Hypotensive Effects of Potassium and Magnesium

by

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ABSTRACT

Despite recent strides for awareness, treatment, and control of hypertension, prevalence remains high with estimates suggesting one third of Americans have hypertension. The hypotensive effects of potassium and magnesium have been known and administered in a clinical setting for nearly a century. The purpose of this study was to examine the effectiveness of taking a potassium/magnesium supplement to help reduce blood pressure in individuals with mildly-moderately elevated blood pressure. In this randomized, controlled crossover trial, potassium and magnesium supplementation was explored among healthy adults with mildly elevated blood pressure in Phoenix, Arizona. Subjects (n = 12) were randomly assigned to ingest either the treatment chewy bar (217 mg potassium/day; 70.8 mg magnesium/day) or a placebo chewy bar for four weeks. For the subsequent four weeks, subjects ingested the other corresponding chewy bar. Systolic (SBP), diastolic (DBP), and average blood pressure values were not significantly different between the two groups ($p = 0.645$, $p = 0.464$ and $p = 0.939$, respectively). Baseline mean blood pressure was 121.0/75.7 mm Hg. The 12 subjects (8 females, 4 males) had a mean age of 29.3 years old and a mean BMI of 26.2. After four weeks, the treatment group had a slightly higher SBP (118.3 ± 13.3 mm Hg) than the control group (116.5 ± 17.8 mm Hg); however, DBP was lower in the treatment group (71.7 ± 12.4 mm Hg) than the control group (73.0 ± 10.0 mm Hg). In conclusion, daily supplementation of potassium and magnesium (217.2 mg/day and 70.8 mg/day, respectively) did not significantly lower blood pressure in adults with mildly-moderately elevated blood pressure.

DEDICATION

First, I dedicate this thesis to my mother. Thank you for encouraging me to always do what I love doing, and to not settle for otherwise. I sure have found that passion of mine. Thank you for the constant love and support through my most challenging days. You have helped me more than you will ever know, and I would not be where I am today if it were not for your love and help. I love you and appreciate all that you have done, sacrificed, and continue to do for me.

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CHAPTER 1

INTRODUCTION

Problem

Considered one of the highest and most preventable causes of death, hypertension affects one out of three Americans and was responsible for approximately one out of every five deaths among U.S. adults in 2005 (1-3). Such a burden on the American health care system is evident from an estimate of lifetime risk among middle-aged and the elderly, for developing hypertension, which is a staggering 90% (4). This enormous demand on our health care system urges for more effective and/or concomitant methods of treatment for high blood pressure. Despite the fact that the past two decades have shown progress in the United States for awareness, treatment and control of hypertension, coincidentally the overall prevalence of hypertension has also increased (2). This alarming trend highlights not only a national success in campaigns to heighten awareness, treatment and control; but iteratively and more importantly, a growing urgency for alternative or concomitant approaches to combat overall prevalence of hypertension and pre-hypertension.

Considering the enormity of the burden on the health care system, focusing more on preventive measures to decrease prevalence of pre-hypertension and hypertension has tremendous potential to improve public health outcomes. Even modest reductions (i.e. 1,200 mg/day decrease) in population-wide sodium intake have been estimated to annually reduce approximately 32,000-66,000 strokes, 54,000-99,000 heart attacks, and 44,000-92,000 overall deaths (5). Additionally, this population-wide reduction would decrease annual health care costs an estimated $10-$24 billion (5).

Beyond the mainstay of pharmaceuticals for treatment, there is an abundance of evidence identifying the relationship between many dietary factors and effects on blood pressure (6-8). The most common non-pharmaceutical remedy for hypertension is the Dietary Approaches to Stop Hypertension dietary pattern ("DASH diet"). Evidence supporting the efficacy of the DASH diet is strong enough that this dietary pattern is a recommendation in national guidelines (5,7-11). Compared to a standard American diet, one of the notable characteristics of the DASH diet is that the recommended food components generally have lower values of sodium and higher values of potassium and magnesium. More specifically, The DASH Diet emphasizes intake of fruits, vegetables, and low-fat dairy products; includes poultry, fish, nuts, seeds, and whole grains; and limits the amount of red meats, sweets, and sugar-sweetened beverages. Basing one's dietary pattern on these foods, the DASH Diet offers rich sources of potassium, magnesium, calcium, and dietary fiber; while limiting the intakes of total fat, saturated fats, and sodium (8). Contrasting from The DASH Diet, over 60% of people in the United States are below the Recommended Dietary Allowance (RDA) for magnesium and less than 10% of the citizenry consume at least the Adequate Intake (AI) for potassium, according to NHANES (National Health and Nutrition Examination Survey) data collected between 2001-2008 (12).

There is a large range of dosage values of potassium and magnesium used for a variety of heterogeneously designed studies. The study that was used to base the dosage values of the present study on, conducted by Wu et al, found a reduction of 7.83 mm Hg systolic blood pressure ($p < 0.01$) and 3.67 mm Hg diastolic blood pressure ($p < 0.05$) on four weeks of potassium-magnesium supplementation (13). Compared to other

supplementation studies demonstrating blood pressure lowering effects of potassium and magnesium, these were the lowest dosage values used. For instance, other controlled trials demonstrating significant blood pressure reduction from potassium supplementation ranged from 1,170 mg/day and even exceeding ten times the amount used in the present study, with dosage values of 3,000 and even 4,680 mg/day, with no reported adverse health outcomes (14-16). Efficacious dosages of magnesium used in other controlled trials range from 368 mg/day to 600 mg/day (18, 19). Despite Wu and colleagues demonstrating efficacy with these relatively low dosage values, these results were measured in a hypertensive population.

Although the antihypertensive effects of potassium and magnesium have been documented and used in clinical settings for nearly a century (19, 20), inconsistent clinical trial findings and the high degree of correlation between multiple dietary factors has unfortunately yielded insurmountable evidence for adopting recommendations in non-clinical settings, such as for pre-hypertensive individuals. However, it is possible that a functional food (i.e. potassium and magnesium supplement) can help lower blood pressure for individuals with mild hypertension and ultimately can reduce dependence on pharmaceuticals.

Proposed Research

Currently, the primary choice of treatment for hypertension in the U.S. is pharmacotherapy (7). With medications as the most prominent treatment option, interventions aimed at modifying lifestyle and dietary factors are often ignored. The objective of this randomized, placebo-controlled crossover trial was to measure the effects of daily potassium and magnesium supplementation (217.2 mg/day and 70.8

mg/day, respectively) in adults with mildly-moderately elevated blood pressure over an 8-week period. Additionally, this study will contribute to the pool of evidence that suggests that potassium and magnesium supplementation can potentially reduce or in some instances replace pharmacotherapy as a sole method of treating hypertension.

Rather than focus on greater awareness and compliance to a didactic but proven effective dietary model (i.e. DASH diet) (21) for hypertension, this study aimed to explore the effectiveness and feasibility of taking a potassium/magnesium supplement (i.e. functional food) to help attenuate pre-/hypertension. It was hypothesized that daily supplementation of potassium and magnesium (217.2 mg/day and 70.8 mg/day, respectively) over a 4-week period will lower blood pressure in comparison to a placebo in adults with mildly elevated blood pressure in Phoenix, Arizona.

Definition of Terms

Antihypertensive: causes a decrease in blood pressure

Cardiovascular Disease: one of many diseases of the heart and/or blood vessels

Diastolic/diastole: referring to the time between heartbeats, in a period of relaxation

Hypertension: elevated blood pressure, greater than or equal to 140 mm HG systolic blood pressure and 90 mm HG diastolic ($\geq$ 140/90 mm Hg)

Hypotensive: the ability to lower blood pressure

Kaliuretic: promotes increased urinary excretion of potassium

Natriuretic/Natriuresis: promotes increased urinary excretion of sodium

Nephron: microscopic functional unit of the kidney

Renal: of or relating to the kidney

Systolic/systole: blood pressure while the heart is contracting

Delimitations and Limitations

Limitations of this study include the number of subjects recruited; only 12 subjects were recruited because the study was unfunded. Although participants were instructed to complete compliance calendars, compliance with protocol is another limitation of the study. Recording accurate dietary intake data was another limitation, which was related to limited reliability and validity using The Food Processor dietary analysis database. Additionally, other potential stressors not accounted for in participants' lives are potential limitations.

Participation in this study was delimited to adults (>18 and <80 years old) who reported no past heart disease, renal insufficiency, liver disease (or other organ pathology), pregnancy/lactation, or medication use for hypertension. Additionally, participation was delimited to those who had a recorded systolic blood pressure (SBP) reading (mean of two readings) of >115 mm Hg and <139 mm Hg; and a diastolic blood pressure (DBP) of >70 and <90 mm Hg. Therefore, results of this study will not be generalizable to other individuals or populations.

CHAPTER 2

LITERATURE REVIEW

Blood Pressure Overview

Current State of Hypertension

Although health officials in the United States have made recent strides for awareness, treatment, and control of hypertension, the ongoing rise in prevalence of hypertension (2) suggests that the conventional education and common remedies for hypertension may not be sufficient. The attention placed on hypertension along with more opportunities to be screened for high blood pressure have increased diagnosis of the condition, however nearly 1/3 of those who are hypertensive are still unaware of their condition and over 1/3 of hypertensive patients are not taking treatment (2). Regardless, the need to effectively reduce the overall prevalence and the costly outcomes associated with hypertension is paramount.

Hypertension is strongly linked with risk of cardiovascular disease (CVD) [22] and stroke as well as other chronic conditions including cerebrovascular disease, ischemic heart disease, and renal failure (23). In 2005, research suggests that approximately 400,000 of the 2.5 million deaths (~16% of total deaths) in the United States were associated with high blood pressure (1). More recently, between 2007 and 2010 the American Heart Association reports estimates that 33% of adults ≥ 20 years old had hypertension (24). Numerous physiological and genetic factors influence blood pressure; yet, dietary factors may have the most substantial effect on maintaining homeostasis of blood pressure. Many nutrients and herbal preparations have been shown to influence blood pressure; however, three essential minerals, potassium,

magnesium, and sodium, are particularly important in regulating and maintaining healthy blood pressure (18-20). This review will (1) briefly outline the biological mechanisms involved in blood pressure homeostasis; (2) examine the physiological and genetic factors that influence risk for hypertension; and (3) discuss dietary factors that can reduce blood pressure, focusing primarily on the roles of sodium, potassium, and magnesium in blood pressure control.

Biological Factors

Kidney's role in blood pressure

Regarding the etiology of hypertension, an important biological system to consider is the urinary system and the vital role that the kidneys serve to regulate intake and outputs of the said minerals being investigated. Generally speaking, excessive sodium intake equates to the kidneys working excessively to rid the body of what is not needed. The kidneys' "salt handling" ability is believed to be a primary factor for overall cardiovascular health (25). For instance, kidney transplant recipients who were hypertensive and previously diagnosed with terminal nephrosclerosis (i.e. progressive hardening of the kidney blood vessels) consistently experienced decreased blood pressure, reaching normal ranges after receiving a kidney from normotensive donors (26).

From 1900 to modern day, all antihypertensive treatment methods either directly or indirectly affect renal sodium handling (27). Treatment methods including pharmacotherapy and even the earlier practice of sympathectomy all affect sodium handling of the kidneys either directly by inhibiting renal transport proteins, or indirectly by regulation of endocrine/neuroendocrine pathways (27). One major

contributor to renal physiology and blood pressure research, Dr. Arthur Guyton, suggests that long-term changes in blood pressure occur primarily from the kidney's diminishing ability to excrete sodium. Decreased ability of the kidneys to excrete sodium leads to greater retention of sodium and subsequently increases in extracellular fluid volume, resulting in elevated blood pressure (28).

Prolonged elevated blood pressure induces progressive and subtle renal micro-vascular injury. One large-scale observational cohort study, including 4,365 older adults, investigated the individual associations of each blood pressure component (i.e. systolic, diastolic, and pulse pressure) with declining renal function (29). Glomerular filtration rate (GFR) was used as an indicator of renal function; measured at baseline, year 3, and at year 7. Increased diastolic blood pressure was not significantly associated with decreasing renal function; however, both systolic blood pressure and pulse pressure were significantly associated with renal function. For every10-mm Hg increase of systolic blood pressure and pulse pressure, there was a faster decline in GFR by 0.13 ml/min/year and 0.15 ml/min/year, respectively (29).

The intricate renal endocrine mechanisms implicated in salt handling and hypertension entail interactions among a variety of hormones. Some of these hormones are renin, angiotensin II, aldosterone, atrial natriuretic peptide (ANP), and antidiuretic hormone (ADH). Primarily, it is the effects on peripheral vascular resistance by which these hormones induce changes in blood volume and diameter of blood vessels, ultimately affecting mean arterial blood pressure. Dysfunction of these hormones has been proposed as a result of genetic predisposition and addressing such hormonal imbalances has been the primary target of pharmacotherapy for hypertension (30).

Fluctuations of blood pressure hormones often occur in response to changes in blood volume. Select blood vessels have volume receptors that detect fluctuations in blood volume and consequently either contract or stretch, which equates to changes in sympathetic tone of the blood vessels (31). The kidney's role in regulating blood pressure is primarily the result of the three hormones renin, angiotensin, and aldosterone; commonly referred to as the renin-angiotensin-aldosterone system [RAS] (32).

The ability of the kidneys to regulate blood pressure is illustrated nicely with the efficaciousness of common blood pressure drugs such as angiotensin-converting enzyme (ACE) inhibitors and angiotensin receptor blockers (32), which all act by regulating renal hormones. In response to decreases in blood pressure, the sympathetic nervous system reacts by secreting renin. The enzyme renin works on a plasma protein called angiotensinogen by cleaving off a portion called angiotensin I. ACE works by converting angiotensin I to angiotensin II, which is a hormone that has a variety of effects on kidney function and blood pressure (31). Angiotensin II causes vasoconstriction resulting in increased blood pressure. Additionally, angiotensin II stimulates: sodium and water reabsorption, sense of thirst, and secretion of both aldosterone and antidiuretic hormone that promotes water retention (31). ACE inhibitors work by decreasing the amount of angiotensin II produced. The dysregulation of these RAS hormones have a direct and often unnoticed impact on blood pressure.

Aldosterone is secreted in response to increased renin in the blood vessels and is one of the primary hormones that mediate hormonal responses of the kidneys to high potassium intakes (33). High dietary intake of potassium increases plasma potassium

levels, and has been shown to stimulate aldosterone secretion. Despite this indirect kaliuretic effect of increased aldosterone, resulting from high dietary potassium intakes, this is only pronounced when aldosterone is at supraphysiological levels (34).

Antidiuretic Hormone (ADH) secretion by the pituitary gland is stimulated by dehydration and elevated blood osmolarity. This hormone works by increasing the permeability of the collecting ducts of the nephrons and therefore increasing the amount of water that the kidneys replace back in the fluid being filtered and back to blood circulation. At supraphysiological levels, ADH causes vasoconstriction and is thus sometimes referred to as "vasopressin". However, ADH primarily functions as a safety mechanism to help prevent dehydration and does not normally fluctuate enough to be relevant for dietary factors of blood pressure control (31).

On the contrast, secretion of atrial natriuretic peptide (ANP) from the heart is stimulated in response to elevated blood pressure and results in increased excretion of salt and water. This natriuretic effect of ANP reduces blood volume and blood pressure by several mechanisms. ANP decreases blood pressure by dilating the afferent arteriole and constricting the efferent arteriole, which results in increased glomerular filtration rate; inhibiting secretion of antidiuretic hormone (ADH), renin and aldosterone; and by inhibiting sodium reabsorption by the collecting duct (31).

Arterial Stiffness and the Augmentation Process

Research demonstrates that arterial stiffness and hypertension are positively associated with age (35, 36). With aging, the composition of structural proteins (i.e. collagen and elastin) changes and has a direct effect on stiffness of arteries. Particularly, these changes occur in major "conduit arteries", including the aorta, iliac,

carotid, femoral, and brachial arteries (35). The primary function of these conduit arteries is to serve as a compliant and low resistant pathway from the heart to supply blood to all of the living tissues. Healthy, pliant conduit arteries are necessary considering that the distension of the aortic artery upon the contraction of the heart, acts in a rhythmic manner called "pressure pulse wave" (35). The elastic fibers that comprise arteries serve to help deliver blood to distal tissues − in a 'ripple-like' manner− without requiring excessively elevated systolic blood pressure. Therefore, the stiffer the conduit arteries, the greater the systolic pressure needed in order to produce adequate blood flow (35).

The structural changes in conduit arteries are primarily related to the changing proportional composition of elastin and collagen fibers. Compared to more distal arteries and arterioles, the more proximal the arteries to the heart, the greater the proportion of elastin to collagen fibers. With ageing, elastin fibers are fragmented and degraded, and ultimately not sufficiently regenerated. This structural degradation is superseded by increased proportion of considerably less-compliant collagen fibers (36).

A recently published randomized, placebo-controlled crossover study investigated the effects of sodium and potassium supplementation, independently, on blood pressure and arterial stiffness (15). This study included 36 untreated pre-hypertensive (69% subjects had baseline SBP > 140) adults, and subjects followed a controlled diet with relatively low sodium and potassium for a 1-week run-in period. Subjects were then randomly assigned to a 4-week interval supplementation of 3,000 mg sodium, 3,000 mg potassium, or a placebo, and then complete the corresponding other two 4-week intervals subsequently. At baseline and at each 4-week visit,

measures of office blood pressure, 24-hour ambulatory blood pressure and indicators (i.e. pulse wave velocity and augmentation index) of arterial stiffness were assessed. The active intervention of sodium resulted in a significant increase in office blood pressure by 7.5/3.3 mm Hg and 24-hour ambulatory blood pressure by 7.5/2.7 mm Hg. Potassium supplementation resulted in a significant decrease in 24-hour ambulatory blood pressure by 3.9/1.6 mm Hg. However, sodium or potassium supplementation had no significant effect on pulse wave velocity or augmentation index (15).

Genetic factors

Salt handling is largely dependent on genetic predisposition. Genetic research has identified numerous genetic mutations in severely hypertensive families, which are responsible for encoding proteins involved in regulating ion channels and transporters in the nephrons of the kidneys. These genetic mutations affect cellular transport of minerals, increase renal reabsorption and blood volume, and ultimately lead to an increase in blood pressure (26). Although these genetic mutations ("single nucleotide polymorphisms") do not imply a causative factor for hypertension, they must be considered because of the impact on individual predisposition to hypertension.

Regarding the important role that the renin-angiotensin-aldosterone system (RAS) (discussed earlier) has on regulating blood pressure, variants of the genes that encode the elements of this system have been hypothesized as key factors for the genetic predisposition of hypertension. Ji and colleagues conducted a case-control study that examined the association between genetic variants (i.e. polymorphisms) that encode RAAS genes and hypertension (37). This study compared 905 hypertensive cases to 905 normotensive controls among the Han Chinese population. It was reported

there was significantly higher Body Mass Index (BMI), serum triglycerides and total cholesterol in the hypertensive group, compared to the 905 controls. Further, this study identified multiple polymorphisms in the RAAS genes that may indicate genetic susceptibility to hypertension. From the 41 single nucleotide polymorphism (SNPs) that were genotyped, six were demonstrated to associate with hypertension. More importantly, the hypertensive cases had significantly higher odds ratio of having these six identified SNPs. Given that this study was conducted with a large and relatively homogenous sample of the Han Chinese, the findings of this study are unique because they limit possible influence of environmental factors and confounders for hypertension. However, the unique design of this study also suggests these findings may be limited to those of the Han Chinese population and not generalizable to the citizens of the United States. The results of Ji and colleagues study suggest that genetic polymorphisms do have an impact on blood pressure regulation and individual susceptibility for hypertension.

Olives and colleagues conducted a study using data from National Health and Nutrition Examination Survey (NHANES), a nationally representative cross-sectional survey (2). NHANES collects data on markers of self-reported health every two years, Olives et al used data from 1999-2008. Compared to white, Hispanic and other races, blacks tend to have the greatest prevalence of hypertension in the United States. However, according to NHANES data, black Americans also have the greatest awareness and treatment of hypertension compared to other races (2). One study investigated the effects of variant potassium intake levels on blood pressure and sodium retention in healthy adults, between 21-35 years old, with normal renal function (38). It

was reported that black subjects had significantly less excretion of both potassium and sodium after a potassium-supplemented diet, as compared to white subjects. Additionally, black subjects had significantly less excretion of sodium after following a potassium-restricted diet as compared to white subjects. However, this study is limited by the small size of the sample; there were only 21 subjects (11 black, 10 white).

High sodium intakes in blacks are associated with greater increases in blood pressure as compared to whites. This effect is partially explained by the finding that blacks have fundamental differences in the kidney's ability to maintain sodium homeostasis (39). Luft and colleagues investigated sodium sensitivity in normotensive subjects who were deemed at higher risk for developing hypertension (39). This study focused on black subjects, primarily those at least 40 years of age and who were related (first-degree) to hypertensive patients. Furthermore, this study documents lower plasma levels of renin and aldosterone in blacks compared to whites ($p < 0.05$). This is an interesting finding considering that renin and aldosterone both elevate blood pressure.

Dietary Factors

Current Antihypertensive Paradigm- The DASH Diet

Many studies identify dietary factors that are important for managing blood pressure. Factors to consider include: sufficient intakes of fruits, vegetables, and low-fat dairy; inclusion of fish, poultry, whole grains, and nuts; and only modest intakes of sugar-sweetened beverages, meats, total fats, and saturated fats (8, 9, 40). These dietary factors are encompassed within the tenets of the DASH (Dietary Approaches to Stop Hypertension) Diet, which is an internationally accepted remedy for attenuating hypertension and has been proven effective in studies for nearly two decades (23).

The DASH Diet is incorporated within guidelines for managing blood pressure from the U.S. Government, the International Society of Hypertension (ISH), and the World Health Organization (WHO) (23). Although the DASH Diet offers a definitive and effective tool for managing hypertension, the public is often reluctant to initiate or maintain significant dietary changes, even for the sake of bettering individuals' health. One study suggested that the degree of adherence to the DASH Diet was relative to the effect that it had on reducing blood pressure (41). This study reaffirmed the notion that the DASH diet is particularly effective when compliance to dietary guidelines is high, and only moderately to non-effective when adherence is minimal. The finding that a common lack of adherence to an effective therapeutic model seems to imply that a simpler and less didactic dietary approach would facilitate greater success of managing blood pressure for such individuals who do not comply well with multiple instructions and guidelines.

Distinguishing characteristics of the DASH Diet are the favorable proportion to fruits, vegetables, legumes, and low-fat dairy products; moderate levels of saturated fats and minimal amounts of red meats and sweets/added sugars; as well as inclusion of whole grains, poultry, fish, and nuts (7). The DASH Diet also limits saturated fats, cholesterol, and salt (9). Comparing the DASH diet to the "Standard American Diet" (SAD Diet) (42), a notably contrasting characteristic is the proportion of dietary minerals consumed. For instance, the SAD Diet includes highly refined and processed foods. Such processed food and food products tend to be high in sodium and low in potassium and magnesium. On the contrast, the DASH dietary pattern includes foods that are rich in potassium, magnesium and calcium. Dietary Guidelines for Americans

in 2015 recommend limiting sodium intake to less than 2,300 mg/day. This Tolerable Upper Limit (UL) of 2,300 mg/day, established for sodium was based on the finding that excessive sodium intake promotes high blood pressure and may promote further decline in individuals with kidney disease (43). However, average intakes of sodium exceed this recommendation by approximately 50% with estimates of average intakes exceeding 3,330 mg/day of sodium (44). This discrepancy of mineral intakes between the two diets is concerning and it was expressed with the Institute of Medicine's release of a report in 2010 titled *Strategies to Reduce Sodium Intake in the United States.*

Appel and colleagues (8) succinctly conclude a major problem that one primary challenge we currently face is to develop and implement more effective strategies for public health and clinical practice that promote sustainable dietary changes to prevent and treat hypertension. This is an enormous task, requiring cooperative development and implementation among government officials, healthcare providers, researchers, and the general public (8). Ultimately, convoluted problems do not yield quick solutions; alternative solutions must be considered for greater control and prevention of hypertension.

Effects of Minerals on Blood Pressure

Interrelationship of Sodium and Potassium

The biological interrelationship of sodium and potassium and their effects on blood pressure was first investigated in the 1920's. Specifically, the natriuretic effect of potassium salts (e.g. potassium citrate, potassium bromide and potassium chloride) and the hypertensive effects of sodium were identified (19). Furthermore, this early 20th century study discussed the likelihood of potassium-poor and sodium-excess diets

being the primary cause in rising prevalence of hypertension in North America. Considering that sodium is the most abundant extracellular ion and that potassium is the primary intracellular ion, these two essential minerals should first be explored simultaneously to help elucidate antihypertensive mechanisms.

Evidence and estimates of ancestral salt intake, suggest that in ancient civilizations *and* even in non-industrialized regions of the globe today, individuals subsist on low sodium intakes such as <1g/day (compared to intakes exceeding 3.3 grams/day in the United States). Even more relevant is the fact that many of these non-industrialized and isolated populations also generally lack hypertension (25). Ancestral and even modern non-industrialized populations rely on non-processed foods that are generally lower in sodium and rich in potassium among other essential minerals such as magnesium. In contrast, sodium-rich diets − that are primarily based on processed foods are deficient in potassium and magnesium − are common in industrialized nations like the United States. This stark contrast of mineral intake in industrialized nations occurred primarily over the past century as the result of potassium losses from processing foods and artificial salting for use as a preservative and to increase palatability of foods. (44).

Cook and colleagues (45) measured the combined effects of potassium and sodium intake on later development of cardiovascular disease (CVD). This 10-15 year trial included information from 2,275 participants and included a total of 193 CVD events. Although there were no significant trends observed in CVD risk between quartiles of sodium and potassium excretion individually, there was a significant trend between quartiles of the sodium-potassium ratios (RR= 1.00, 0.84, 1.18, and 1.50, p-

trend=0.04) (45). These findings suggest that the ratio of sodium to potassium is a greater determinant of hypertension and CVD than intake levels of sodium or potassium alone.

Further, one major international study investigated the relationship between sodium and potassium excretion in 10,079 adults aged 20-59 and found that the ratio of sodium to potassium was more strongly associated with both systolic blood pressure ($p < 0.001$) and diastolic blood pressure ($p < 0.05$) than sodium or potassium alone (46). Conducted from 1988-1990, this 'Intersalt' study demonstrated a significant and positive relationship between sodium levels and both systolic and diastolic blood pressure (46). The Intersalt study was a novel undertaking since it used standardized methodology at 52 separate locations in 32 countries. However, the study was limited by 24-hour urine collection used to estimate electrolyte excretion. Thus, within-person variability was a major limitation of this study.

There is an abundance of evidence suggesting the hypertensive effects of excessive dietary sodium intake (7-9, 46). One prominent cohort study conducted in Japan found a positive association between sodium intake and mortality from stroke. Specifically, comparing those in the highest quintile (3,105 ± 414 mg/day) to those in the lowest quintile (1,150 ± 345 mg/day) of sodium intake, there was a 55% greater risk of total stroke (47). The estimated mean sodium intake from food frequency questionnaire was 1,909 mg/day; however, the estimated mean intake was 3,841 mg/day from four 3-day dietary records included in the validation study. In this same cohort, there was an inverse association between potassium intake and mortality from total cardiovascular disease. Although that study is a bit outdated, being conducted

from 1988-1990, these findings are notable because of the large study population (58,730 Japanese men and women) with subjects generally consuming high intakes of dietary sodium comparable to the average American intake.

Current and past literature richly reflects the importance of consuming adequate potassium while simultaneously decreasing sodium intake to reduce hypertension and associated cardiovascular outcomes. Therefore, an alternative and indirect approach to decreasing sodium levels is to promote sodium excretion; which leads to the importance of the natriuretic effect of potassium and adequate dietary consumption of potassium as a preventive means from hypertension.

Potassium

Considering the multiple roles potassium serves that are related to blood pressure homeostasis, the primary antihypertensive mechanisms to explore are the induced vascular relaxation and the natriuretic effect of potassium. The antihypertensive effects of potassium were first identified nearly a century ago, and were applied in clinical settings thereafter (19). One widely known clinical practice of promoting potassium intakes was that of Dr. Walter Kempner at Duke University in the 1940's. Despite concerns of nutrient deficiencies, Dr. Kempner's Rice-Fruit Diet demonstrated success with treating congestive heart failure and severe hypertension (48). Hallmarks of Kempner's Rice-Fruit Diet include an abundance of potassium and extremely low levels of sodium. This diet was extensively used throughout the 1940's, and was not only successful at managing hypertension, but also proved effective at reducing obesity, stopping hemorrhaging, heart and kidney failure, and even attenuating diabetes and arthritis.

In 2003, the National High Blood Pressure Education Program Coordinating Committee (JNC 7) recommended that adequate consumption of dietary potassium (3,500 mg/day) be suggested for primary prevention of hypertension. The American Heart Association published new guidelines in 2006 that recommended increasing potassium intake to 4,700 mg/day (8). However, because of an insufficiency of dose-response trials with potassium supplementation, there have yet to be any specific levels of dietary potassium recommended for those who are aiming to manage hypertension (8).

Research suggests salt sensitivity is attenuated, and can even be alleviated when supplemental potassium intake levels are sufficient. That is, potassium supplementation has demonstrated an impressive, dose-dependent inhibitory effect on sodium sensitivity in normotensive individuals (16). Sodium sensitivity was defined as an elevation in mean arterial blood pressure (≥ 3 mm Hg) after a salt loading administration. Changes in mean arterial blood pressure (MAP) after salt loading ≤ 10 mm Hg were considered moderate salt sensitivity; changes >10 mm Hg were considered severe salt sensitivity. Morris and colleagues used a metabolically controlled diet protocol, starting with a basal low-sodium (approximately 345 mg/day [15 mmol/day]) and moderately deficient potassium (approximately 1,176 mg/day [30 mmol/day]) diet for 6 weeks, compiling data from 41 case studies (38 healthy normotensive men). During weeks 3-6, sodium chloride was loaded (5,750 mg/day [250 mmol/day]); during weeks 4-6, potassium was supplemented at mid- or high-normal levels (approximately 2,745 mg and 4,704 mg, respectively [70 and 120 mmol/day, respectively]). The mid-normal level of potassium decreased moderate salt sensitivity, while the high-normal level eliminated moderate

sodium sensitivity and decreased severe salt sensitivity. Of importance, this study included healthy normotensive adult males; mostly black males (24 blacks, 14 white). These findings are important considering that black males are more salt sensitive and have a higher prevalence of hypertension (16). Furthermore, this research reinforces the notion that it is the proportional intake of potassium to sodium that is more of a determinant of blood pressure than the individual intakes of potassium or sodium.

Vascular effects of Potassium

Indices of vascular function are commonly used measures of risk of stroke and heart diseases. One tool to assess vascular function is flow-mediated dilation (FMD). A noninvasive measure, FMD utilizes ultrasound waves to measure endothelial function and is used as an indicator of mortality risk from cardiovascular diseases. In a prospective study, Gokce and colleagues concluded that in patients with peripheral artery disease, diminished FMD of the brachial artery is a predictor of cardiovascular incidences (49). In a randomized crossover study, Blanch et al found that, compared to a low potassium diet, a high potassium diet improved postprandial FMD, which indicates improved endothelial function (50).

Zhou et al (51) investigated effects of vascular changes on hypertensive Dahl salt-sensitive rats fed various diets: a high-sodium diet, high-sodium/potassium-supplemented diet, or a standard rat chow diet. Zhou and colleagues reported significant increases in blood pressure among the rats fed a high-sodium diet. This hypertensive effect was diminished by potassium supplementation. Further, supplementation of potassium enhanced vascular relaxation and increased urinary excretion of nitrite (NO_2^{-}) and nitrate (NO_3^{-}). It was deduced that vascular relaxation

observed in the potassium-supplemented rats was the result of increased endothelial nitric oxide (NO). Although the mechanism of increased NO resulting from potassium supplementation remains unclear, it was suggested that this might be the result of increased production of NO in the endothelial cells (51).

Endothelial cells produce NO and production of this signaling molecule is important because of the vasodilator effects, which result in reduced vascular resistance and blood pressure (52). Taddei and colleagues investigated whether supplementation of intra-arterial potassium affected acetylcholine-induced vasodilation in hypertensive and normotensive patients (52). This study found that infusion of potassium enhances the acetylcholine-dependent vasodilation of the endothelium, thus decreasing peripheral resistance and blood pressure. It was reported that potassium administration enhanced endothelial function in hypertensive patients, but not in normotensive patients. However, one limitation of this study is the modality of potassium administration. The acute intravenous administration of potassium may not have the same effects as dietary supplementation of potassium. Therefore, these findings may not be relatable to supplementation of dietary potassium; though they do help elucidate mechanisms by which increased potassium intake, by any mode of ingestion, reduces blood pressure.

Natriuretic effect of Potassium

Long-term high potassium dietary intakes are shown to increase density of sodium and potassium secretory channels in Sprague-Dawley rats (53). Electrophysiological data on isolated components of the functional units (i.e. nephrons) of rat kidneys demonstrate that increased dietary intake of potassium results in upregulation of active potassium and sodium channels, ultimately increasing urinary

excretion of both minerals (53). Given that this natriuretic/kaliuretic effect is known to result from long-term high potassium intake, Palmer and colleagues examined whether these channel fluctuations were implicated in the short-term, daily maintenance of potassium homeostasis. Sprague-Dawley rats in the treatment group were administered a high-potassium diet (10% potassium chloride) for 6 hours. After just 6 hours, the density of sodium channels in the cortical collecting tubules was 0.08 channels/μm^2 for controls and 1.0 channels/μm^2 after high-potassium diet. Despite the potential of this finding being a mechanistic explanation for natriuresis, there are limitations to these findings. Firstly, only a small segment of the nephron, the cortical collecting tubule, was investigated and therefore does not account for other segments of in the nephrons where secretion and reabsorption occur. Further, these studies were conducted postmortem and it is presumed that the cells remain polarized and that the documented clustering of sodium and potassium channels was not possibly the result of slight physical damage to cells during isolation of the tissues.

The natriuretic effect of potassium is directly related to the intake of sodium. One study found that the greater the sodium intake was, the greater the subsequent excretion of sodium following potassium supplementation (54). Van Buren and colleagues studied the effect that the same dosage of potassium load (approximately 3,920 mg [100 mmol]) had on varying sodium intake levels and the subsequent urinary excretion of minerals. Compared to the low and moderate levels of sodium intake, the subjects with the highest level of sodium intake demonstrated the highest level of sodium excretion after potassium loading. Additionally, this study demonstrated that in those who had a sodium-restricted diet (i.e. low sodium levels), potassium

administration actually caused retention of sodium rather than natriuresis. These findings further illustrate the interdependent effects of potassium and sodium and highlight the importance of adequate potassium intake in those who consume high sodium diets and have high blood pressure.

Effects of potassium deficiency

Inadequate levels of dietary potassium cause the kidneys to reserve sodium and water, resulting in increased blood volume and blood pressure. Gallen and colleagues investigated the effects of potassium-restricted diets on sodium retention and blood pressure including 21 subjects with normal renal function and taking no medications. This study concluded that after restricting potassium intake, healthy/normotensive participants with normal renal function and taking no medications increased renal retention of sodium, chloride, and potassium; ultimately leading to expansion of blood volume and increased blood pressure (38). Additionally, this study found that the antinatriuretic effect of a potassium-restricted diet is immediate as this was evident in the first 24 hours of urinary electrolyte excretion. This effect was gradually dissipated over the subsequent 8 days.

Krishna and colleagues conducted a double blind, randomized, crossover study investigating the effects of potassium restriction on blood pressure (55). This study included 12 hypertensive subjects that were placed on a 10-day isocaloric diet, who also maintained a constant intake of sodium. Compared to the higher potassium group, the group that adhered to a potassium-depleted diet had significantly higher systolic and diastolic blood pressure (7 mm Hg and 6 mm Hg, respectively). Converse to the natriuretic effects of potassium discussed earlier, the potassium-restricted group had

significantly decreased excretion of sodium. Therefore, a potassium deficient diet promotes retention of sodium, which may exacerbate essential hypertension.

These findings that a low potassium diet promotes retention of sodium and increased blood volume and pressure is important considering that the average potassium intake in the United States is far below the Adequate Intake (AI). Self-reported dietary data gathered from the National Health and Nutrition Examination Survey (NHANES III), adult men average about 2,900-3,200 mg/day and adult women average about 2,100-2,300 mg/day potassium. Even more concerning is the estimate that only about 10% of men and 1% of women are consuming at least the AI of potassium (4,700 mg/day) (8).

High-potassium, low-sodium salt substitutes

More recently, a randomized controlled trial published in 2014 demonstrated efficacy of a low-sodium, high-potassium salt substitute reducing blood pressure among a hypertensive Tibetan population (56). Similar to Americans, Tibetans have a high prevalence of hypertension and have estimated intakes of sodium exceeding four times the World Health Organization's (WHO) recommended intake. This 3-month intervention included 282 Tibetans aged 40 years and above and included subjects who reported already taking antihypertensive medications in the prior month. The salt-substitute treatment was comprised of 65% sodium chloride, 25% potassium chloride, and 10% magnesium sulfate; the control group received three months worth of 100% sodium chloride to prepare foods with. Both the treatment and control groups had marked decreases in systolic and diastolic blood pressure, however the treatment group had statistically significant greater decreases in both systolic and diastolic blood

pressure. Compared to the control group, the net reduction of blood pressure in the treatment group was -8.2 mm Hg systolic and -3.4 mm Hg diastolic (all $p < 0.05$) (56).

Another randomized, double-blinded study investigated the feasibility and hypotensive effect of replacing table salt with an alternative salt ("Smart Salt") rich in potassium and magnesium (57). This study included 45 subjects and lasted 8 weeks. Comparing the subjects' 24-hour daily urinary sodium levels, there were significant decreases in the treatment group (those who used Smart Salt®) compared to those in the regular salt (control) group. Additionally, it was reported that the Smart Salt group observed a mean decrease of 7.5 ± 10.1 mm Hg in systolic blood pressure (SBP) and a mean decrease of 2.7 ± 4.5 mm Hg in diastolic blood pressure (DBP). This finding was interesting since the regular salt (control) group observed a marginal increase in blood pressure. That is, 3.8 mm Hg higher in SBP and 1.5 mm Hg higher in DBP. However, there was only statistical significance between these two groups' SBP values at week 3 ($p = 0.028$) and not a statistically significant ($p = 0.076$) difference upon finishing the 8-week intervention. Smart Salt® is novel because it offers consumers a dietary product to replace table salt while reducing salt intake, all while purportedly not sacrificing flavor that consumers come to expect with table salt. That is, Smart Salt® is composed of 50% sodium chloride, 25% potassium chloride, and 25% magnesium salts (57).

Magnesium

Magnesium's biological roles are diverse, and intracellular magnesium is needed to facilitate over 300 different enzymatic reactions (43). More specifically, this essential mineral is needed for proper metabolism of carbohydrates, fats, and protein. It is also necessary for the synthesis of nucleic acid (DNA), activation of amino acids, and

transcription of DNA and RNA. Due to the vast number of functional roles magnesium has in biochemical reactions, there are numerous mechanisms by which magnesium may have direct or indirect influence on blood pressure.

Like potassium, the hypotensive effects of magnesium have been known and used in clinical settings for approximately a century (20). Pharmacological doses of magnesium sulfate were first used to induce hypotension among hypertensive patients in clinical settings in 1925 (20). Due to its role in numerous biological processes, magnesium has different mechanisms of action in blood pressure management than potassium. A primary mechanism to consider is that magnesium promotes dilation of the blood vessels (18).

Regarding more preventive approaches to high blood pressure, a meta-analysis conducted in 2002 which investigated the effects of magnesium supplementation on blood pressure concluded the detection of a dose-dependent response to reducing blood pressure among normotensive and hypertensive persons (58). This meta-analysis compiled 20 different randomized trials and 14 out of these 20 were investigating hypertensive populations, while only 6 of these studies investigated effects in normotensive populations. Magnesium dosages in the included studies were between 10-40 mmol/day (i.e. approximately 243 mg – 972 mg). The dose-dependent effect from the meta-analysis overall was reported that for each increase of 10 mmol/day of magnesium, there was a decrease of 4.3 mm Hg systolic BP (95% CI 6.3 to 2.2; $P <$ .001) and of 2.3 mm Hg diastolic BP (95% CI 4.9 to 0.0; $P = .09$). Considering the large range of magnesium dosages and difference in populations' blood pressure ranges, there were only marginal yet notable decreases in blood pressure.

Effect of magnesium on vascular tone

Magnesium is a vasodilator, and therefore lowers blood pressure by decreasing peripheral resistance (18). It is proposed that magnesium's role in controlling cellular sodium pump (on all cell membranes) activity has a direct influence in transporting sodium and potassium across cell membranes, which affects blood pressure regulation. Variant levels of extracellular magnesium induce such effects. Magnesium also affects the reactivity of the cells by influencing the amount of sodium and calcium that are exchanged (18). Ultimately, it is this altered reactivity that affects vascular tone and consequently, blood pressure.

Although magnesium is not precisely involved in the biochemical course of vascular contraction, it is a calcium antagonist, and has an indirect role on vascular contraction that must be further explored. Kh and colleagues measured the effects of magnesium supplementation on blood pressure and calcium handling in hypertension-induced rats (59). Deoxycorticosterone acetate (DOCA) was used to induce hypertension in the rats of the treatment group, to compare the effects of magnesium supplementation to those in the control group, who were normotensive. In the magnesium-supplemented/DOCA-induced hypertension group, rats had significantly lower concentrations of intracellular calcium (59). This finding is important because of the role that calcium has in maintaining vascular tone.

A study in 2010 investigated the effects that oral magnesium supplementation had on vascular function in elderly diabetics (17). After one month of supplementation, those in the treatment group had significantly improved endothelial-dependent vasodilation, while there were no significant changes in vasodilation for those in the

control group. It must be noted that the study population was composed of elderly (i.e. ≥ 65 years old) diabetic patients. There were 30 subjects in the treatment group, who took 368 mg/day of magnesium (4.5 g/day magnesium pidolate). There were no significant decreases in blood pressure, but endothelial-dependent flow-mediated dilation (post-ischemic, induced from tourniquet) of the brachial artery improved in the treatment group. Specifically, the significant vascular changes measured in the treatment group went from 3.3% ± 3.6% to 8.4% ± 3.9% ($p < 0.01$) (17). There were no significant changes within the control group. The results of this study demonstrated that magnesium supplementation in elderly diabetic patients resulted in improved endothelial function of the brachial artery.

These findings are generally congruent with other studies investigating magnesium-induced vasodilation. This relaxation effect of the blood vessels is purportedly mediated by the release of nitric oxide (NO) from the endothelium (60). It is NO acting as a mediator, which promotes vasodilation and in turn decreases peripheral resistance in blood arteries, and ultimately decreases blood pressure (61). Sodium retention from high sodium intake lowers biological synthesis of NO. Fujiwara and colleagues (61) illustrated this by showing that following increased intakes of salt ("salt loading"), plasma levels of NOx (nitrate + nitrite) decreased accordingly and were increased after subsequent salt restriction. However, a limitation of this study was that it included only hypertensive patients and these findings may not be applied to pre-hypertensive individuals.

Magnesium, sodium, and insights of Deep Sea Water

One study measured the effects of oral magnesium supplementation in hypertensive populations and reported that 1g of magnesium oxide/day (600 mg Mg/day) lead to a significant decrease in both blood pressure and blood lipid concentrations (18). The 4 weeks of magnesium supplementation resulted in a decrease of mean blood pressure from 111±6 to 102±6 mm Hg ($p < 0.001$); the subsequent 4-week placebo resulted in increase of mean blood pressure back up to 108±5 mm Hg ($p < 0.001$). Another interesting finding of this study was that those with higher sodium in erythrocytes (red blood cells) had greater hypotensive effects after treatment of magnesium. This finding reinforces the notion that those with higher intakes of sodium will have greater hypotensive responses to supplementation of magnesium (in addition to potassium). However, both the small sample size (only 21 males) and including only hypertensive individuals limit data interpretation from this study.

With the recently passed Affordable Healthcare Act emphasizing the importance of primary prevention of diseases, there is a greater focus on prehypertension. In 2013, Rodriquez-Moran and colleagues investigated the association between low serum magnesium values and prehypertension in otherwise healthy adults (62). This was a cross-sectional study that included 175 healthy men ($n = 59$) and women ($n = 116$), aged between 20-65 years. There was a statistically significant difference in serum magnesium levels between the prehypertensive group and the group without prehypertension. Those with prehypertension had lower magnesium levels and higher triglyceride levels than non-prehypertensive individuals.

Recent research brings greater attention to the mineral composition and potential salubrious effects of deep-sea water. Although the primary mineral in surface seawater is sodium and includes lesser amounts of magnesium and potassium, deep-sea water (DSW) has a predominant composition of magnesium and potassium. A recent study investigated the effects that DSW had on blood pressure and blood lipids in spontaneously-hypertensive rats (63). The seawater mineral was obtained from the Pacific Rim off of Taiwan and the composition was primarily magnesium. Three different concentrations of diluted DSW were composed for the treatment groups: 3.75 mg Mg/kg DSW (0.1x DSW); 37.5 mg Mg/kg DSW (1 x DSW); and 75 mg Mg/kg DSW (2 x DSW). Just half way through the 8-week study, there were significant decreases in systolic and diastolic arterial blood pressure in two groups (1 x DSW and 2 x DSW) compared to the control group. At the end of the 8-week study, even the lowest (0.1 x DSW) of the three concentrations resulted in significant decreases in both systolic and diastolic arterial pressure compared to the control group. Furthermore, the two stronger concentrations of DSW resulted in significantly decreased cholesterol levels and lipid accumulation in the liver. This study is novel because of the unique utilization of an abundant natural resource (i.e. DSW) and the resulting data that gives further credence to the claim that desirable and proportional intake of essential minerals is a key factor in blood pressure management.

Research Deficiencies

The antihypertensive mechanisms of the primary dietary remedy for hypertension, the DASH Diet, have yet to be fully elucidated (10, 40). Among the most commonly proposed mechanisms is the increased nitric oxide bioavailability that has

been observed from the DASH Diet (40). Additionally, the natriuretic effect associated with the diet is commonly associated with concurrent high intake levels of potassium, magnesium, and calcium (64). However, more randomized controlled trials with larger sample sizes and longer follow-up periods and a better understanding of molecular pathways are needed to further elucidate the specific mechanisms of the DASH Diet.

There is inconsistency of findings among different randomized clinical trials (RCTs) and meta-analyses, regarding hypotensive efficacy of potassium and magnesium supplementation, in various populations. One recent meta-analysis (65), published in 2006, investigated the hypotensive effects of potassium supplementation and found inconsistent findings between six RCTs. No statistically significant decreases in blood pressure were found in the review of six RCTs. The most notable outcome of this recent meta-analysis however, was the dissimilarity among the findings of different trials. Another limitation of this meta-analysis was that it included studies that had minimal duration of follow-up visits and two of the six studies had small sample sizes. Therefore, this study concluded that the evidence on potassium supplementation for hypertension is inconclusive. In contrast, a less recent and perhaps more significant meta-analysis (66) reviewed 20 trials and found a statistically significant decrease in both systolic and diastolic blood pressure from potassium supplementation. This article concluded that recommending increased potassium intakes should be considered for the prevention and treatment of hypertension. However, one major limitation of this review was that it included trials with very limited follow-up periods, such as only four days.

Regarding magnesium, a recent meta-analysis concluded there was not strong enough evidence to indicate the usage of magnesium supplementation for adults to

manage essential hypertension (67). Deficits of knowledge that impede update of magnesium recommendations for those with hypertension and pre-hypertension are related to absence of a standard type and dosage of supplement:, short intervention trials, and overall heterogeneity of subject populations. However, the antihypertensive effects of magnesium may be more pronounced in pre-hypertensive persons rather than those with essential hypertension.

In summary, the most prominent deficit of knowledge pertains to consistency of findings. That is, the general lack of uniformity between many of the study designs and the populations being investigated makes it challenging to draw strong conclusions about the effectiveness and dose-related responses to supplementation of potassium and magnesium. This problem is particularly difficult to elucidate because of the synergistic effects of the minerals being investigated and because of the great variance between intake levels of blood pressure-regulating minerals seen between different population groups. Additionally, due to the many intricate renal mechanisms involved in biological balance of the aforementioned minerals, further understanding of precise hypotensive mechanisms of potassium and magnesium requires a profound understanding of renal physiology.

CHAPTER 3

METHODS

Subjects and study design:

Subject selection

Subjects were considered eligible for the study if they were over 18 years old, under 80 years old, had stable medication use, who had mildly/moderately elevated blood pressure (i.e. Systolic blood pressure: 115-140 mm Hg and/or Diastolic blood pressure: 70-90 mm Hg), and had no unresolved medical conditions. Unresolved medical conditions were defined as any medical diagnoses such as food allergies, type 2 diabetes, thyroid problems and cancer; and that had not already been treated by a physician.

Subjects were excluded if they had any of the following: heart disease, renal insufficiency (or other organ pathology), medication use for hypertension, systolic blood pressure >140 or <115 mm Hg, diastolic blood pressure >70 or <90 mm Hg, nut allergies, or if they were pregnant or lactating. A nut allergy was an exclusion criterion because the treatment/placebo was made with peanut butter.

Recruitment

Subjects were recruited from a college campus and surrounding community in metropolitan Phoenix via online advertisements in winter 2013 and spring 2014. List serves were obtained from the Arizona State University campus community. The dispersed advertisement informed that this was an 8-week randomized controlled trial designed to examine whether a nutrient preparation improves blood pressure in adults with mildly to moderately elevated blood pressure (see appendix A). Interested

individuals were invited to complete an online screening questionnaire (see appendix B). Those who qualified and were still interested in participating were then invited to meet with study investigators at Arizona State University (in the Arizona Biomedical Collaborative [ABC] building on the Downtown Phoenix Campus) to discuss the trial. Each subject provided written consent at the initial visit and the study received approval by the Arizona State University Institutional Review Board (see appendices C and D, respectively).

Chewy Bar Treatments

Each treatment serving was 18.5 grams, had 77.5 calories, and contained 10 grams peanut butter (Jif Natural Peanut Butter, The J.M. Smucker Company, Orville, OH), 5 grams jelly (Smucker's Seedless Strawberry Jam, The J.M. Smucker Company, Orville, OH), and 2 grams all-purpose (AP) flour (see appendix E). In addition to these base ingredients that comprised the placebo recipe, the treatment chewy bar also contained 217.2 mg potassium (417 mg of Potassium Chloride Powder, NOW Supplements, Bloomingdale, IL) and 70.8 mg magnesium (943 mg of MagCitrate Powder, Designs for Health Inc, Suffield, CT). All participants were instructed to consume one chewy bar each day, at the same time of the day, and to not consume any other drinks (except water) or foods at least 30 minutes before or after the chewy bar. Participants recorded daily consumption of the chewy bar on a compliance calendar to measure the adherence to the prescribed regimen. For ingredient calculations, see Appendix E.

The mineral dosage values used in this study were based on similar controlled trial that used the same dosage values and found significantly lower blood pressure on potassium-magnesium supplementation (13). See Chapter 5 for further discussion.

Protocol and Procedures

A crossover, randomized, double-blinded placebo controlled trial design was used (for Methods Flow Chart, see appendix F). Subjects were screened for inclusion and exclusion criteria through an online screening questionnaire (see appendix B). Those who fit the subject criteria were invited to visit the test location one week prior to the start of the trial. At visit 1, participants were asked to record their dietary intake for three days in order to assess baseline dietary intake of sodium, potassium, and magnesium. This 3-day food record was returned at visit 2. After visit 1, participants were stratified by age, gender, BMI, and blood pressure and randomly assigned to one of two groups: potassium and magnesium supplement chewy bar or a control chewy bar for the first four weeks.

At baseline (visit 2), participants came in for a second visit and they were informed of the treatment protocol and provided with the corresponding chewy bars. Baseline measures of height, weight, waist circumference, physical activity and blood pressure were collected at visit 2. Four weeks later, participants came in for visit 3. At visits 3 and 4, body weight and blood pressure were collected, physical activity questionnaire (see appendix G), and a 24-hour food recall were administered and participants were provided with the second batch of chewy bars. For the subsequent four weeks, the subjects ingested the other corresponding chewy bar (i.e. potassium/magnesium or control). Participants were instructed not to change diet or exercise habits during the trial. Additionally, participants were instructed to not eat within 30 minutes before or after ingesting the chewy bar and not to consume milk along with the chewy supplements in order to allow for optimal absorption of minerals.

If medication and/or supplement use changed during the trial, participants were asked to notify the study investigators.

The chewy bars were prepared in a commercial kitchen at Arizona State University, on the Downtown Phoenix campus. In addition to the minerals used in the treatment chewy bars, these bars were prepared using a peanut butter, jelly, and flour base of ingredients (see Appendix E). These bars were then weighed and pressed into ice cube trays, frozen, portioned and stored in the freezer until given to participants. Separate batches (i.e. one for treatment, one for control) of chewy bars were made on two occasions, in sufficient quantity to give each participant four weeks of daily servings.

Measurements

At each visit, body weight was recorded using a calibrated Tanita scale, from Tanita Corporation (Arlington Heights, Illinois; model TBF-300A). Blood pressure was measured at rest after sitting quietly for 5 minutes using a calibrated blood pressure cuff from Omron Healthcare (Bannockburn, Illinois; model HEM-907XL). Three blood pressure values recorded at each visit. The first reading was disregarded, and the mean values of the 2nd and 3rd blood pressure readings were used as the recorded dependent variable. Dependent (paired) sample T-tests were used to compare the mean difference of blood pressure changes between the finish of the participants' 4 weeks adhering to the treatment chewy and the finish of the 4 weeks adhering to the control chewy. In order to better control for confounding effects of blood pressure fluctuations, participants were asked two questions each visit to identify any abnormal stressors: 1) were there any stressful incidences today or last night; 2) did you sleep okay last night.

Participants were asked to reschedule appointment if they answered yes to either of these two questions.

Participants completed a three-day 24-hour diet record prior to baseline visit (provided at visit 1 [consent visit]) and completed two 24-hour diet recalls, one each at both visits 3 and 4. The investigator administered the diet recalls with the participants at visits 3 and 4. Dietary intake was recorded in order to measure any differences in potassium, magnesium, or sodium intakes between the two experimental groups. Dietary data was then analyzed using the online application, The Food Processor. This program was used to approximate dietary intake of sodium, potassium, and magnesium.

Statistical Analysis

SPSS (Statistical Package for the Social Sciences) v. 22 was used to run descriptive and inferential statistical analysis. Results were expressed as the mean ± standard deviation (SD). All data were checked for normality. The Shapiro-Wilk test was used to check normality due to the small sample size (n = 12). Skewed data (i.e. baseline potassium and magnesium intakes; sodium and potassium intakes after treatment; and magnesium intake after control) were normalized by being log-transformed or transformed to square root values. The paired sample T-test was used to compare the difference between mean values of the two conditions. For the normally distributed data, a Pearson correlation was used to test for correlation. For the non-normally distributed data that could not be transformed to a normal distribution (i.e. systolic blood pressure of the two groups after treatment), the Wilcoxon test was used. A p value < 0.05 indicated significant differences between the groups.

CHAPTER 4

RESULTS

Recruitment

A total of 34 people completed the online screening questionnaire. Of these people, 28 qualified to participate in this study and were solicited. After meeting with all 28 respondents for further screening and blood pressure readings, only 12 subjects qualified and consented to participate (8 females, 4 males). The 16 respondents who did not qualify at initial screening visit were disqualified for having mean blood pressure readings outside of the inclusion criteria ranges (i.e. systolic blood pressure: 115-140 mm Hg; diastolic blood pressure: 70-90 mm Hg). The majority of the disqualified respondents were disqualified for having blood pressure below the inclusion criteria. Participant characteristics at baseline (visit 2) are displayed in Table 1.

Descriptive Statistics

The mean age of subjects was 29.3 years old. Subjects had a mean Body Mass Index of 26.2, which is classified as slightly overweight. At baseline visit, mean systolic blood pressure was 121.0 ± 12.3 mm Hg and mean diastolic blood pressure was 75.7 ± 8.8 mm Hg, which was on the lower end of the blood pressure inclusion criteria ranges.

Table 1: Descriptive Characteristics of all Study Participants at Baseline (n = 12)

Characteristics	Minimum	Maximum	Mean (± Std. Dev.)
Age (years)	18	53	29.3 ± 10.9
Weight (lbs.)	101.8	240.2	174.6 ± 41.7
Body Mass Index (kg/M^2)	19.6	34.8	26.2 ± 4.7
Height (inches)	65.5	76.0	68.5 ± 3.0
Activity Level-(METS)	17	99	64.8 ± 29.6
Systolic BP (mm Hg)	108.5	153.0	121.0 ± 12.3
Diastolic BP (mm Hg)	62.0	94.0	75.7 ± 8.8
Energy intake (calories/day)	839.0	2,849.0	1,824.1 ± 593.8
Sodium intake (mg/day)	1,157.0	4,695.0	2,721.1 ± 1,120.3
Potassium intake (mg/day)	751.0	3,594.0	1,653.4 ± 834.3
Magnesium intake (mg/day)	96.0	415.0	179.3 ± 101.2

Treatment Adherence

All 12 subjects completed the 8-week study, for a 100% retention rate. Ten of twelve subjects returned the compliance calendars at the end of the study. Based on these 10 calendars, compliance for ingestion of daily treatment was 88.2% and compliance for ingestion of daily control was 92.8%. There was no significant

difference between the compliance rates of the two groups ($p = 0.133$). See Table 2. During the study there were no complications reported by subjects.

Systolic, Diastolic, and Average Blood Pressure Values

When subjects were asked at each visit if they had encountered any abnormal stressful situation in the past 24 hours, the following was noted. At one visit, one subject responded that they had taken an exam just prior to the visit, though did not consider this abnormal stress. Another subject responded at one visit, that they had been taking Sudafed for a cold for the past week. Another subject reported that they had taken a yoga class just prior to the study visit, which potentially had an antihypertensive effect. Because these subjects did not consider the aforementioned situations abnormally stressful, they were not asked to reschedule the visit. The visits proceeded according to protocol.

Contrary to what was anticipated, the treatment group had a slightly higher systolic blood pressure (118.3 ± 13.3 mm Hg) than the control group (116.5 ± 17.8 mm Hg); however, diastolic blood pressure was slightly lower in the treatment group (71.7 ± 12.4 mm Hg) compared to the control group (73.0 ± 10.0 mm Hg). However, there were no statistical differences found in systolic or diastolic blood pressures between the treatment and control chewies ($p = 0.645$ and $p = 0.464$, respectively). Additionally, there was no significant difference in average blood pressure blood pressure values between the treatment and control chewies ($p = 0.939$). There was however a significant difference ($p = .030$) for body weights between the treatment and control chewies. See Table 2.

Table 2. Blood Pressure, Weights, and Physical Activity Levels

	Treatment (Mean ± SD)	Control (Mean ± SD)	Significance (2-tailed)
Systolic BP (mm Hg)	118.3 ± 13.3	116.5 ± 17.8	0.964*
Diastolic BP (mm Hg)	71.7 ± 12.4	73.0 ± 10.0	0.464
Average BP (mm Hg)	95.0 ± 10.8	94.8 ± 12.7	0.939
Physical activity (METS)	67.0 ± 37.2	66.7 ± 44.3	0.953
Weight (kilograms)	81.6 ± 5.6	81.0 ± 5.6	0.030**
Caloric intake (24-hour recall)	1,836.5 ± 452.4	1,802.2 ± 827.0	0.912
Body Mass Index (kg/ m^2)	26.34 ± 4.50	26.31 ± 4.44	0.783
Compliance (percentage of trial days, chewy ingested)	88.2 ± 15.5	92.8 ± 5.8	0.133
All values represent mean ±Standard Deviation (SD) *Non-parametric data, Wilcoxon test was used **Significant difference in mean blood pressure value between the treatment and control chewies were determined at the .05 level.			

Body Weight and Body Mass Index

Subjects had a mean Body Mass Index (BMI) of 26.2 ± 4.7, at baseline. Body weight was significantly higher following the treatment period, compared to the control period ($p = 0.030$) (179.5 ± 12.3 lbs. and 178.1 ± 12.3 lbs., respectively). However, there was not a significant difference of BMI between the treatment and control chewies ($p = 0.783$).

Physical Activity Questionnaire

At visits 2, 3 and 4 (weeks 0, 4 and 8, respectively) the participants completed a physical activity questionnaire (see Appendix G). The mean value of approximated weekly METS for the treatment group was 67.0 ± 10.7 and 66.7 ± 12.8 for the controls.

There was no significant difference of physical activity between the treatment and control chewies (p = 0.953).

Potassium, Magnesium, and Sodium Intakes

The mean values of sodium, potassium, and magnesium intakes during the treatment period were 3,746 ± 714 mg/day, 2,403.3 ± 1,030.4 mg/day and 280.1 ± 104.8 mg/day, respectively. During the control period nutrient intakes were 4,484 ± 800 mg/day, 1,681 ± 310 mg/day and 261± 73 mg/day, respectively. Sodium, potassium, and magnesium intake levels were log transformed to normalize the data for both the treatment period (Shapiro-Wilk test of normality p = 0.700, 0.295 and 0.401 respectively) and the control period (Shapiro-Wilk test of normality p = 0.785, p = 0.358 and 0.390). There were no significant differences for the dietary intakes of sodium, potassium and magnesium between the two groups (p = 0.552, p = 0.068 and p = 0.826, respectively) See Table 3.

Table 3. Dietary intakes of sodium, potassium, and magnesium

	Treatment	Control	Significance (2-tailed)
Sodium (mg/day)	3,745.8 ± 2.474.9	4,484.4 ± 2,772.6	0.552
Potassium (mg/day)	2,403.3 ± 1,030.4	1,680.7 ± 1,074.1	0.068
Magnesium (mg/day)	280.1 ± 104.8	261.4 ± 254.0	0.826

All values are represented in mg/day as Mean ±SD.
*Significant difference in mean blood pressure value between the treatment and the control periods were determined at the .05 level using log transformed values.

CHAPTER 5

DISCUSSION

Although great progress has been made in the past two decades for awareness, treatment and control of hypertension, the continual rise in prevalence of hypertension demands alternative approaches to combat this arterial disease (2). Despite the recognized antihypertensive effects of potassium and magnesium in clinical settings for nearly a century, actualizing the potential utility of these minerals to manage prehypertension has been challenging to achieve (19, 20). Barriers that impede updating guidelines to promote increasing intake of potassium and magnesium include inconsistency of research findings, heterogeneous study designs and sample populations, and short duration of intervention trials (65-67).

Furthermore, there are various effects induced from a variety of types (i.e. elemental compounds) of potassium and magnesium supplements. For instance, Keith and colleagues compared the natriuretic effects of different potassium salts: potassium citrate, chloride, bicarbonate, acetate, and potassium nitrate (68). All potassium salts compared produced significant natriuretic effects, however potassium nitrate had the most significant natriuretic effect compared to the other salts. Because of such heterogeneity in study designs, there is not a standard recommendation for supplementing these nutrients for the purpose of lowering blood pressure.

Rather than focus on treatment once essential hypertension develops, this study examined the utility of potassium and magnesium supplementation for pre-hypertensive individuals. This study therefore suggests an alterative and non-didactic approach for consumers to manage slightly elevated blood pressure before considering medications.

This relationship was researched to ascertain whether daily supplementation of potassium and magnesium would decrease blood pressure in individuals with mildly elevated blood pressure.

Blood Pressure

After four weeks of supplement intake, there were no significant differences in systolic (SBP) or diastolic blood pressures (DBP) ($p = 0.964$ and 0.464, respectively) between those in the treatment group (mean = 118.3 mm Hg SBP, 71.7 mm Hg DBP) and those in the control group (mean = 116.5 SBP, 73.0 mm Hg DBP). According to these results it appears that the treatment (217.2 mg/day potassium and 70.8 mg/day magnesium) did not have any significant antihypertensive effects.

It is possible that potassium and magnesium do have antihypertensive effects in a population with mild-moderately elevated blood pressure, however the dosage in the present study was not adequate for this effect. The dosage of potassium for those in the treatment group was only 5% (daily) of the Adequate Intake (AI) value for potassium, while the daily dose of magnesium was only 18% for males, 23% for females of the Recommended Dietary Allowance (RDA). The study that was used to base the present study's dosage values on, conducted by Wu et al, found a reduction of 7.83 ± 1.87 mm Hg SBP ($p < 0.01$) and 3.67 ± 1.03 mm Hg DBP ($p < 0.05$) on four weeks of potassium-magnesium supplementation (13). These significant results were from a population of 35 subjects (10 males, 25 females) with a mean age of 58 ± 7 years, living in the Shaanxi district, China. Unlike the population of the present study, Wu and colleagues included subjects who were concomitantly taking blood pressure medications. Furthermore, Wu and colleagues found significant results with this

particular dosage of potassium-magnesium supplementation in a hypertensive population, with mean baseline blood pressure of 142.3 mm Hg SBP, 82.5 mm Hg DBP (13). This is important to note considering that baseline blood pressure of the present study was approximately 15% and 8% lower for systolic and diastolic blood pressure, respectively, compared to Wu and colleagues baseline characteristics. Other research suggests that the antihypertensive effects of potassium are largely influenced by the baseline blood pressure (14). That is, the greater the baseline blood pressure values, the greater the antihypertensive effect of potassium and magnesium.

Mullen and colleagues conducted a randomized, double blind placebo controlled study, comparing the antihypertensive effect of different potassium salts (i.e. potassium chloride and potassium citrate) with a population of 24 normotensive adult males (69). There were no significant changes of blood pressure after supplementation of potassium chloride or potassium citrate among these normotensive subjects. The daily dosage of potassium chloride/citrate was approximately 2,940 mg/day (75 mmol/day). Average intakes of potassium in modern societies are approximately 2,745-3,136 mg/day (70-80 mmol/day), therefore Mullen's study approximately doubled daily potassium intake in these normotensive males and there was no significant changes to blood pressure (33). Despite showing promising results in a normotensive population, the efficacious value used in Mullen and colleagues' study exceeded 10 times the amount used in the present study.

On the contrast, Braschi and colleagues tested blood pressure effects of different potassium salts (i.e. potassium chloride and potassium citrate) on normotensive individuals, with less than 50% of the amount of potassium used in Mullen and

colleagues' study (69) and found hypotensive results from supplementation of both potassium citrate and potassium chloride (14). In this 6-week, randomized double blind placebo controlled trial, subjects were given approximately 1,180 mg/day (30 mmol/day) of potassium citrate, potassium chloride, or a placebo. The findings indicate that supplementation of both potassium salts were effective at lowering mean arterial blood pressure in normotensive individuals. Compared with the control group, six weeks of supplementation of potassium chloride, potassium citrate resulted in a decrease of -4.70 mm Hg and -5.22 mm Hg, respectively. There was not a significant difference between the antihypertensive effects of the two salts.

However, the more interesting finding is that there was a significant correlation ($p = 0.007$) found between SBP and the change in SBP from baseline to end of the trial. This illustrated that the reduction in SBP values after the 6-week trial was greater in those with higher SBP values. Concluding Braschi and colleagues' study, the antihypertensive effect of potassium supplementation was independent of the type of potassium salts, and was greater in those with elevated SBP at baseline.

Despite Braschi and colleagues' study administering a higher potassium dosage than the present study, the finding of the relationship between baseline SBP and change in SBP after potassium supplementation potentially indicates why there was no significant changes in blood pressure found with the given relatively low dosages of the current study (i.e. only 5% daily Adequate Intake for potassium and approximately 20% of Recommended Dietary Allowance for magnesium). Considering the blood pressure range of this current study (inclusion criteria: SBP- >115 mm Hg and <139 mm Hg; DBP- >70 mm Hg and <90 mm Hg) and the relatively low dosages of

potassium and magnesium supplementation, there were no significant changes in blood pressure of individuals with slightly elevated blood pressure. Although, the aim was to recruit a population with mild-moderately elevated blood pressure, the population that was recruited had a baseline blood pressure only slightly above optimal (121.0/75.7 mm Hg).

Compared to other efficacious studies of both potassium and magnesium supplementation individually, the dosage values used in the present study were the lowest. For instance, other controlled trials demonstrating significant hypotensive effects on potassium supplementation varied from dosages of 1,170 mg/day and even exceeding ten times the amount used in the present study, with dosage values of 3,000 and even 4,680 mg/day, with no reported adverse health outcomes (14-16). Similarly, hypotensive dosages of magnesium used in other controlled trials range from 368 mg/day to 600 mg/day (17, 18).

Body Weight

After four weeks of following supplement protocol, body weight after the treatment period was significantly higher than body weight after the control period ($p = 0.030$) (179.5 ± 12.3 and 178.1 ± 12.3 pounds, respectively). With less than one kilogram, the difference in body weight may be related to fluid status, which is not detected on the Tanita scale that was used to measure body weight and body composition. Fluid status naturally fluctuates in humans, throughout the day. For instance, if one subject is more hydrated than another subject with the same body composition, the individual that is more hydrated will be read as having a leaner body

composition, due to the ability of water to hasten the electrical current that is used in bioelectrical impedance.

Physical Activity

There was no significant difference between the physical activity levels of the treatment and control periods ($p = 0.953$). There were no differences in physical activity levels anticipated between the two groups because all participants were asked to maintain physical activity patterns for the duration of the 8-week study.

Sodium, Potassium, and Magnesium Intakes

The mean daily intake of sodium, for all participants after the four weeks of ingesting the chewy bars (including treatment and placebo) was 4,115 mg/day. These high sodium intakes are greater than some estimates of the average daily intake in the United States, which approximate daily sodium intakes at 3,500 mg/day (7). A Tolerable Upper Limit (UL) has been established for sodium and recommends limiting sodium intake to less than 2,300 mg/day. The UL for sodium was established based on the finding that excessive sodium promotes hypertension and is problematic for individuals with kidney disease (43). There was no significant difference in the approximated dietary intake of sodium between the two groups ($p = 0.552$).

The high sodium intake of the subjects in the present study, in conjunction with the lack of significant blood pressure changes between the treatment and control is an interesting finding given that there is a correlation between sodium intake and the natriuretic effect of potassium supplementation (54). One study investigated the effect that the same dosage of potassium load (3,920 mg/day) had on varying sodium intake levels. Compared to the low and moderate levels of sodium intake, the subjects with the

highest level of sodium intake demonstrated the highest level of sodium excretion after potassium loading (54). However, the dosage of potassium in the present study was considerably lower; therefore the dosage of potassium was not likely an adequate quantity to cause any significant changes in blood pressure.

The average dietary intake of potassium, for all participants after the four weeks of ingesting the chewy bars was 2,042 mg/day. This average intake is less than half of the Adequate Intake (AI) for potassium, which is 4,700 mg/day for adults. Further, the dosage of potassium given in the current study was less than 5% of the AI value. Although there was not a significant difference of potassium intakes between the treatment and control ($p = 0.068$), there was a 43% increase in potassium intake approximated in the treatment group. However, these dietary data have considerable limitations that need to be mentioned. The dietary analysis database used (The Food Processor), like other dietary databases, does not provide all the possible food products available in order to reliably select the most valid food products and measure accurate dietary profile information.

The average magnesium intake for all participants after the four weeks of ingesting the chewy bars was approximately 271 mg/day. These intake values equate to approximately 50%-75% of the Recommended Dietary Allowance (RDA) for magnesium. Another important limitation is that these dietary data were measured from a food record and from two separate 24-hour food recalls. These limited measures of dietary intake are only brief appraisals of subjects' diets, and is not necessarily representative of subjects' average dietary intakes.

CHAPTER 6

CONCLUSION

Data from this research suggest that daily supplementation of potassium and magnesium (217.2 mg/day and 70.8 mg/day, respectively) does not significantly decrease blood pressure in individuals with mildly-moderately elevated blood pressure. Increased blood pressure is strongly linked with risk of cardiovascular disease and stroke (22) as well as other chronic conditions including cerebrovascular disease, ischemic heart disease, and renal failure (23).

These results may not be completely representative of the population at large because a small sample size (n = 12) was used, only college students and university staff were included in the sample, and baseline blood pressures were only mildly elevated (baseline mean blood pressure = 121/75 mm Hg). Research demonstrates that the antihypertensive effects of potassium and magnesium are potentiated with greater baseline blood pressure values (14). Furthermore, the dosage levels of potassium and magnesium (<5% Adequate Intake and approximately 20% Recommended Dietary Allowance, respectively) in the current study were not substantial with regard to the aforementioned Dietary Reference Intakes of each of the two essential minerals and baseline blood pressures.

Additional research is needed to determine if higher dosages of potassium and magnesium will have a significant impact on individuals with mild-moderately elevated blood pressure. Future studies should use a larger sample size; explore the antihypertensive effects of larger doses of potassium and magnesium, and the effect of these minerals on variant levels of sodium intake.

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APPENDIX A

TRIAL ADVERTISEMENT

ARE YOU CONCERNED ABOUT YOUR *BLOOD PRESSURE*?

School of Nutrition & Health Promotion
ASU

THE NUTRITION PROGRAM AT ASU IS RECRUITING HEALTHY ADULTS (18-80 years of age) FOR A BLOOD PRESSURE TRIAL.

THIS STUDY WILL EXAMINE WHETHER A NUTRIENT PREPARATION IMPROVES BLOOD PRESSURE IN ADULTS WITH SLIGHTLY ELEVATED BLOOD PRESSURE

Participation will include:

- Enrolling in a 12-week trial with daily supplement ingestion and 5 visits to the test site (located at the Nutrition Laboratories at the Downtown ASU campus)
- Consuming a nutrient preparation daily for 12 weeks
- Recording dietary intake using an online program on 5 occasions
- Maintaining normal diet and activity patterns

INTERESTED?? Please visit our recruitment site:
https://www.surveymonkey.com/s/ASUBloodPressureStudy

APPENDIX B

ONLINE SCREENING QUESTIONNAIRE

1. Please provide your email address:
2. Please select your gender
3. What is your age?
4. Please enter your height and weight
5. Do you have (or think you have) food allergies?
6. Do you take any dietary supplements daily? (e.g. vitamin, mineral, and/or herb)
    a. If yes (please specify)
7. Has a doctor ever told you that you have high blood pressure?
8. Do you know your blood pressure
    a. If yes, what was the last reading you remember?
9. Do you have any active disease state that is currently requiring medication?
10. If female, are you pregnant, lactating, or do you anticipate becoming pregnant?
11. If you smoke, please select how many cigarettes you smoke per day
12. How many times per week do you participate in vigorous, highly intense exercise?
13. Are you following a special diet (such as vegetarian or gluten-free)?
    a. If yes, please specify
14. Will you be able to maintain your current diet and physical activity patterns for a consecutive 8 weeks?
15. Are you willing and able to travel to either the ASU Campus in Tempe or the ASU Downtown Campus to meet with research investigators on five separate occasions? (<20 minutes/visit)

APPENDIX C

CONSENT FORM

Adults Needed for ASU Supplement Trial

Title of research study: Hypotensive Effects of Potassium and Magnesium

Why am I being invited to take part in a research study?

ASU School of Nutrition and Health Promotion professor Dr. Carol Johnston and graduate student Jason Pawloski invite you to take part in a research study. ASU Nutrition Program is inviting healthy adults (18 years of age) who have mildly elevated blood pressure to be part of a research trial.

Why is this research being done?

The purpose of this research is to determine if a mineral preparation (potassium and magnesium) significantly decreases blood pressure for individuals with mildly elevated blood pressure. Additionally, this research aims to contribute to the building base of evidence suggesting that potassium and magnesium supplementation can reduce or replace blood pressure medications.

Today, the primary treatment methods for hypertension ("high blood pressure") in the U.S. are prescribed medications. Although there is already a proven effective, nationally recommended dietary remedy (the DASH Diet) for lowering hypertension, knowledge of and adherence to this diet are not substantial enough to decrease the overall prevalence of hypertension.

Potential benefits resulting from this research include new evidence supporting the effectiveness and feasibility of simply taking a mineral supplement (a 'functional food') to help decrease hypertension rather than relying on medications.

How long will the research last?

The research trial will last 8 weeks and will entail a total of 4 visits with investigators. Each visit will last approximately 15-20 minutes.

What happens if I say yes, I want to be in this research?

It is up to you to decide whether or not to participate. If you decide to participate, the 4 office visits will be conducted in the following sequence:

Screening visit, start of research trial (week 0), week 4, and week 8.

At each visit, blood pressure and body weight will be recorded. The visits will be conducted in the ABC Building (on 5th street and Van Buren) on the ASU Downtown Phoenix Campus and you will be meeting with investigator Jason Pawloski.

What happens if I say yes, but I change my mind later?

You can leave the research at any time it will not be held against you. If you decide to leave the research, contact the investigator so the investigator can withdraw you from the study.

Who can I talk to?

If you have questions, concerns, or complaints, or think the research has hurt you, talk to the research team at: Dr. Carol Johnston (602) 827-2265, carol.johnston@asu.edu; or Jason Pawloski (623) 293-2518, jrpaulso@asu.edu.

This research has been reviewed and approved by the Bioscience IRB ("Institutional Review Board"). You may talk to them at (480) 965-6788 or research.integrity@asu.edu if:

- Your questions, concerns, or complaints are not being answered by the research team.
  - You cannot reach the research team.
  - You want to talk to someone besides the research team.
  - You have questions about your rights as a research participant.
  - You want to get information or provide input about this research.

Your signature documents your permission to take part in this research.

Signature of participant	Date
Printed name of participant	
Signature of person obtaining consent	Date
Printed name of person obtaining consent	
Printed name of person witnessing consent process	

APPENDIX D

INSTITUTIONAL REVIEW BOARD APPROVAL

APPROVAL: MODIFICATION

Carol Johnston
SNHP – Nutrition
602/827-2265
CAROL.JOHNSTON@asu.edu

Dear Carol Johnston: On 2/5/2014 the ASU IRB reviewed the following protocol:

Type of Review: Modification
Title: The hypotensive effect of hawthorn berry extract in healthy adults with mild hypertension
Investigator: Carol Johnston
IRB ID: STUDY00000015
Funding: None
Grant Title: None
Grant ID: None
Documents Reviewed:
• Consent, Category: Consent Form;
• consent (hawthorn berry), Category: Consent Form;
• Consent (minerals), Category: Consent Form;
• updated hawthorn berry consent, Category: Consent Form;
• CONSENT FORM4minerals.pdf, Category: Consent Form;
• Protocol, Category: IRB Protocol; • Mood measure, Category: Measures (Survey questions/Interview questions /interview guides/focus group questions);
• Sleep measure, Category: Measures (Survey questions/Interview questions /interview guides/focus group questions);
• Calendar, Category: Participant materials (specific directions for them);
• Verbal script/online ad, Category: Recruitment Materials;
• Verbal script/online ad (minerals), Category: Recruitment Materials:
• Online survey, Category: Recruitment materials/advertisements /verbal scripts/phone scripts; • Health history questionnaire, Category: Screening forms;
• Checksheet, Category: Screening forms;
• Herbalist diploma, Category: Vitaes/resumes of study team;

The IRB approved the modification.
When consent is appropriate, you must use final, watermarked versions available under the "Documents" tab in ERA-IRB.
In conducting this protocol you are required to follow the requirements listed in the INVESTIGATOR MANUAL (HRP-103).
Sincerely,

IRB Administrator
Continuing Review – 2014 (00000015) Hypotensive effect of Hawthorne Berry Extract
This protocol covers two blood pressure intervention trials involving natural supplements: Hawthorne berry extract and potassium/magnesium. 25 participants completed the Hawthorne berry extract trial and 12 participants completed the

potassium/magnesium trial. We will leave the protocol open for future enrollment (although this is unlikely). Data are currently being entered into spreadsheets for analyses. Preliminary data from the potassium/magnesium trial indicate a non-significant reduction in blood pressure due to the active treatment.
There were no complications reported by participants. In the next year, an honors thesis and an MS thesis should be completed based on this research.

APPENDIX E

CHEWY BAR INGREDIENTS CALCULATIONS

Potassium/Magnesium calculations (treatment only):

Treatment dosage: 217.2 mg/day potassium and 70.8 mg/day magnesium
Total participants expected- 30-40 (~15-20/group)

Potassium needed:

217.2mg/day X 15 (subjects) X 56 (i.e. 8 weeks) = 182,448mg/1000 = 182.5 grams
182.5 g/ 28 (g/oz.) = 6.52 oz.

Test- trial batch **(per serving calc.)-**

365mg (supp. serving)/ 217.2mg K = 1.68;
So, 0.7 (supp. dosage)/ 1.68 = 0.417g powder needed/dosage

K+ Supp. powder needed/batch:

15 (subjects) x 56 days x 0.417g = **350.28 grams** (for 8 week batch, 15 subjects)
23.35g/subject batch

Magnesium needed:

70.8 mg/day X 15 (subjects) X 56 (8 weeks) = 59,472mg/1000 = 59.5 grams
59.5 g/ 28 = 2.125 oz./16 = 0.13 pounds of Magnesium/treatment batch

Test- trial batch **(per serving calc.)-**

300mg Mg (supp. serving)/ 70.8mg Mg = 4.24;
So, 4,000 mg (supp. dosage)/ 4.24 = 0.943 grams powder needed/dosage

Mg+ supp. powder needed/batch-

15 (subjects) x 56 days x 0.943g = **792.12 grams** (8 wk. batch, 15 subjects)
52.81g/subject

Non-mineral Ingredients calculations (treatment and placebo):

Peanut Butter needed

10g/serving x 30 subjects x 56 days (8 weeks) = 16,800g

16,800/28 = 600 oz.

600oz./ 16 = **37.5lbs. Peanut Butter**

1.25lbs. /subject batch
567g

Jelly needed

5g/serving x 30 subjects x 56 days = 8,400g

8,400g/28= 300 oz.

300oz./16= **18.75 lbs. Jelly**

0.625lbs. /subject batch
283.5g

AP Flour

2g/serving x 30 x 56 = 3,360g

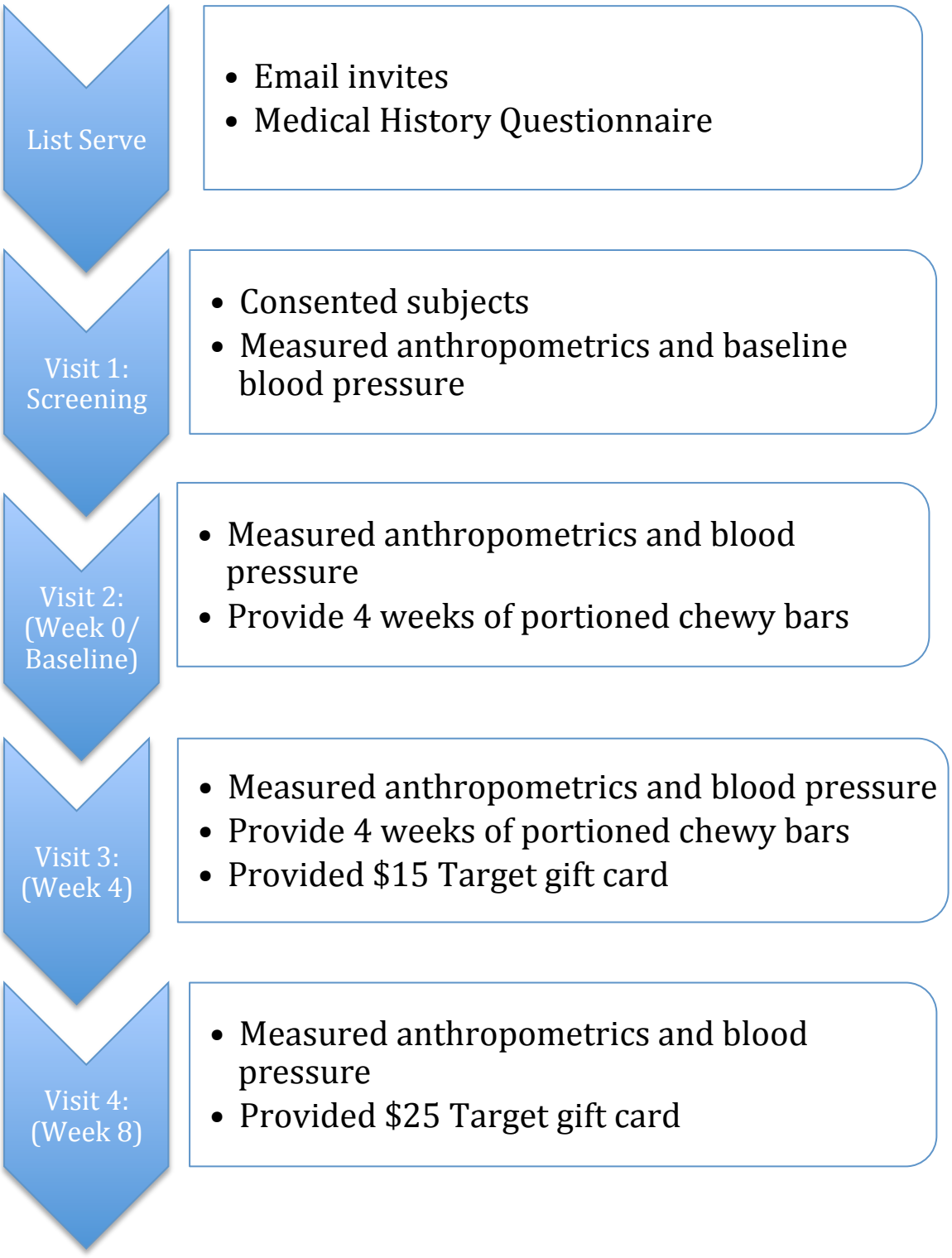
3,360g/28= 120oz.

120oz./16= **7.5 lbs. AP flour**

0.25lbs. /subject batch
113.4g

APPENDIX F

METHODS FLOW CHART


List Serve
Email invites
Medical History Questionnaire
Visit 1: Screening
Consented subjects
Measured anthropometrics and baseline blood pressure
Visit 2: (Week 0/ Baseline)
Measured anthropometrics and blood pressure
Provide 4 weeks of portioned chewy bars
Visit 3: (Week 4)
Measured anthropometrics and blood pressure
Provide 4 weeks of portioned chewy bars
Provided $15 Target gift card
Visit 4: (Week 8)
Measured anthropometrics and blood pressure
Provided $25 Target gift card

APPENDIX G

PHYSICAL ACTIVITY QUESTIONNAIRE

ID# _____ Date of baseline visit (visit #2) _____

Please circle **the number of times** you did the following kinds of exercises **for more than 15 minutes** last week.

- **Mild exercise** (minimal effort):

Easy walking, golf, gardening, bowling, yoga, fishing, horseshoes, archery, etc.

Times per week: 0 1 2 3 4 5 6 7 8 9 10 11 12 13 14+

- **Moderate exercise** (not exhausting):

Fast walking, easy bicycling, tennis, easy swimming, badminton, dancing, volleyball, baseball, etc.

Times per week: 0 1 2 3 4 5 6 7 8 9 10 11 12 13 14+

- **Strenuous exercise activities** (heart beats rapidly):

Running, jogging, hockey, football, soccer, squash, basketball, cross country skiing, judo, roller skating, vigorous swimming, vigorous long distance bicycling, etc.

Times per week: 0 1 2 3 4 5 6 7 8 9 10 11 12 13 14+

APPENDIX H

SAMPLE SIZE CALCULATIONS

	1	2	3	4
Author	Sarkkinen et al	Wu et al	Patki et al	Naismith DJ, Braschi A.
Year	2011	2006	1990	2003
SBP Decrease (mm Hg)	7.5 ± 10.1	7.83 ± 11.1	11.2 ± 11.2	8.4 ± 22.1
DBP Decrease (mm Hg)	2.7 ± 4.5	3.67 ± 1.03	12.2 ± 5.0	6.46 ±
n per group (number of subjects in study)	22	35	37	30
Calculated n per group	30	33	17	110
Age Range (years)	25 - 75	41 - 70	49.9 ± 7.6	22-65
Subject state	BMI: 23-40; SBP: 130-159; DBP: 85-99	EH= SBP ≥140 DBP ≥90	EH= Mean BP- SBP: 155 DBP: 100.4	"Apparently healthy"
Study design/ duration	RCT; 8 weeks	RCT; 4 weeks	Crossover; 8 weeks	RCT; 6 weeks