
<td>197</td>
</tr>
<tr>
<td>K$^+$</td>
<td>28.4</td>
</tr>
<tr>
<td>K$^+$ diluted</td>
<td>8.3</td>
</tr>
<tr>
<td>Cl$^-$</td>
<td>141</td>
</tr>
</tbody>
</table>
Demographic Results

The characteristics of the study population are shown in Table 3. Out of 142 participants, 99 were female (69.7%) and 43 were male (30.3%). The mean age of the population was 61.1 ± 11.5 years old, with females (\(\bar{x} = 59.3\)) on average being significantly younger than males (\(\bar{x} = 64.5\)), t-test value \(p = 0.021\). Systolic BP, body weight and height were similar between sexes, whereas, BMI was significantly higher (\(p = 0.002\)) and waist-to-hip ratio was significantly lower (\(p < 0.001\)) in females compared to males, although an average values were within the normal ranges. Complete urine samples and BP measurements were collected from a total of 135 participants. Average urine electrolyte excretion values for \(\text{Na}^+\) and \(\text{K}^+\) are expressed as estimated 24HU electrolyte values with standard deviations: 170.6 ± 89.3 mmol/L, 82.0 ± 54.0 mmol/L, respectively. Chloride values are expressed as mean SU values since there is no literature which calculates an estimated 24HU \(\text{Cl}^-\) from overnight urine collection. Study population mean for \(\text{Cl}^-\) was calculated to be 87.7 ± 42.1 mmol/L. The normal ranges for adults for the three electrolytes should fall into the following ranges: \(\text{Na}^+ 40 – 220\) mmol/L; \(\text{K}^+ 25 – 125\) mmol/L; \(\text{Cl}^- 110 – 250\) mmol/L (University of Rochester Medical Center, n.d.). Females had higher mean values for each of the urine electrolytes than males, but only \(\text{K}^+\) excretion was statistically significant (Figure 5) (Mann-Whitney Rank Sum Test, \(p = 0.016\)). The \(\text{Na}^+/\text{K}^+\) ratio in females was lower than males, but not significantly lower (\(p = 0.057\)).
Table 3. Characteristics of study population. Values are expressed as means ± standard deviation or percentage as appropriate. An * indicates a significant difference between females and males (p < 0.05)

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>Men</th>
<th>Women</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample size, n</td>
<td>142</td>
<td>43 (30.3)</td>
<td>99 (69.7)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>61.1 ± 11.5</td>
<td>64.5 ± 12.0</td>
<td>59.3 ± 10.8*</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>151.1 ± 28.9</td>
<td>152.0 ± 24.8</td>
<td>150.7 ± 30.7</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>58.6 ± 14.2</td>
<td>58.4 ± 12.5</td>
<td>58.9 ± 15.0</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>155.2 ± 9.0</td>
<td>162.3 ± 8.1</td>
<td>151.8 ± 7.2</td>
</tr>
<tr>
<td>Body Mass Index (kg/m²)</td>
<td>24.8 ± 6.0</td>
<td>22.6 ± 5.0</td>
<td>25.9 ± 6.2*</td>
</tr>
<tr>
<td>Waist-to-hip ratio</td>
<td>0.92 ± 0.35</td>
<td>0.97 ± 0.41</td>
<td>0.89 ± 0.32*</td>
</tr>
<tr>
<td>Estimated 24HU Na⁺ (mmol/L)</td>
<td>170.6 ± 89.3</td>
<td>168.4 ± 69.4</td>
<td>173.3 ± 97.0</td>
</tr>
<tr>
<td>Estimated 24HU K⁺ (mmol/L)</td>
<td>82.0 ± 54.0</td>
<td>68.7 ± 53.8</td>
<td>87.2 ± 52.8*</td>
</tr>
<tr>
<td>SU Cl⁻ (mmol/L)</td>
<td>87.7 ± 42.1</td>
<td>83.3 ± 29.3</td>
<td>90.0 ± 46.5</td>
</tr>
<tr>
<td>Estimated daily Na⁺ (g/day)</td>
<td>3.93 ± 2.0</td>
<td>3.86 ± 1.6</td>
<td>4.03 ± 2.2</td>
</tr>
<tr>
<td>Estimated daily K⁺ (g/day)</td>
<td>3.21 ± 2.1</td>
<td>2.85 ± 2.2</td>
<td>3.39 ± 2.1</td>
</tr>
<tr>
<td>Na⁺/K⁺ ratio (for estimated daily intake, g)</td>
<td>1.57 ± 1.12</td>
<td>1.89 ± 1.5</td>
<td>1.43 ± 0.9</td>
</tr>
</tbody>
</table>

Figure 5. Electrolyte excretion for females and males in the study population. Values represent mean ± standard error.
**Electrolyte Excretion Comparison by Hypertensive Category**

Electrolyte excretion values were categorized based upon the BP of each participant and sex as follows: normotensive (n = 17), pre-hypertensive (n = 43), hypertensive stage I (n = 33) and hypertensive stage II (n = 49). Hypertensive individuals, on average, showed the highest amount of Na\(^+\), K\(^+\) and Cl\(^-\) excretion when compared to normotensive and pre-hypertensive participants (Table 4). However, when hypertensive stage I vs. stage II are subdivided, there is no trend. Only Na\(^+\) excretion showed a significant difference between hypertensive and normotensive p < 0.05 (t-test p = 0.028).
Table 4. Mean systolic BP and urine electrolyte excretion means for normotensive, pre-hypertensive and combined hypertensive stage I & II categories. Hypertensive I & II is also subdivided into Stage I and Stage II below. Values are expressed as means ± standard deviation or percentage as appropriate. A * indicates significantly different values when compared to normotensive values.

<table>
<thead>
<tr>
<th>Category</th>
<th>Total</th>
<th>Men</th>
<th>Women</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\bar{x} \pm S.D.$</td>
<td>$\bar{x} \pm S.D.$</td>
<td>$\bar{x} \pm S.D.$</td>
</tr>
<tr>
<td>Normotensive (%)</td>
<td>17 (12.0)</td>
<td>5 (11.0)</td>
<td>12 (12.5)</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>109.1 ± 8.2</td>
<td>108 ± 7.7</td>
<td>109.3 ± 8.6</td>
</tr>
<tr>
<td>Estimated 24HU Na$^+$ (mmol/L)</td>
<td>123.2 ± 85.0</td>
<td>163.8 ± 83.8</td>
<td>125.8 ± 91.7</td>
</tr>
<tr>
<td>Estimated 24HU K$^+$ (mmol/L)</td>
<td>61.0 ± 44.6</td>
<td>40.0 ± 15.7</td>
<td>66.4 ± 47.9</td>
</tr>
<tr>
<td>SU Cl$^{-}$ (mmol/L)</td>
<td>65.5 ± 30.6</td>
<td>74.7 ± 32.0</td>
<td>66.3 ± 31.8</td>
</tr>
<tr>
<td>Pre-hypertensive (%)</td>
<td>43 (30.3)</td>
<td>10 (21.7)</td>
<td>33 (34.4)</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>129.7 ± 5.8</td>
<td>128.0 ± 5.6</td>
<td>130.2 ± 5.8</td>
</tr>
<tr>
<td>Estimated 24HU Na$^+$ (mmol/L)</td>
<td>172.9 ± 95.0</td>
<td>178.7 ± 84.1</td>
<td>171.1 ± 99.2</td>
</tr>
<tr>
<td>Estimated 24HU K$^+$ (mmol/L)</td>
<td>94.6 ± 67.0</td>
<td>86.9 ± 94.9</td>
<td>96.9 ± 57.7</td>
</tr>
<tr>
<td>SU Cl$^{-}$ (mmol/L)</td>
<td>86.2 ± 42.3</td>
<td>85.5 ± 33.7</td>
<td>86.5 ± 45.1</td>
</tr>
<tr>
<td>Hypertensive Stage I &amp; II</td>
<td>82 (57.7)</td>
<td>31 (67.4)</td>
<td>51 (53.1)</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>170.3 ± 21.4</td>
<td>164.0 ± 18.3</td>
<td>174.2 ± 22.4</td>
</tr>
<tr>
<td>Estimated 24HU Na$^+$ (mmol/L)</td>
<td>178.2 ± 85.3*</td>
<td>165.8 ± 64.1</td>
<td>185.9 ± 94.9</td>
</tr>
<tr>
<td>Estimated 24HU K$^+$ (mmol/L)</td>
<td>79.1 ± 46.4</td>
<td>67.4 ± 36.9</td>
<td>87.2 ± 50.0</td>
</tr>
<tr>
<td>SU Cl$^{-}$ (mmol/L)</td>
<td>92.6 ± 42.8</td>
<td>84.0 ± 28.2</td>
<td>97.9 ± 48.8</td>
</tr>
<tr>
<td>Hypertensive Stage I I</td>
<td>33 (23.2)</td>
<td>15 (34.9)</td>
<td>18 (18.2)</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>150.8 ± 7.3</td>
<td>148.2 ± 7.0</td>
<td>152.9 ± 6.9</td>
</tr>
<tr>
<td>Estimated 24HU Na$^+$ (mmol/L)</td>
<td>189.1 ± 83.8</td>
<td>176.7 ± 60.3</td>
<td>199.5 ± 99.8</td>
</tr>
<tr>
<td>Estimated 24HU K$^+$ (mmol/L)</td>
<td>89.2 ± 56.4</td>
<td>77.2 ± 44.2</td>
<td>99.3 ± 64.3</td>
</tr>
<tr>
<td>SU Cl$^{-}$ (mmol/L)</td>
<td>96.7 ± 41.3</td>
<td>92.0 ± 28.8</td>
<td>100.7 ± 49.9</td>
</tr>
<tr>
<td>Hypertensive Stage II</td>
<td>49 (34.5)</td>
<td>16 (37.2)</td>
<td>33 (33.3)</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>183.7 ± 17.0</td>
<td>178.8 ± 11.8</td>
<td>186.1 ± 18.9</td>
</tr>
<tr>
<td>Estimated 24HU Na$^+$ (mmol/L)</td>
<td>170.7 ± 86.4</td>
<td>155.7 ± 67.9</td>
<td>178.5 ± 92.9</td>
</tr>
<tr>
<td>Estimated 24HU K$^+$ (mmol/L)</td>
<td>72.2 ± 37.2</td>
<td>58.3 ± 26.8</td>
<td>80.8 ± 39.7</td>
</tr>
<tr>
<td>SU Cl$^{-}$ (mmol/L)</td>
<td>89.7 ± 44.1</td>
<td>76.5 ± 26.3</td>
<td>96.4 ± 48.4</td>
</tr>
</tbody>
</table>
When the estimated 24HU Na⁺, K⁺, and SU Cl⁻ electrolyte excretion were compared, all individuals, regardless of BP, have normal excretion levels for Na⁺ (40 – 220 mmol/L) and K⁺ (25 – 125 mmol/L), but are under the normal excretion levels for Cl⁻ (110 – 250 mmol/L) (Figure 6, Table 4). Analysis of each electrolyte excretion value showed that there is a significant difference between normotensive and hypertensive stage I individuals for estimated 24HU Na⁺ (p= 0.015) and SU Cl⁻ excretion (p = 0.037). While not significant (p = 0.053), these same individuals also showed a difference in estimated 24HU K⁺ excretion values.

Figure 6. Electrolyte excretion levels of estimated 24HU Na⁺, K⁺ and SU Cl⁻ for different hypertensive stages. Values represent mean ± standard error.
When data were separated by sex there were no statistically significant differences between hypertensive categories for any electrolyte within the same sex or between males and females of the study cohort (Figure 7). Generally, there is an increase in electrolyte excretion as BP increases, with the exception of hypertensive stage II individuals. This may be due to the small sample size of the overall study. The estimated 24HU K⁺ excretion for males does not follow this trend.

Normotensive females and those with stage I hypertension did show differences between 24HU Na⁺ and SU Cl⁻ values (p = 0.050) and between normotensive and stage II (p = 0.059), although these were not statistically significant. The biggest difference between males and females was observed in hypertensive stage II individuals for K⁺ 58.3 ± 26.8 vs. 80.8 ± 39.7, p = 0.089 (Table 4).
Figure 7. Mean electrolyte excretion levels of estimated 24HU Na⁺, K⁺ and SU Cl⁻ for different hypertensive stages for females (a) and males (b). Values represent means ± standard error.
Electrolyte Excretion Comparison by Age

Individuals in each of the hypertensive categories varied in age from 40 to >70 years (Table 5). The most hypertensive stage I & II individuals ($n = 28$; female 16, male 12) are ≥70 years old, followed by individuals 50 – 59 ($n = 24$; female 17, male 7), 60 – 69 ($n = 17$; female 12, male 5), and 40 – 49 ($n = 11$; female 7, male 4). On average, there were 12 pre-hypertensive individuals (dominated by women due to larger female sample size overall, $n = 7$) in all age categories except 70 and above. There were equal number ($n = 5$) of normotensive individuals in age groups 40 – 49 and 50 – 59 (females only), which decreased to 3 individuals in age 60 – 69 and 2 individuals (only males) in the group above 70.

When mean systolic BP values and age were compared, in general a positive linear correlation exists for both males and females, as expected, with the exception of females aged 60 – 69 (Figure 8). Lower mean systolic BP value for females of ages 60 – 69 might be related to a smaller overall sample size when compared to women ages 50 – 59. Differences of systolic BP between male and female matched age categories were not significant ($p > 0.05$). Differences of systolic BP within the same sex, indicated that 40 – 49 year old females have significantly lower BP than females of ages 50 – 59 ($p = 0.048$), 60 – 69 ($p = 0.047$) and ≥70 ($p = 0.002$), whereas males ages 50 – 59 have significantly lower systolic BP than males ≥70 years old ($p = 0.045$). There was a large difference in systolic BP between males age 40 – 49 and males ≥70 years old, however it was not significant ($p = 0.081$).
**Table 5.** Mean systolic BP and urine electrolyte excretions for overall study population, males and females, divided by age. Values are expressed as means of urinary electrolytes (mmol/L) ± standard deviation. A* indicates significant differences between male and same age female.

<table>
<thead>
<tr>
<th>Age Group</th>
<th>Total</th>
<th>Men</th>
<th>Women</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>142</td>
<td>43</td>
<td>99</td>
</tr>
<tr>
<td>Age 40-49</td>
<td>30 (21.1)</td>
<td>6 (14.0)</td>
<td>24 (24.2)</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>137.3 ± 23.4</td>
<td>116.7 ± 6.0</td>
<td>136.8 ± 25.6</td>
</tr>
<tr>
<td>Estimated 24HU Na⁺ (mmol/L)</td>
<td>164.0 ± 83.0</td>
<td>175.7 ± 56.1</td>
<td>164.9 ± 89.5</td>
</tr>
<tr>
<td>Estimated 24HU Na⁺ (mmol/L)</td>
<td>85.1 ± 52.2</td>
<td>85.5 ± 54.2</td>
<td>83.4 ± 52.3</td>
</tr>
<tr>
<td>SU Cl⁻ (mmol/L)</td>
<td>82.5 ± 36.6</td>
<td>89.7 ± 32.0</td>
<td>82.0 ± 38.0</td>
</tr>
<tr>
<td>Age 50-59</td>
<td>41 (28.9)</td>
<td>11 (25.6)</td>
<td>30 (30.3)</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>151.4 ± 32.4</td>
<td>129.4 ± 18.1</td>
<td>154.5 ± 35.8</td>
</tr>
<tr>
<td>Estimated 24HU Na⁺ (mmol/L)</td>
<td>186.2 ± 96.6</td>
<td>203.1 ± 78.3</td>
<td>181.2 ± 101.7</td>
</tr>
<tr>
<td>Estimated 24HU Na⁺ (mmol/L)</td>
<td>97.5 ± 70.4</td>
<td>93.8 ± 88.6</td>
<td>96.8 ± 64.0</td>
</tr>
<tr>
<td>SU Cl⁻ (mmol/L)</td>
<td>96.7 ± 48.4</td>
<td>96.3 ± 29.2</td>
<td>96.1 ± 53.6</td>
</tr>
<tr>
<td>Age 60-69</td>
<td>33 (23.2)</td>
<td>9 (20.9)</td>
<td>24 (24.2)</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>149.0 ± 27.0</td>
<td>145.1 ± 26.8</td>
<td>150.4 ± 27.5</td>
</tr>
<tr>
<td>Estimated 24HU Na⁺ (mmol/L)</td>
<td>159.3 ± 88.0</td>
<td>121.2 ± 52.3</td>
<td>173.6 ± 95.1</td>
</tr>
<tr>
<td>Estimated 24HU Na⁺ (mmol/L)</td>
<td>72.7 ± 48.8</td>
<td>40.1 ± 27.2*</td>
<td>84.9 ± 49.8</td>
</tr>
<tr>
<td>SU Cl⁻ (mmol/L)</td>
<td>83.8 ± 41.2</td>
<td>65.2 ± 22.8</td>
<td>90.8 ± 44.7</td>
</tr>
<tr>
<td>Age 70 and above</td>
<td>37 (26.1)</td>
<td>17 (39.5)</td>
<td>20 (20.2)</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>162.5 ± 25.9</td>
<td>168.7 ± 24.9</td>
<td>162.5 ± 26.0</td>
</tr>
<tr>
<td>Estimated 24HU Na⁺ (mmol/L)</td>
<td>173.3 ± 85.9</td>
<td>167.8 ± 72.1</td>
<td>178.0 ± 97.8</td>
</tr>
<tr>
<td>Estimated 24HU Na⁺ (mmol/L)</td>
<td>71.4 ± 33.7</td>
<td>65.3 ± 28.8</td>
<td>76.5 ± 37.3</td>
</tr>
<tr>
<td>SU Cl⁻ (mmol/L)</td>
<td>87.2 ± 38.9</td>
<td>81.6 ± 31.1</td>
<td>92.0 ± 44.7</td>
</tr>
</tbody>
</table>
Figure 8. Mean systolic BP values of males and females divided by age. Values represent mean systolic BP (mmHg) ± standard error. Color coded asterisks mark significant differences between age groups for females (red) and males (black).

Analysis of mean electrolyte excretion indicates both males and females of all ages excrete normal levels of estimated 24HU Na\(^+\) and K\(^+\), but below normal urinary electrolyte levels of SU Cl\(^-\) (Figure 9, Table 5). Men between 60 – 69 years of age excrete the lowest levels of Na\(^+\) (121.2 ± 52.3 mmol/L) and SU Cl\(^-\) (65.2 ± 22.8 mmol/L) when compared to females (173.6 ± 95.1 mmol/L and 90.8 ± 44.7 mmol/L, respectively) and males of all ages (168.4 ± 69.4 mmol/L and 83.3 ± 28.3 mmol/L), however these differences are not statistically significant. Also, males 60 – 69 years old excrete the lowest levels of estimated 24HU K\(^+\) (40.1 ± 27.2 mmol/L) although still within normal ranges. There is a significant difference in estimated 24HU K\(^+\) excretion between 60 – 69 years old males when compared to matched females (p = 0.007) (Table 5). This significant difference in estimated 24HU K\(^+\) values can also be seen when comparing the
60-69 male group to all age female (p = 0.004) and all age male (p = 0.025) individuals (Table 5).

Individuals who are 50 – 59 have the highest recorded electrolyte excretion values for all three ions, Na\(^+\), K\(^+\) and Cl\(^-\) (Figure 9, Table 5). Males in this age group also have the highest estimated 24HU Na\(^+\) (203.1 ± 78.3 mmol/L) overall. Although excreted electrolytes are higher in the 50 – 59 age group for both males and females, the systolic BP readings are not (Figure 8). These results may imply that age is a larger contributing factor to high systolic BP than Na\(^+\), K\(^+\) and Cl\(^-\) levels.

**Figure 9.** Mean electrolyte values of estimated 24HU Na\(^+\), K\(^+\) and SU Cl\(^-\) for study population divided by age. Values represented as means ± standard error.
Linear Regression Analysis – Sodium

When the relationship between individual Na⁺ excretion and individual systolic BP was statistically assessed by linear regression, there was no strong correlation (r = 0.0935) between estimated 24HU Na⁺ excretion and systolic BP (Figure 10a). Interestingly with increasing age the correlation between systolic BP and Na⁺ excretion decreases when stratified by both sex and age. When categorized by each age demographic, again there is no strong correlation between observed Na⁺ excretion and systolic BP (Figures 11 and 12). Females do exhibit a slight positive correlation between estimated 24HU Na⁺ excretion and systolic BP that is not observed in males, r = 0.0983 vs. r = 0.0172 respectively (Figures 11 and 12).
Figure 10. Relationship between estimated 24HU Na⁺ excretion (mmol/L) and systolic BP (mmHg). (a) overall study population; (b) individuals ages 40-49; (c) individuals ages 50-59; (d) individuals ages 60-69; (e) individuals ages 70 and above.
Figure 11. Relationship between estimated 24HU Na⁺ excretion (mmol/L) and systolic BP (mmHg) for females. (a) overall female population; (b) individuals ages 40-49; (c) individuals ages 50-59; (d) individuals ages 60-69; (e) individuals ages 70 and above.
Figure 12. Relationship between estimated 24HU Na⁺ excretion (mmol/L) and systolic BP (mmHg) for males. (a) overall male population; (b) individuals ages 40-49; (c) individuals ages 50-59; (d) individuals ages 60-69; (e) individuals ages 70 and above.
Linear Regression Analysis – Potassium and Chloride

When individual estimated 24HU K\(^+\) and SU Cl\(^-\) excretion values were analyzed, there was no statistically significant correlation between excreted ions and systolic BP (r = –0.0678 and r = 0.0126 respectively) (Figure 13a). Interestingly the relationship between systolic BP and estimated 24HU K\(^+\) levels, is consistent with other work in that K\(^+\) has a negative correlation with systolic BP, even though the correlation is very small. Spot Cl\(^-\) excretion values demonstrate a similar trend to Na\(^+\) excretions. This was not unusual in that for most individuals Cl\(^-\) intake comes from table salt (NaCl). After analyzing estimated 24HU K\(^+\) and SU Cl\(^-\) excretion values for each sex separately (Figures 13b, 13c), it seems that males contribute negatively to the overall K\(^+\) and systolic BP correlation (males r = –0.194, females r = –0.0417) while females contribute positively to the correlation for Cl\(^-\) and systolic BP (males r = 0.0005, females r = 0.144).
Figure 13. Relationship between electrolytes excretion (mmol/L) for estimated 24HU $\text{K}^+$ and SU $\text{Cl}^-$ vs. systolic BP (mmHg). (a) overall population; (b) female population; (c) male population.
Electrolyte Excretion Comparison by BMI

When the ion excretion data was stratified based upon four BMI (underweight (n = 11), normal weight (n = 63), overweight (n = 29) and obese (n = 24)), overweight individuals of both sexes have the highest average systolic BP values: males 166.3 mmHg, females 160.6 mmHg, followed by obese individuals with systolic BP of 150.0 mmHg and 154.2 mmHg for males and females, respectively (Figure 14). The lowest average systolic BP values are seen in normal weight and underweight individuals of both sexes. There is a statistically significant difference between normal weight and overweight individuals (p = 0.013) (Figure 14), although no significant differences between systolic BP between males and females in matched BMI category or between different BMI categories within the same sex, with the exception of normal weight and overweight females (p = 0.038). While the systolic BP difference between normal weight and overweight male individuals was also high, it was not significant (p = 0.1). All four BMI groups of males and females, based on the systolic BP, fall within hypertensive I & II categories.
Figure 14. Mean systolic BP values of males and females divided by BMI. Values represent mean systolic BP (mmHg) ± standard error. Asterisk mark significant differences between BMI groups for females (red).

Individuals were evaluated divided based upon their BMI, analyzed for electrolyte excretion and compared to one another for each of the excreted ions (Figure 15, Table 6).

On average, underweight and overweight individuals excreted similar levels of Na\(^+\) and Cl\(^-\), but less than obese individuals. Individuals with higher BMI, on average were excreting higher levels of estimated 24HU Na\(^+\) and SU Cl\(^-\) with the exception of underweight individuals. Significant differences were observed between normal weight and obese individuals for estimated 24HU Na\(^+\) (149.5 ± 78.7 vs. 219.2 ± 86.4) and SU Cl\(^-\) excretions (76.4 ± 36.8 vs. 96.2 ± 44.2), p < 0.001 for both. Although not statistically significant (p = 0.058), the difference of K\(^+\) level between normal weight and obese individuals was large (76.6 ± 57.3 vs. 84.2 ± 37.7). Normal weight individuals vs.
underweight and normal weight vs. overweight individuals did not show statistically
significant differences for SU CI⁻, p = 0.051 and p = 0.090 respectively.

Figure 15. Mean electrolyte excretion levels of estimated 24HU Na⁺, K⁺ and SU CI⁻ for
different BMI categories. Values represent means ± standard error.

Stratification of the study cohort by sex and comparison of mean excreted ions
between males and females of matched BMI, indicated that the only significant
difference was between normal weight individuals for estimated 24HU K⁺ excretion (p =
0.025, Table 6). While, underweight individuals excreted very different Na⁺ values
(males 205.9 ± 77.7 vs. females 110.4 ± 84.2, p = 0.109), the difference was not
statistically significant (Table 6). This difference might be influenced by a very small
female size in the underweight category (n = 3).
Table 6. Mean systolic BP and urine electrolyte excretion means for underweight, normal weight, overweight and obese individuals. Values are expressed as number of individuals (n) or as means ± standard deviation.

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>Men</th>
<th>Women</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( \bar{x} \pm S.D. )</td>
<td>( \bar{x} \pm S.D. )</td>
<td>( \bar{x} \pm S.D. )</td>
</tr>
<tr>
<td>Underweight</td>
<td>11</td>
<td>8</td>
<td>3</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>148.0 ± 30.8</td>
<td>147.1 ± 27.3</td>
<td>150.1 ± 46.1</td>
</tr>
<tr>
<td>Estimated 24HU Na(^+) (mmol/L)</td>
<td>179.8 ± 87.4</td>
<td>205.9 ± 77.7</td>
<td>110.4 ± 84.2</td>
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<tr>
<td>Estimated 24HU K(^+) (mmol/L)</td>
<td>80.5 ± 48.2</td>
<td>84.2 ± 55.2</td>
<td>70.5 ± 27.4</td>
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<tr>
<td>SU Cl(^-) (mmol/L)</td>
<td>100.0 ± 32.5</td>
<td>98.5 ± 29.4</td>
<td>104.0 ± 47.1</td>
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<tr>
<td>Normal weight</td>
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<td>40</td>
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<tr>
<td>Systolic BP (mmHg)</td>
<td>148.5 ± 27.4</td>
<td>147.8 ± 25.4</td>
<td>148.9 ± 28.9</td>
</tr>
<tr>
<td>Estimated 24HU Na(^+) (mmol/L)</td>
<td>149.5 ± 78.7</td>
<td>145.8 ± 63.2</td>
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<td>Estimated 24HU K(^+) (mmol/L)</td>
<td>76.6 ± 57.3</td>
<td>62.9 ± 63.9</td>
<td>84.5 ± 52.4</td>
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<tr>
<td>SU Cl(^-) (mmol/L)</td>
<td>76.4 ± 36.8</td>
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<tr>
<td>Overweight</td>
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<tr>
<td>Systolic BP (mmHg)</td>
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<td>166.3 ± 14.4</td>
<td>160.6 ± 24.7</td>
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<tr>
<td>Estimated 24HU Na(^+) (mmol/L)</td>
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<td>Estimated 24HU K(^+) (mmol/L)</td>
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<td>102.4 ± 65.4</td>
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<tr>
<td>SU Cl(^-) (mmol/L)</td>
<td>96.6 ± 48.9</td>
<td>29.3 ± 13.6</td>
<td>100.2 ± 52.3</td>
</tr>
<tr>
<td>Obese</td>
<td>24</td>
<td>5</td>
<td>20</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>148.1 ± 33.8</td>
<td>158.0 ± 24.0</td>
<td>154.2 ± 38.9</td>
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<td>Estimated 24HU Na(^+) (mmol/L)</td>
<td>219.2 ± 86.4</td>
<td>191.6 ± 66.0</td>
<td>224.8 ± 89.0</td>
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<tr>
<td>Estimated 24HU K(^+) (mmol/L)</td>
<td>84.2 ± 37.7</td>
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<td>82.9 ± 38.1</td>
</tr>
<tr>
<td>SU Cl(^-) (mmol/L)</td>
<td>96.2 ± 44.2</td>
<td>96.8 ± 27.7</td>
<td>111.4 ± 46.3</td>
</tr>
</tbody>
</table>

Analysis of electrolyte excretion between the same sex individuals with different BMI determined a couple significant differences for males but not for females (Figure 16b). Underweight and normal weight males excreted significantly different levels of estimated 24HU Na\(^+\) and SU Cl\(^-\) \((p = 0.037, p = 0.046\), respectively\), while overweight and obese males had significantly different estimated 24HU K\(^+\) excretion levels \((p = \)
0.048). Underweight males excreted the highest electrolyte values when compared to normal weight, overweight and obese males.

Analysis of female 24HU Na⁺ excretion indicated a positive correlation between Na⁺ and increasing BMI (Figure 16a, Table 6). Underweight females excreted the lowest Na⁺ values (110.4 ± 84.2) when compared to normal weight (151.7 ± 87.1), overweight (196.5 ± 110.0) and obese (224.8 ± 89.0). However comparison between two BMI groups (underweight vs. overweight and underweight vs. obese) indicated no significance, p = 0.168, p = 0140 respectively. Analysis of normal weight and obese female excretion of Na⁺ was not significant, p = 0.086, likely due to small sample size of underweight females. A similar trend between mean excretion levels and BMI was also seen in estimated 24HU K⁺ with the exception of obese females that did not excrete the highest values. Normal weight and obese females had the highest difference between 24HU K⁺ excretions (p = 0.079).
Figure 16. Mean electrolyte excretion levels of estimated 24HU Na⁺, K⁺ and SU Cl⁻ for different BMI categories for (a) females and (b) males. Values represent means ± standard error.
DISCUSSION

This study analyzed urinary excretion levels of Na\(^+\), K\(^+\) and Cl\(^-\) in a hypertensive population from the Kasigau region of western Kenya. After analyzing the cohort, we conclude that the overall study population does not show a strong positive correlation between systolic BP and Na\(^+\) consumption. While salt consumption may not be the primary mechanism driving hypertension in this population, it still likely influences this multifactorial disease, since within the population there are clear trends between Na\(^+\) excretion levels and increasing age, BMI, and hypertension states.

Aging is one of the main non-modifiable risk factors that progressively lowers physiological functions of all organ systems (Buford 2016; Franceschi et al., 2008). In our study, increasing age groups had increasing systolic BP but did not show a significant correlation with Na\(^+\) and K\(^+\) excretion as expected. Age related hypertension is a result of vascular changes over time (Park et al., 2015). Increased systolic BP in the elderly is associated with arterial stiffening that is a result of elastic fiber degradation and vascular fibrosis (Park & Lakatta, 2012). Arterial stiffening happens naturally over time, however, it can be accelerated when other risk factors are in play, such as obesity, high salt intake, diabetes, activation of RAS and oxidative stress. With the aging population worldwide it is important to address modifiable risk factors in order to reduce the prevalence of hypertension.

Recent studies have shown that oxidative stress and inflammation can lead to hypertension (Park and Lakatta, 2012; Rodrigo et al., 2011; Vaziri and Rodriguez-Iturbe,
2006; Vaziri, 2008) via activation of NADPH oxidase activity and reduction of nitric oxide (NO) availability (Park and Lakatta, 2012; Rodrigo et al., 2011). NADPH oxidase is stimulated by several hormones: endothelin-1 (ET-1), urotensin and Ang-II (Rodrigo et al., 2011). Ang-II, a vasoconstrictor of the RAS, upregulates NADPH oxidase which in turn increases ROS causing oxidative stress as a result of imbalanced antioxidants and ROS (Rodrigo et al., 2011). In this way, inflammation stimulates oxidative stress, which partly stimulates inflammation through redox-sensitive effectors leading to a “vicious cycle” ultimately resulting in endothelial dysfunction (Figure 17). Investigating RAS, oxidative stress, and inflammatory markers will be an important next step in evaluating the mechanism of hypertension in our study population.

**Figure 17.** Cycle of aging, inflammation, and oxidative stress in relationship to hypertension. Adapted from Buford (2016).
Additionally, oxidative stress can be caused by environmental factors such as air pollution and particulate matter (PM). According to the WHO (2012), up to 8 million people die every year due to exposure to air pollution, the largest environmental risk factor on a global scale. About fifty percent of the deaths that occur are attributed to indoor or household air pollution (HAP) caused by heating and cooking using solid fuels like coal, wood or biomass waste (WHO, 2014a). Open fire stoves are widely used in LMICs with the majority (~90 %) used in rural households without appropriate ventilation (Gordon et al., 2014, Kajdan et al., 2015). Combustion of solid fuels releases carbon dioxide and PM, which can lead to pulmonary disease, cancer, CVD and high BP (Brook et al., 2010; Gordon et al., 2014; Majdan et al., 2015). PM air pollutants can affect an individual’s BP directly and indirectly. Indirect effects of PM include inflammation and oxidative stress responses, which cause an increase in BP (Brook et al., 2010), and indirect effects of PM include low birthweight of babies due to PM exposure to pregnant women (Gordon et al., 2014). Low birthweight is known to be inversely associated with BP (as cited in Intapad et al., 2014). Hence, individuals who participated in our study might have been predisposed to high BP upon birth which is exacerbated by being exposed to PM throughout their lifetime. Testing the exposure to carbon dioxide and PM in each participants household might provide an explanation for increased systolic BP in the population.

As mentioned in the results section, urinary electrolyte excretion values show a positive trend with BMI, with the exception for underweight individuals (Figure 15). This could be considered a confirmation that overweight and obese individuals tend to consume more salt than normal weight individuals. Larger caloric intake and food higher
in salt concentration along with an unbalanced diet overall, is a risk for hypertension. A study by Kaduta et al. (2011) demonstrated a link between education level, socioeconomic status and obesity. People living in rural areas follow diets less due to an unbiased self-image, than those living in urban areas. Less variance in available food also contributes to a simple diet, and indeed previous work from our lab has shown an inverse correlation between income and health in the villages of Kasigau (Williams, 2012).

This study also showed that there is a significant difference in BMI between women and men (25.9 kg/cm\(^2\) vs. 22.6 kg/cm\(^2\) respectively) which is similar to other reports from sub-Saharan African countries in which higher BMI in women is associated with a higher prevalence of hypertension and CVDs (BeLue et al., 2009). Obesity in women is considered to also be a cause of a high prevalence of metabolic syndrome, which is linked to lack of education, changes in hormonal secretion due to aging, and low socio-economic status (Kaduka et al., 2012; Mugo, 2016). Women living in rural areas tend to consume more carbohydrates compared to women in urban areas due to limited resources, leading to higher caloric intake and obesity among women in rural areas (Mugo, 2016; Steyn et al., 2012).

Since this study takes place in rural Kenya, it is important to also consider how social-behavioral factors may be impacting hypertension. Ethnic black women whose BMI is over 25kg/cm\(^2\) do not consider themselves overweight (Kruger et al., 2005; Malaza et al., 2012). Women who are underweight or normal weight are often looked down upon in countries, where HIV prevalence is high, because thinness can be
associated with the disease (Kruger et al., 2005; Malaza et al., 2012). Malaza et al. (2012) demonstrated that obesity is significantly higher in HIV-uninfected individuals when compared to HIV-infected. Additionally, HIV-uninfected women have significantly more hypertension when compared to HIV-infected women. In many sub-Saharan countries overweight and obese women are considered to be attractive, successful and are treated with respect, a perspective that is encouraging both obesity and hypertension.

Urine analysis of our population revealed a very high overall Na+/K+ ratio (1.57 ± 1.12). According to WHO guidelines, the recommended adult Na+ and K+ intake per day is 2.0 g and 3.5 g respectively (Na+/K+ ratio of ~0.55) (WHO, 2012a; WHO, 2012b). However, the current global Na+ consumption is estimated to be more than double the recommended value, thus the reduction of salt intake and increase in K+, would greatly reduce the risk of CVD (WHO, 2012a; WHO, 2012b). While our analysis of electrolyte excretion and systolic BP did not show a strong correlation between dietary Na+ intake, our study cohort does have a high Na+ intake overall (3.92 ± 2.1 g/day). While greater than WHO recommended guidelines, global analysis of Na+/K+ ratios from ~100,000 individuals from 18 countries revealed that only ~10% of individuals consume less than 3.0 g of Na+ per day (Mente et al., 2014; WHO, 2012b). Among people who consumed both >5 g of Na+ and <1.9 g of K+ per day, systolic BP can increase as high as 12 mmHg for each 1g increment of Na+ excretion (Mente et al., 2014). A lower consumption of Na+ (3 – 5 g/day and < 3 g/day) was correlated with lower systolic BP increment (1.74 mmHg per gram and 0.74 mmHg per gram, respectively). A short term study by The Dietary Approaches to Stop Hypertension (DASH) showed that reducing Na+ intake from
3.5 to 2.5 g per day can reduce systolic BP by 2.1 mmHg (Sacks et al., 2001). Analysis of ~100,000 people from 17 countries showed an association between high K⁺ excretion and reduced risk of CVD and mortality (O’Donnell et al., 2014). Both of the before mentioned studies support the fact that electrolyte consumption can influence BP. It should be noted that even though this Kenyan cohort appears to excrete Na⁺ and K⁺ values within normal ranges, we did not measure creatinine levels in the urinary samples. Normalizing our data to creatinine could provide a more accurate estimation of 24 h salt intake and possibly alter the given conclusions.

Based on the data provided we conclude that people living in Kasigau do not consume large quantities of dietary Na⁺. However, determining whether or not the population is salt-sensitive cannot be concluded at this point. Nevertheless, education on salt consumption and low Na⁺ diet trials could be conducted in order to confirm that salt consumption is a modifiable risk factor. Additionally expanding this study to look at the role of HAP and oxidative stress-linked hypertension is a logical next step. Having another cohort in Kenya, from an urban area, would also be beneficial in comparing the trends and risk factors identified in this study. Finally, in our study population ~57% of individuals have high prevalence of hypertension, however a study by Hendriks et al. (2012) indicates that a majority (83%) of people in Kenya are unaware of their disease. Thus, continuity of the PiC:MiK program is essential for increasing awareness of hypertension as well as other NCDs in the community. The relationship with local physicians and community members who help the program and research will help the local people take ownership of their health and wellness.
REFERENCES


APPENDIX A: HSRB Approval

INSTITUTIONAL REVIEW BOARD
OFFICE OF RESEARCH INTEGRITY

DATE: March 26, 2014

TO: Nancy Rice, PhD
FROM: Western Kentucky University (WKU) IRB

PROJECT TITLE: [S99990-1] The Molecular Epidemiology of Essential Hypertension in Kenya
REFERENCE #: IRB 14-387
SUBMISSION TYPE: New Project

ACTION: APPROVED
APPROVAL DATE: March 26, 2014
EXPIRATION DATE: March 26, 2015
REVIEW TYPE: Expedited Review

Thank you for your submission of New Project materials for this project. The Western Kentucky University (WKU) IRB has APPROVED your submission. This approval is based on an appropriate risk/benefit ratio and a project design wherein the risks have been minimized. All research must be conducted in accordance with this approved submission.

This submission has received Expedited Review based on the applicable federal regulation.

Please remember that informed consent is a process beginning with a description of the project and insurance of participant understanding followed by an implied consent form. Informed consent must continue throughout the project via a dialogue between the researcher and research participant. Federal regulations require each participant receive a copy of the consent document.

Please note that any revision to previously approved materials must be approved by this office prior to initiation. Please use the appropriate revision forms for this procedure.

All UNANTICIPATED PROBLEMS involving risks to subjects or others and SERIOUS and UNEXPECTED adverse events must be reported promptly to this office. Please use the appropriate reporting forms for this procedure. All FDA and sponsor reporting requirements should also be followed.

All NON-COMPLIANCE issues or COMPLAINTS regarding this project must be reported promptly to this office.

This project has been determined to be a Minimal Risk project. Based on the risks, this project requires continuing review by this committee on an annual basis. Please use the appropriate forms for this procedure. Your documentation for continuing review must be received with sufficient time for review and continued approval before the expiration date of March 26, 2015.

Please note that all research records must be retained for a minimum of three years after the completion of the project.

If you have any questions, please contact Paul Mooney at (270) 745-2129 or irb@wkul.edu. Please include your project title and reference number in all correspondence with this committee.
APPENDIX A: HSRB Approval (continued)

WESTERN KENTUCKY UNIVERSITY

Institutional Review Board
Continuing Review Report

If this is your third year for your Continuing Review Requests, please complete a new application.

Name of Project: The Molecular Epidemiology of Hypertension in Kenya
Name of Researcher: Nancy A. Rice, Ph.D.
Department: Biology

How many total subjects have participated in the study since its inception? #259

How many subjects have participated in the project since the last review? #76

Is your data collection with human subjects complete? ☐ Yes X No

1. Has there been any change in the level of risks to human subjects? ☐ Yes ☐ No (If “Yes”, please explain changes on a separate sheet).

2. Have informed consent procedures changed so as to put subjects above minimal risk? ☐ Yes ☐ No (If “Yes”, please describe on a separate sheet).

3. Have any subjects withdrawn from the research due to adverse events or any unanticipated risks/problems? ☐ Yes ☐ No (If “Yes”, please describe on a separate sheet).

4. Have there been any changes to the source(s) of subjects and the Selection criteria? ☐ Yes ☐ No (If “Yes”, please describe on a separate sheet).

5. Have there been any changes to your research design that were not specified in your application, including the frequency, duration and location of each procedure? ☐ Yes ☐ No (If “Yes”, please describe on a separate sheet).

6. Has there been any change to the way in which confidentiality of the Data is maintained? ☐ Yes ☐ No (If “Yes”, please describe on a separate sheet).

7. Is there desire to extend the time line of the project? ☐ Yes ☐ No

On what date do you anticipate data collection with human subjects to be completed? July 2017

INSTITUTIONAL REVIEW BOARD
APPROVED

WKU IRE# 14-387
Approval - 5/5/2016
End Date - 3/26/2017
Expedited
Original - 3/26/2014
APPENDIX B: University of Nairobi Ethics Review Board Approval

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KENYATTA NATIONAL HOSPITAL
P O BOX 20723 Code 00202
Tel: 725376
Fax: 725372
Telegram: MEDSUP, Nairobi

Ref: KNH-ERC/IA/85

Prof. Nancy A. Rice
Principal Investigator
Department of Biology
Western Kentucky University
Bowling Green, KY 42101-1080 USA
Email: Nancy.rice@WKU.edu

Dear Prof. Rice

REVISED RESEARCH PROPOSAL: “INVESTIGATING THE MOLECULAR EPIDEMIOLOGY OF HYPERTENSION IN EAST AFRICA” (P509/07/2016)

This is to inform you that the KNH- UoN Ethics & Research Committee (KNH- UoN ERC) has reviewed and approved your above revised proposal. The approval period is from 23rd February 2017 – 22nd February 2018.

This approval is subject to compliance with the following requirements:

a) Only approved documents (informed consents, study instruments, advertising materials etc) will be used.
b) All changes (amendments, deviations, violations etc) are submitted for review and approval by KNH-UoN ERC before implementation.
c) Death and life threatening problems and serious adverse events (SAEs) or unexpected adverse events whether related or unrelated to the study must be reported to the KNH-UoN ERC within 72 hours of notification.
d) Any changes, anticipated or otherwise that may increase the risks or affect safety or welfare of study participants and others or affect the integrity of the research must be reported to KNH- UoN ERC within 72 hours.
e) Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period. (Attach a comprehensive progress report to support the renewal).
f) Clearance for export of biological specimens must be obtained from KNH- UoN ERC for each batch of shipment.
g) Submission of an executive summary report within 90 days upon completion of the study. This information will form part of the data base that will be consulted in future when processing related research studies so as to minimize chances of study duplication and/ or plagiarism.

For more details consult the KNH- UoN ERC website http://www.erc.uonbi.ac.ke

Protect to discover
APPENDIX B: University of Nairobi Ethics Review Board Approval (continued)

Yours sincerely,

PROF M. L. CHINDIA
SECRETARY, KNH-UoN ERC

c.c. The Principal, College of Health Sciences, UoN
The Director, CS, KNH
The Assistant Director, Health Information, KNH
The Chair, KNH-UoN ERC
Co-investigators: Dr. Hellen Nyambura Karuki, Dr. Fred Bukachi
APPENDIX C: Urine Collection Instructions (English)

How to Provide a Clean Catch Urine Sample

Instructions

1. **Wash** hands with soap and water, rinse, and dry or use a provided towelette.

2. **Clean.** Wipe the genitalia area with the provided antiseptic towelette.

3. **Void.** Pass the first portion of urine into the toilet, then pass a portion of remaining urine into the specimen cup. The cup should be help to avoid contact with any skin and clothing. Keep the fingers away from the rim and inner surface of the container.

4. **Transfer.** When voiding is completed, use provided pipette to transfer the continent into the designated Falcon tube, close the tube, and put the tube in the provided bag.
JINSI YA KUPEANA SAMPULI YA MKOJO SAFI KUFANYIA

UTAFITI

Maelezo

1. KUNAWA
   Tafadhali nawa mikono yako vizuri kwa kutumia sabuni na maji uliyopewa. Safisha vizuri alafu kausha mikono kwa kutumia kitambaa maalum ulichopewa.

2. KUSAFISHA.
   Tafadhali safisha kwa makini sehemu yako ya siri ya kutoa mikojo kwa kutumia kifutio maalumu ulichopewa.

3. KUKOJOA
   Kojoa mikojo ya kwanza ndani ya choo alafu weka kiwango kidogo cha mikojo ya mwisho ndani ya chupa uliyopewa. Tafadhali uhakikishe kuwa chupa hiyo haitashikana na ngozi ya mwili wako au nguo ulizovaa. Chunga usiguse ncha ya chupa au ndani ya chupa kwa kutumia vidole vyako.

4. UHAMISHO
   Tafadhali tumia mrinja wa kuvutia mkojo uliopewa, kuvuta mkojo kutoka kwa chupa iliyoebaba sampuli na kuweka mkojo huo ndani ya chupa ya pili uliopewa. Eka chupa hiyo ya pili iliyo na sampuli ya mkojo ndani ya mfuko uliopewa.
<table>
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<th>Abbreviation</th>
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<tr>
<td>24HU</td>
<td>24 hour urine</td>
</tr>
<tr>
<td>ACE</td>
<td>Angiotensin converting enzyme</td>
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