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The Role of Sugar-Sweetened Beverage Intake and Vitamin D in Elevated Systolic Blood Pressure

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THE ROLE OF SUGAR-SWEETENED BEVERAGE INTAKE AND VITAMIN D IN ELEVALED SYSTOLIC BLOOD PRESSURE

A Thesis Presented

by

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ABSTRACT

THE ROLE OF SUGAR-SWEETENED BEVERAGES AND VITAMIN D

IN ELEVATED SYSTOLIC BLOOD PRESSURE

SEPTEMBER 2017

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High sugar-sweetened beverage (SSB) intake and poor vitamin D status have both been associated with increased risk of elevated systolic blood pressure (SBP) in previous research. However, these associations have never been investigated in the same study population, leaving the question of a possible interaction uninvestigated. One potential mechanism for an interaction is that SSB intake may increase serum uric acid (UA) and UA may interfere with utilization of vitamin D. This study examined these relationships in a sample of men and women (n=2,875) aged 20-74 using data collected in the 2003-2006 NHANES survey. No statistically significant association was found between SSB intake and risk of elevated SBP (defined as SBP>120mmHg) in whole group analysis. In subgroup analysis by gender, women (n=1,550) showed a 68% (OR: 1.68, 95% CI: 1.12-2.50, p-value 0.011) increased risk of elevated SBP in the highest SSB intake quartile (mean intake of 3.27 servings/day) compared to the lowest (mean intake of 0.03 servings/day) after adjustment for age, race, BMI, alcohol use, physical activity, and smoking, but no association was found in men (n=1,325). A statistically significant association was found between 25(OH)D and SBP, with a 30% decrease in risk of elevated SBP (OR: 0.70, 95% CI: 0.55-0.90, p-value 0.005) for those in the highest serum 25(OH)D group (>75nmol/L) compared to the lowest (<50nmol/L) in the fully adjusted model. However, no association was found between SSB intake and serum UA. Assessing potential effect

modification between SSB and vitamin D in their impact on blood pressure using a multiplicative term and stratified analysis did not provided evidence of an interaction effect.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

INTRODUCTION

For a disease that affects one in three American adults, hypertension (HTN) is elusive. Largely asymptomatic, it hides in plain sight while earning the moniker “the silent killer” by contributing to the death of around 1,000 Americans every day (CDC, 2016). There are two things that make hypertension so difficult to combat: the underlying cause of primary hypertension is unknown and only about half of people diagnosed with hypertension are meeting their blood pressure targets, despite the over 75 drug treatments available (CDC, 2016). In secondary hypertension, such as obvious injury to the kidneys, hypertension is a symptom of another disease state and will resolve when the underlying cause is treated. When the underlying cause is not known, it is called essential or primary hypertension, but the dichotomy is a false one. The goal of hypertension research is to understand the underlying cause behind cases that are currently considered essential so that they, too, can be resolved. Myriad sub-types of hypertension may eventually be found within the current umbrella term. Each of these discoveries will hopefully also move more patients out of the resistant hypertension category, as understanding the causes will lead to better, more targeted prevention and treatment strategies.

Poor vitamin D status has been linked to HTN in cross-sectional and cohort studies. A 2011 meta-analysis found that the risk of developing HTN was 27% lower among persons in the highest verses the lowest category of serum 25-hydroxyvitamin D (Odds Ratio (OR): 0.73, 95% confidence interval (CI): 0.63–0.84) (Burgaz, 2011). Mechanisms proposed for this protective effect are that the active form of vitamin D has been shown to reduce renin secretion and smooth muscle proliferation in vascular tissue (Forman, 2008). However, the story changes with

attempts to lower blood pressure (BP) through vitamin D supplementation. Meta-analysis of 46 randomized placebo-controlled clinical trials found no effect of supplementation on BP (Beveridge, 2015). One possible cause of this unexpected outcome would be if some other factor were involved in the relationship between vitamin D and hypertension. Without accounting for this other factor, analysis of change in BP relative to change in vitamin D levels might reveal no relationship.

A possibility for that missing factor could be sugar-sweetened beverages (SSBs). Many cross-sectional and cohort studies that examined SSB intake found a positive association between higher intake and increased blood pressure or the risk of developing hypertension (HTN) (Barrio-Lopez, 2013; Bremer, 2009; Cohen, 2012; Duffey, 2010; Ejtahed, 2015; Mirmiran, 2015; Nguyen, 2009; Sayon-Orea, 2015; Wang, 2013). A 2015 meta-analysis of 6 cohort studies found a 12% higher risk of HTN (Risk Ratio 1.12, 95% CI: 1.06-1.17) for highest (≥1 SSB/day) versus lowest (none) quantile of SSB intake (Jayalath, 2015). Studies that examined the effect of reducing SSB intake have shown a blood pressure lowering effect or reduced risk of developing HTN (Chen, 2010; Sayon-Orea, 2015). One mechanism by which SSB intake is thought to raise BP is through the ability of high fructose intake to induce an increase in serum UA concentration (Perez-Pozo, 2010; Nakagawa, 2006; Sanchez-Lozada, 2007, Wang, 2012). Mild hyperuricemia has been shown to induce BP elevation in animal models by increasing renin production and decreasing nitric oxide production, thereby disrupting endothelial function (Khosla, 2005; Mazzali, 2001). These mechanisms are suggestively similar to those found for low vitamin D status.

The purpose of this study was to explore the association between SSB, vitamin D status and BP and determine whether these factors in combination modify the main associations with

blood pressure. Could good vitamin D status protect against the impact of high SSB intake on BP? Or could high SSB intake nullify the positive effect of vitamin D repletion? The possibility that uric acid is the mediator in that interaction was also investigated. To our knowledge, the relationship between SSBs, vitamin D and HTN has not been explored previously.

This study examined these relationships in a sample of men and women (n=2,875) aged 20-74 using data collected in the 2003-2006 NHANES survey. No statistically significant association was found between SSB intake and risk of elevated SBP (defined as SBP>120mmHg) in whole group analysis. In subgroup analysis by gender, women (n=1,550) showed a 68% (OR: 1.68, 95% CI: 1.12-2.50, p-value 0.011) increased risk of elevated SBP in the highest SSB intake quartile (mean intake of 3.27 servings/day) compared to the lowest (mean intake of 0.03 servings/day) after adjustment for age, race, BMI, alcohol use, physical activity, and smoking, but no association was found in men (n=1,325). A statistically significant association was found between 25(OH)D and SBP, with a 30% decrease in risk of elevated SBP (OR: 0.70, 95% CI: 0.55-0.90, p-value 0.005) for those in the highest serum 25(OH)D group (>75nmol/L) compared to the lowest (<50nmol/L) in the fully adjusted model. These findings are generally consistent with previous research. However, no association was found between SSB intake and serum UA, which was unexpected, as the possible ability of high SSB intake to increase serum UA has been widely speculated to be a mechanism by which SSBs might increase BP. Assessing potential effect modification between SSB and vitamin D in their impact on blood pressure using a multiplicative term and stratified analysis did not provided evidence of an interaction effect. As the proposed mechanistic link between SSB intake and vitamin D was UA, finding no interaction effect is consistent with the finding of no association between SSBs and UA.

CHAPTER 2

LITURATURE REVIEW

2.1 Blood Pressure Regulation

2.1.1 Renin-Angiotensin-Aldosterone System (RAAS)

The RAAS is the principal means of blood pressure regulation. Blood pressure is monitored in the kidneys by the macula densa cells of the juxtaglomerular-complex. These cells are sensitive to the pressure of blood coming through the afferent arteriole of the nephron. If pressure is too low, macula densa cells release the enzyme renin into the blood stream. Renin converts angiotensinogen to angiotensin I, which is then converted to angiotensin II by angiotensin converting enzyme (ACE) found in the lungs. Angiotensin II is a vasoconstrictor, so it has an immediate effect on blood pressure. It also travels to the adrenal glands and triggers the release of aldosterone, which then completes the circuit, returning to the kidneys where it acts on the distal nephron, signaling for an increase in epithelial sodium channels placed into the apical surface. (Mironova, 2015) Water is then able to travel down its concentration gradient by osmosis back into the blood stream. The resulting increase in blood volume boosts blood pressure, returning it to the normal range, which, in turn, signals the macula densa cells to stop secreting renin and the RAAS is shut down. While this whole-body view of the RAAS is the most widely accepted model, some argue that all of the steps of the RAAS system can also take place exclusively in the kidney. They state that small amounts of all of the molecules involved, such as ACE and aldosterone, are made intrarenally, arguing that this mechanism plays a role in hypertension. (Wadei, 2012)

The renin-angiotensin-aldosterone system is a key mechanism for long-term blood pressure regulation that is very effective at correcting low blood pressure by increasing blood volume. However, RAAS is a poor way to correct high blood pressure, since the only way it can act to reduce blood pressure is to turn off. Once RAAS is shut down, new epithelial sodium channels will stop being placed into the collecting ducts, but the existing ones will continue to function until they break down. In effect, the signal that blood pressure is too high can only be acted on at the speed of protein turnover. (Mironova, 2015) This may be surprising, given the number of anti-hypertensive medications that focus on interrupting RAAS. These drugs are effective in patients where the homeostatic disregulation that is resulting in hypertension is that the RAAS is stuck in the "on" position. For some reason, the juxtaglomerular-complex is continuing to secrete renin, telling the body that blood pressure is too low when it is in fact above the normal range. Another facet of this mechanism is paracrine signaling in the collecting duct, with ATP used as the signal molecule, a type of purinergic signaling. This purinergic signal should block the epithelial sodium channels to prevent sodium reabsorption when blood pressure is high. Defects in this purinergic signaling system in the kidneys also interfere with blood pressure regulating effects of pressure natriuresis, discussed below. (Mironova, 2015)

2.1.2 Pressure Natriuresis (PN)

In addition to the RAAS system, blood pressure is controlled by the pressure natriuresis response, which takes place entirely in the kidneys. When blood pressure rises, it causes an increase in water and sodium excretion by the kidneys. This in turn lowers fluid volume throughout the body and returns blood pressure to the normal range. A feature to note about PN is that it inhibits the renin-angiotensin-aldosterone system. If it is functioning properly,

pressure natriuresis is also one way that the body can compensate for an overactive RAAS. This was demonstrated in a study were dogs were infused with excess aldosterone. The dogs whose PN system was uninterrupted were able to maintain normal sodium balance in spite of the excess aldosterone. (Granger, 2002)

Despite tight homeostatic control of renal blood flow and glomerular filtration rate, renal perfusion pressure increase is associated with increases in renal interstitial hydrostatic pressure (RHIP). (Granger, 2002; Ivy, 2014) The increase in pressure is thought to be transmitted into the interstitium via the vasa recta, the blood vessels that run parallel to the loop of Henle. Blood flow in the vasa recta is not tightly controlled so it increases with increased renal perfusion pressure. This causes fluid in the medulla of the kidney to be less able to enter the vasa recta, resulting in higher renal interstitial hydrostatic pressure. (Granger, 2002; Ivy, 2014)

Another way PN may be regulated is through nitric oxide production. When renal perfusion pressure rises, intrarenal nitric oxide production increases, which leads to an increase in water and sodium excretion by inhibiting tubular sodium reabsorption. (Granger, 2002) As a powerful vasodilator, the increase in nitric oxide also increases renal blood flow and RHIP. It is unclear exactly how RHIP enhances pressure natriuresis, but there is speculation that it may result from the release of certain prostaglandins, changes in tight junction permeability to sodium, or redistribution of apical sodium transporters. (Granger, 2002)

One of the key benefits of pressure natriuresis for blood pressure regulation is that it responds in direct relationship to sodium levels so that it should be able to compensate for any amount of variation in sodium intake. However, in some people this is clearly not the case, a phenomenon often termed salt sensitivity, leading to speculation by some researchers that HTN results from a shift in the PN relationship so that it does not respond until higher pressures.

(Wadei, 2012) Salt sensitivity is more common in older people, African Americans and people who developed HTN at an early age. It is also associated with obesity where there is "disturbance of insulin secretion typical of the metabolic syndrome." (Wadei, 2012) Salt sensitive people may have a normal glomerular filtration rate or even have normal daytime blood pressure readings. But they are more likely to have a less marked, or even absent, diurnal difference in their blood pressure and reduced rates of sodium excretion over night. They also have a higher risk of certain complications of hypertension, such as micoralbuminuria, cardiovascular complications and left ventricular hypertrophy. (Wadei, 2012; Ivy, 2014)

2.2 Sugar-Sweetened Beverages and Blood Pressure

Cross-sectional studies of adults have generally supported an association between SSBs and hypertension, although the results of some studies have not achieved statistical significance. Table 2.1 summarizes the findings of studies conducted with adult subjects. Dhingra and colleagues (2007) conducted a cross-sectional study using data gathered as part of the Framingham Heart Study. The primary outcome of interest in the study was metabolic syndrome, but the individual components, one of which is hypertension, were also analyzed separately. They defined elevated blood pressure as >135/85mmHg or on antihypertensive medication and compared those meeting that criteria who were consuming greater ≥1 SSB per day to those drinking <1 per day. They found an 18% increased risk of elevated BP (OR: 1.18, 95% CI, 0.96 to 1.44), which was not statistically significant. The model was adjusted for age, sex, physical activity, smoking, dietary intake of saturated fat, trans fat, fiber, magnesium, total calories, and glycemic index.

A 2011 study by Brown and colleagues examined data collected as part of the International Study of Macro/Micronutrients and Blood Pressure (INTERMAP), which was gathered in 10 communities in the US and the UK. SSB consumption was assessed by 24-hour recall and BP was directly measured. Their analysis of 2,696 subjects found an increase of 1.05mmHg (95% CI: 0.50-1.60) in systolic blood pressure(SBP) per 1 serving (355mL) per day in a model adjusted for energy, urinary sodium, potassium, dietary alcohol, cholesterol, polyunsaturated and saturated fatty acids, weight and height. However, these results were also not statistically significant. Artificially sweetened beverage intake was also analyzed and found to have an inverse relationship with blood pressure (Brown, 2011). The following year, Kim and colleagues had similar findings from a study looking at data from the 2003-2006 cycles of the National Health and Nutrition Examination Survey (NHANES). Participants with a previous diagnosis of HTN were excluded, leaving 3,044 subjects over the age of 19, 357 of whom were found to have BP in the hypertensive range at the NHANES examination. SSB intake was assessed by food frequency questionnaire (FFQ) and blood pressure was directly measured. The fully adjusted model, which accounted for age, gender, NHANES period, BMI, total caloric intake, race, pack years of smoking, alcohol drinking, sodium/potassium intake ratio, physical activity, levels of education and history of diabetes, looked at the odds ratio of HTN by level of SSB intake. They found an odds ratio of 1.21 (0.81-1.81) for 1 time per month-<3 times per week, 1.39 (0.86-2.24) for 3 times per week-<1 times per day, 1.26 (0.80-1.98) for 1-<3 times per day, and 1.50 (0.84-2.68) for ≥3 times per day compared to the reference group of <1 time for month with a p-value of 0.33 for the trend (Kim, 2012). As with Brown et al's study, the findings are suggestive of a connection between SSBs and HTN but they are not statistically significant.

A more recent cross-sectional study from 2015 was conducted by Ejtahed and colleagues in Iran. The main focus of the study was the relationship between SSBs and metabolic

Table 2.1: Observational studies of SSBs and BP in adults.

First Author	Year	Country	Population	SSB categories	BP categories	OR	Stat. Sig.
Cross-sectional Studies							
Brown	2011	U.S. and U.K.	Adults, Age 40-59, n=2696	1 (355mL) serving/day	SBP up 1.05mmHg		NS
Dhingra	2007	U.S.	6039 person-observations, 3470 in women; mean age 52.9 years	≥1/day vs. <1 per day	>135/85 mmHg or anti-HTN meds. vs. Normal BP	1.18	NS
Kim	2012	U.S.	Adults, ≥19 yrs, n=3044	<1/month	HTN vs. normal BP	1.0 (0.53, 1.89)	NS
				1/month to <3/week		1.21 (0.81, 1.81)	
				3/week to <1 /day		1.39 (0.86, 2.24)	
				1/day to <3/day		1.26 (0.80, 1.98)	
				≥3/day		1.50 (0.84, 2.68)	
Ejtahed	2015	Iran	Adults, 19-70 yrs, n=5,852	Quartile 1 mean 2.6 g/day	HTN vs. normal BP	1.0	P for trend=0 .02
				Quartile 2 mean 13.0 g/day		1.03 (0.85, 1.25)	
				Quartile 3 mean 36.1 g/day		1.22 (1.01, 1.48)	
				Quartile 4 mean 144 g/day		1.27 (1.03, 1.55)	
				4^{th} vs. 1^{st} quartile	SBP up 1.8mmHg DBP up 1.7mmHg		<0.001
Cohort Study							
Cohen	2012	U.S.	Adults, n=253,891	≥1/day vs. >1/month	Diagnosed HTN vs. normal BP	1.13 (1.09, 1.17)	<0.05
Meta-Analysis							
Jayalath	2015	U.S. and Spain	n=240,508	≥1/day vs. none	HTN vs. normal BP	1.12 (1.06, 1.17)	<0.05

syndrome and its components. They examined 5,852 adult men and women who were part of the fourth phase of the Tehran Lipid and Glucose Study from 2009-2011. They assessed SSB intake in a slightly unusual way by converting the amount reported on an FFQ into grams. The participants were then divided into quartiles, with the mean intake for each quartile being 2.6, 13.0, 36.1, and 144 grams of SSB per day. As with Kim et al's study, they were looking for people without a previous diagnosis of HTN but with blood pressure in the hypertensive range when measured by the researchers. They found odds ratios by quartile of SSB intake for elevated BP of 1.03 (CI 0.85-1.25), 1.22 (CI 1.01-1.48) and 1.27 (CI 1.03-1.55). The ORs for the third and fourth quartiles are statistically significant. Ejtahed et al also examined BP as a continuous variable and found an increase of 1.8mmHg SBP and 1.7mmHg DBP between the first and fourth quartiles with a p-value <0.001 for both trends (Ejtahed, 2015).

The results of cohort studies have provided more consistent support for an association between SSBs and HTN. Cohen and colleagues (2012) conducted a study using data from 3 large cohorts: The Nurses' Health Study I, the Nurses' Health Study II, and the Health Professionals' Follow-Up Study. The advantage of this approach was that it gave them a very large sample size of 253,891. They were also able to use self-reported diagnosis of hypertension as their outcome variable because the participants, who were all health professionals, were unlikely to misunderstand or misreport a diagnosis. The pooled analysis of these 3 cohorts resulted in a hazard ratio of 1.13 (95 % CI: 1.09–1.17) for those drinking one or more SSBs per day compared to less than one per month. The model was adjusted for many confounders including a diet quality score based on compliance with the DASH diet as well as the more typical items like alcoholic beverage intake, age, BMI, smoking status and physical activity (Cohen, 2012). In 2015, Jayalath and colleagues did a meta-analysis of 6 prospective cohort studies (n=240,508) which examined SSB consumption and HTN. They found a risk ratio of 1.12 (95% CI: 1.06, 1.17) when

comparing the highest SSB intake group (≥1 per day) with the lowest intake (none) group. They also found a dose-response relationship which resulted in a 8.2% increase in risk of HTN with every additional SSB per day from none to ≥1 SSB per day (β = 0.0027, P < 0.001) (Jayalath, 2015).

Adolescents have been the focus of many studies on the relationship between SSBs and HTN or metabolic syndrome, summarized on Table 2.2. Three of the four cross-sectional studies reviewed showed

Table 2.2: Observational studies of SSBs and BP in adolescents.

First Author	Year	Country	Population	SSB categories	BP categories	OR	Stat. Sig.
Cross-sectional Studies							
Bremer	2009	U.S.	Adolescents12-19yrs, n=6967	1 serving per day	SBP up 0.16mm Hg		0.003
Loh	2016	Malaysia	Adolescents 13yrs n=837	Mean daily intake	No stat. sig. difference found in BP		NS
Wang	2013	Canada	Adolescents 8-10yrs, >85th BMI percentile, n=632	100mL	SBP up 1.1mmHg		0.001
Nguyen	2009	U.S.	Adolescents 12-18yrs, n=4867	0 oz/day vs. >36 oz/day	SBP up z-score 0.18 (about 2mmHg)		0.03
Cohort Studies							
Duffey	2010	U.S.	Adolescents n=2639	Highest vs. lowest intake quartile	>130/85 mmHg or anti-HTN meds. vs. Normal BP	1.06 (1.01, 1.12)	0.023
Ambrosini	2013	Australia	Adolescents 14-17yrs, n=1433	Tertiles	No stat. sig. difference found in BP		NS
Mirmiran	2015	Iran	Adolescents 6-18yrs, n=439	Highest vs. lowest intake quartile	HTN vs. Normal BP	2.74 (1.05, 7.19)	P for trend = 0.018

a statistically significant relationship, but the effect size was small ranging from a 0.16mmHg increase in SBP per SSB per day to 2mmHg increase in SBP in the highest compared to the lowest intake groups (Bremer, 2009, Loh, 2016, Wang , 2013, Nguyen , 2009). One study examined the effect in overweight or obese versus normal weight adolescents and found a 1.1mmHg higher mean SBP for the highest SSB intake group in the overweight and obese group but no effect in the normal weight group (Wang, 2013). Cohort studies had more mixed results. One study looked at SSB consumption averaged for years 0-7 of the study and compared it to prevalence of HTN at 20 years of follow up. They found a 6% increase risk of HTN (RR: 1.06, 95% CI: 1.01-1.12, p-value 0.023) for highest versus lowest quartile of SSB intake (Duffey, 2010). A smaller study (n=439) done in Iran found a stronger association when comparing the highest to lowest quartile of intake with an OR of 2.74 (95 % CI: 1.05–7.19) for HTN. However, when the data were analyzed with SSB intake as a continuous variable, the relationship was no longer significant (Mirmiran, 2015). A cohort study done in Australia found no association (Ambrosini, 2013).

Very few intervention studies, summarized on Table 2.3, have examined the impact of lowering SSB intake on HTN. Maersk and colleagues (2012) did a randomized controlled trial primarily looking at the impact of various beverages on liver adiposity in overweight subjects. They found a non-statistically significant increase in BP in subjects fed 1L of sucrose-sweetened beverage per day for six months. However, there were only 10 subjects in the SSB test group (Maesrk, 2012). Some cohort studies have collected data on changes in SSB consumption and the impact on BP. Chen and colleagues (2010) examined the BP-lowering effect of decreased SSB consumption on the PREMIRE cohort, a multi-center trial examining the impact of various lifestyle treatments on patients with pre-hypertension or stage 1 HTN. The wide variety of data collected during the study allowed Chen et al to control for changes in diet quality, as measured by DASH adherence, and weight change along with the normal confounders of age, race, etc.

The tertile with the greatest reduction in SSB consumption showed a reduction in SBP of 9.5±4.3mmHg, reduction in DBP of 6.3±2.9mmHg, with 23.5% of participants in this tertile moving from the hypertensive to normotensive BP range over the 18 months of the study. There was also a statistically significant trend across the tertiles for all of these results (Chen, 2010). Two studies have analyzed data from the SUN cohort, which included data on increase and decrease in SSB consumption over the six year course of the study. Barrio-Lopez and colleagues (2013)

Table 2.3: Assessing effect of change in SSB intake over time.

First Author	Year	Country	Population	SSB categories	BP categories	OR	Stat. Sig.
Randomized Controlled Trial							
Maersk	2012	Denmark	n=10 in each group	1L sucrose-sweetened beverage per day for six months	No stat. sig. difference compared to water group		NS
Cohort Studies							
Chen	2010	U.S.	pre-HTN or stage 1 HTN, n=810	Per 1 serving/day decrease	SBP up 0.7mmHg (0.12, 1.25) DBP up 0.4mmHg (0.02-0.75)		NS
Barrio-Lopez	2013	Spain	Adults, n=8157	Largest increase vs. largest decrease quintile	Self-reported HTN or use of anti-HTN meds.	1.60 (1.3, 2.1)	P for trend < 0.001
Sayon-Orea	2015	Spain	Adults, n=13,843	Largest increase vs. no change	Self-reported HTN or use of anti-HTN meds.	1.26 (1.02, 1.55)	<0.05

were looking at MetS and its components and found that participants with the highest increase in SSB consumption had 60% increased risk developing HTN compared to those in largest decrease quartile (OR: 1.6, 95 % CI 1.3, 2.1; P for trend < 0·001). This study was conducted in

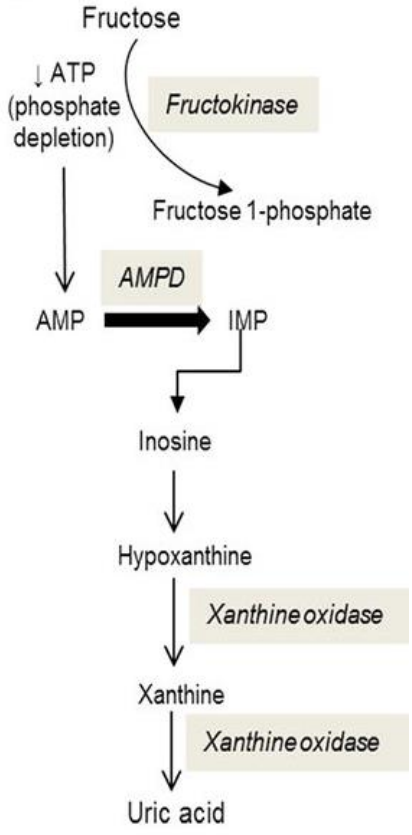
Spain and they attempted to control for adoption of an overall American diet but adding French fries, red meat, fast food and adherence to a Mediterranean dietary pattern to the confounders controlled for, as well as the more typical total energy intake, smoking, physical activity and alcohol intake (Barrio-Lopez, 2013). Two years later, Sayon-Orea and colleagues (2015) conducted a study using the same data set but looking specifically at HTN. Their analysis was slightly different because they looked at the incidence of HTN in participants who increased SSB consumption versus those whose intake did not change, rather than increase compared to decrease in SSB intake. Predictably, the association was also positive but weaker, with a 26% increased risk of HTN (OR: 1.26, 95% CI: 1.02–1.55) (Sayon-Orea, 2015).

The majority of published findings do support a positive association between SSB intake and elevated BP and the risk of HTN, both in adults and adolescents (Barrio-Lopez, 2013; Bremer, 2009; Cohen, 2012; Duffey, 2010; Ejtahed, 2015; Jayalath,2015; Mirmiran, 2015; Nguyen, 2009; Sayon-Orea, 2015; Wang, 2013). Of the studies that did not find a statistically significant association, more than half found a non-significant positive correlation (Brown, 2011; Chen, 2010; Dhingra, 2007; Kim, 2012). The OR for development of elevated BP or HTN ranged between 1.06 (Duffey, 2010) and 2.74 (Mirmiran, 2015) for studies with a statistically significant finding. Large 95% confidence intervals in some of the reported odds ratios (Barrio-Lopez, 2013; Kim, 2012; Mirmiran, 2015) suggest that there may be quite a bit of heterogeneity in individuals' BP response to SSB intake. Those studies which examined BP as a continuous variable found an increase of 0.16mmHg (Bremer, 2009) to 1.1mmHg SBP (Wang, 2013) per additional serving of SSB per day, which suggests that the effect may only be medically significant in heavy users of SSBs.

2.3 Sugar-Sweetened Beverages and Uric Acid

The most frequently discussed mechanism by which SSBs are thought to increase blood pressure is through elevating serum uric acid levels (Brown, 2011; Ejtahed, 2015; Jayalath, 2015; Kim, 2012; Nguyen, 2009). The reason for this is that SSBs are a very rich dietary source of fructose. After ingestion, fructose is transported to the liver where it is phosphorylated by fructokinase to form fructose-1-phosphate, using a phosphate group from an ATP. This reaction is not well regulated and will continue as long as fructose is present. Eventually, it can lead to depletion of ATP, and then ADP and AMP until only the adenosine remains. Adenosine will be broken down by the purine pathway with the final product being uric acid. A key enzyme in this pathway, illustrated in Figure 1, is xanthine oxidase, which catalyzes the final two steps. Humans do not produce the enzyme uricase that would further degrade uric acid into allantoin, which leads to an approximately 10 times higher serum UA than most other mammals. While hyperuricemia is associated with many diseases, especially gout, UA also functions as an important antioxidant and is inversely associated with some disease such as Parkinson's Disease and other neurodegenerative diseases. UA

Figure 2.1: Uric acid metabolism.



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Asghar, Z. A., Thompson, A., Chi, M., Cusumano, A., Scheaffer, S., Al-Hammadi, N., ... & Moley, K. H. (2016). Maternal fructose drives placental uric acid production leading to adverse fetal outcomes. *Scientific reports*, *6*.

is ultimately removed by the kidneys through active excretion and through the gastrointestinal tract (Mandal, 2015).

Uric acid has been shown to have a relationship with hypertension in studies such as one conducted by Sundstrom and colleagues (2005), which examined 3329 middle-aged Framingham Heart study participants and found a 17% increased risk (OR: 1.17, 95% CI: 1.02-1.33) for developing HTN at 4 years of follow-up per 1 standard deviation elevation in baseline UA levels. Another 2005 study by Alper and colleagues looked at 577 participants in the Bogalusa Heart Study. Age at baseline was between 5 and 17 years and subjects were followed for an average of 12 years. They found a strong correlation between UA levels in childhood and both childhood and adult elevated BP, with a stronger association observed in females than males (Alper, 2005). In a randomized controlled trial of allopurinol, a xanthine oxidase inhibitor, Feig and colleagues (2013) investigated whether lowering UA had a BP-lowering effect in 30 adolescents with incident stage 1 HTN. Participants had a 6.3 mmHg (95% CI: 3.8−8.9 mmHg) reduction in mean 24-hour SBP and 4.6 mmHg (95% CI: 2.4−6.8 mmHg) reduction in mean 24-hour DBP during the treatment compared to the placebo phase of this cross-over study. Those reductions are consistent with what could be expected from standard antihypertensive drug therapies (Feig, 2013).

The mechanism for the impact of uric acid on BP has been investigated using animal models. Mazzali and colleagues (2001) developed a rat model to look at mild hyperuricemia, more similar to the UA levels seen in humans than earlier rat models, which resulted in as much as a 10-fold increases in UA, acute kidney injury and death. The hyperuricemic rats in Mazzali's study had a more modest 2-3-fold increase and did show an increase in BP over the study period. Interestingly, the BP difference between hyperuricemic rats and control rats was the

greatest in rats on a modest salt-restricted diet. BP in rats who had been hyperuricemic and where then treated to lower UA levels dropped back down into the normal range. When the kidneys of treated rats were examined, they showed an increase in interstitial collagen deposition, fibrosis and neutrophil infiltration. Hyperuricemic animals were also found to have markedly increased renin and decreased nitric oxide synthase. In hyperuricemic rats given an ACE-inhibitor and the NO-precursor L-arginine, BP was normal and renal injury prevented, suggesting that the RAAS and NO systems are part of the causal pathway for UA-induced HTN (Mazzali, 2001). Khosla and colleagues further elucidated this relationship. They induced hyperuricemia in rats using the same method as in Mazzali et al's study, and found a 40-50% decrease in NO production within one day which lasted throughout the seven days of the experiment. They also preformed and *in vitro* study with bovine endothelial cells and found a dose-dependent decrease in NO production with UA exposure (Khosla, 2005).

While the studies discussed above support relationships between fructose, UA and HTN (Alper, 2005; Feig, 2013; Khosla, 2005; Mandal, 2015; Mazzali, 2001; Sundstrom, 2005;), the question remains of whether intake from SSBs provides enough fructose and produces enough UA to activate this pathway. Animal studies have been used to investigate this relationship by inducing elevated UA and BP through fructose feeding, rather than by inhibiting uricase, and have found a positive correlation (Sanchez-Lozada, 2007, Nakagawa, 2006). However, very high levels of fructose, up to 60% of calories consumed, are used to overwhelm the rats' innate ability to metabolize uric acid. This level of fructose intake is so far in excess of normal human consumption some question the applicability of this data in humans (Ha, 2012). Sanchez-Lozada and colleagues (2007) did include a test group of rats who were fed normal chow but also given ad-libitum access to 10% fructose-sweetened water, which is somewhat more similar to the way it is consumed in humans drinking SSBs. These rats were found to have consumed 38±2% of

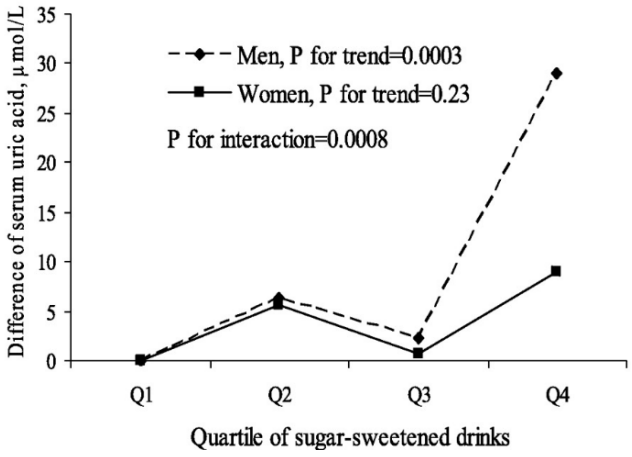
their calories from fructose and showed a statistically significant increase in both UA and BP at the end of the 8 week study, although not as large an increase as the group fed 60% fructose chow.

Experimental studies examining the impact of high levels of fructose intake on UA and BP in humans are fairly rare. Wang and colleagues (2012) did a review and meta-analysis of controlled-feeding trials and found no effect in isocaloric studies but did find a relationship in hypercaloric trials. One example of such a study was done by Perez-Pozo and colleagues in 2010. They conducted a randomized controlled study investigating this relationship by feeding subjects (n=74, all men) 200g of fructose per day for two weeks. Half of the subjects were also treated with allopurinol, which disrupts the action of xanthine oxidase, a critical enzyme in the uric acid pathway. The fructose-only group had a statistically significant increase in their uric acid levels and BP but the fructose-plus-allopurinol group did not have an increase in either (Perez-Pozo, 2010). In their meta-analysis of three hypercaloric trials, including Perez-Pozo's study, Wang et al found a mean increase in UA levels of 0.52 mg/dL (Wang, 2012). These findings suggest that the SSB-UA pathway might be of concern only with higher intakes of SSBs, which are more likely to be excess calories.

Cross-sectional studies have tried to determine whether high SSB intake leads to elevated UA levels or hyperuricemia in free-living humans. Choi and colleagues (2008) examined the relationship between SSB intake and UA levels in 14,317 NHANES (1988-94) participants free of gout and UA lowering medications. They found the highest intake group of SSBs, ≥4 servings per day, had a 0.42mg/dL (95% CI: 0.11, 0.73) higher mean UA level than the reference group (0 servings per day) and an 82% higher risk hyperuricemia (OR: 1.82, P = 0.003 for trend over quintiles). However, the strength of the association varied by gender: men in the highest SSB

group had a mean UA 0.52mg/dL higher than the reference group where women had 0.19mg/dL (Choi, 2008). Gao and colleagues undertook a very similar study with 4,073 participants from the 2001-2002 NHANES survey, and found a 0.22mg/dL mean difference in UA between the highest and lowest SSB intake quartiles. They also found a striking difference between the genders, as shown in Figure 2, reproduced here (Gao, 2007).

Figure 2.2: Difference in UA production in response to SSB intake by gender.



Some studies have looked at the impact of SSBs on both UA and HTN. In a study of U.S. adolescents (n=4,867), Nguyen and colleagues (2009) found that their mean UA was 0.18mg/dL greater in highest intake quartile (>36oz/day) compared to the lowest SSB intake quartile (0oz/day). This study also found higher BP in the highest intake group, as reported earlier in this review. Bobridge and colleagues (2013) conducted a similar study with Australian adolescents (n=814); however, they looked at total fructose intake rather than SSBs alone. They found a significant association between energy-adjusted fructose intake and UA in boys but not girls. While they found no association between fructose intake and BP, they did find an association between UA and BP in boys but not in girls. (Bobridge, 2013)

Evidence from animal studies shows that hyperuricemia, even in a fairly mild form, causes structural changes to the kidney and to renin and NO production that have a significant impact on blood pressure (Khosla, 2005; Mazzali, 2001). Studies that have used allopurinol to interrupt UA production and successfully lower BP further bolster this association (Feig, 2013; Perez-Pozo, 2010). There is also epidemiological and experimental evidence in humans to show that hyperuricemia is associated with hypertension (Alper, 2005; Sundstrom, 2005). However, the effect in large, general population studies is less pronounced than might be expected if extrapolated from animal models (Bobridge, 2013; Nguyen, 2009). Part of this difference could be attributed to the marked difference between the genders, as seen in the studies by Choi , Goa and Bobridge et al.

2.4 Vitamin D and Uric Acid

Vitamin D can be consumed in the diet or it can be synthesized in the skin, where UVB radiation converts 7-dehydrocholesterol to previtamin D, which spontaneously isomerizes to vitamin D_3 at normal body temperatures. Vitamin D_3 is also the form consumed from animal-based dietary sources, whereas plant foods provide vitamin D_2. A vitamin D binding protein transport the vitamin D_3 to the liver, where it is transformed to 25-hydroxyviatmin D (25(OH)D). 25(OH)D is the circulating form of vitamin D and plasma levels increase directly in response to dietary intake or skin synthesis. For this reason, serum 25(OH)D levels are used to determine vitamin D status. Controversy continues on what constitutes adequate serum vitamin D levels, but the estimated average requirement set by the Institute of Medicine remains 16ng/dL(40 nmol/L), which is based on the level needed for bone health. However, 25(OH)D is not known to

have any unique biological function. The kidneys are the main site of activation by the enzyme 1-α hydroxylase to the active form of vitamin D, 1,25-dihydroxyvitamin D (Stipanuk, 2013).

Vitamin D plays a critical role in calcium metabolism and bone health by facilitating absorption of calcium in the intestine, reabsorption in the kidneys and regulation of bone mineralization and remodeling. However, in recent years vitamin D receptors have been found in many other tissues throughout the body. Epidemiological evidence has suggested links with cancer, diabetes, and heart disease etiology and progression (Stipanuk, 2013). Vitamin D impacts blood pressure through its role in renin gene transcription and endothelial function (Min, 2013).

2.4.1 25-hydroxyvitamin D

Numerous studies have focused on the potential association between serum vitamin D levels as 25-hydroxyvitamin D (25(OH)D) and uric acid levels. Table 2.4 shows the results of observational studies that examined this relationship by measuring mean serum UA levels across 25(OH)D categories. In a cross-sectional study of UA and vitamin D status in middle-aged and elderly Chinese Han women (n=1,726), Peng et al found an association in post-menopausal (>55 years) but not pre-menopausal women (<55 years). The risk of elevated UA (>90th percentile) was nearly 2.5 times greater among post-menopausal women with suboptimal vitamin D status (<30ng/mL, <75nmol/L) than in women with sufficient vitamin D status (OR: 2.38; 95% CI: 1.47, 3.87) (Peng, 2012).

Several other studies did not examine the relationship between 25(OH)D and UA as a primary objective but did include measurements of this association among their reported results. Another cross-sectional study conducted by Alemzadeh and colleagues of 152 obese adolescents aged 13-19 years in the United States had the primary objective of ascertaining

whether there was an association between UA and parathyroid hormone levels (PTH) or PTH:25(OH)D ratio. With subjects stratified by vitamin D sufficiency (≥30ng/mL), insufficiency (20-29.9ng/mL) and deficiency, (<20ng/mL), Alemzadeh et al found UA levels increased as 25(OH)D levels decreased (5.4 ± 1.1, 6.0 ± 1.2, and 6.5 ± 1.2mg/dL respectively, p-value of 0.0007) (Alemzadeh, 2016).

Table 2.4: Observational studies of impact of difference in 25(OH)D on UA

First Author	Year	Country	Population	25(OH)D Levels, ng/mL	Serum Uric Acid, mg/dL	OR	Stat. Sig.
Cross-sectional Studies							
Alemzadeh	2016	U.S.	Adolescents, 13-18yrs, obese n=152	Sufficient, ≥30	5.4 ± 1.1		0.0007
				Insufficient, 20-29.9	6.0 ± 1.2		
				Deficient, <20	6.5 ± 1.2		
Peng	2012	China	Women, <55yrs n=858	>30	Normal	1.0 (0.53, 1.89)	NS
				≤30	Elevated, ≥5.3, >90th %tile		
			Women, >55yrs n=868	>30	Normal	2.38 (1.47, 3.87)	0.001
				≤30	Elevated, ≥6.0, >90th %tile		

Studies where vitamin D levels were controlled or manipulated all found no association between vitamin D and UA (Table 2.5). A 1974 animal study conducted by Al-Gauhari and colleagues, which was designed to assess the impact of vitamin D deficiency on protein metabolism, found no statistically significant difference in UA between rats fed a normal diet and a vitamin D-deficient diet and isolated from all sun exposure for 45 days (4.5±0.2 vs 4.8±0.2mg/dL, p-value NS) (Al-Gauhari, 1974). Intervention trials with humans which supplemented vitamin D and then measured uric acid also found no effect. Lind and colleagues

examined the metabolic impacts of supplementation of 1mg of alphacalcidol (1(OH)D) daily on 42 adults with hypertension in Sweden. They found no statistically significant difference in UA

Table 2.5: RTC and intervention studies of impact of difference in 25(OH)D on UA.

First Author	Year	Country	Population	Vitamin D Levels, ng/mL	Serum Uric Acid, mg/dL	Stat. Sig.
Animal Study						
Al-Gauhari	1974	Egypt	albino rats 50% male n=20	Deficient	4.5±0.2	NS
				Non-deficient	4.8±0.2	
Randomized Controlled Trials						
Brazier	2005	France	Women, >65yrs, baseline D ≤12 ng/mL, n=192	Supplement - 28.75 (23.25-35.75) M(IQ)	5.3 (4.0, 6.1)	NS
				Placebo - 10.75 (8.00-14.0)	4.9 (3.9, 6.1)	
Lind	1991	Sweden	Adults w/ HTN n=42, groups matched for age and sex	Treatment (1mg 1(OH)D/ day), before	5.7±1.3	NS
				Treatment, after	5.9±1.2	
				Control, before	5.7±0.8	
				Control, After	5.6±0.8	

levels between either group before and after the study (treatment before 5.7±1.3, after 5.9±1.2; control before 5.7±0.8, after 5.6±0.8mg/dL, p-value NS) or between the treatment and control groups. (Lind, 1991) In a year-long supplement trial, Brazier and colleagues assessed the safety of a calcium and vitamin D supplement (500mg $CaCO_3$, 400 IU cholecalciferol twice daily) in a group of French women over age 65 (n=192). The treatment group had 25(OH)D levels of 28.75ng/mL (23.25-35.75) (median, interquartile range), putting them in the insufficient range, and the placebo group of 10.75ng/mL (8.00-14.0), putting them in the deficient range. These two groups had UA levels of 5.3mg/dL (4.0, 6.1) and 4.9mg/dL (3.9, 6.1) respectively, with a p-value on the t-test of 0.603. Interestingly, more participants were outside the normal range (UA >5.7mg/dL) in the treatment group versus the placebo group (46 (52.3%) vs 32 (37.2%), χ^2 p-

value 0.05) (Brazier, 2005). Other studies which found an association between vitamin D and UA levels found the opposite effect (Alemzadeh, 2016; Peng, 2012).

Looking at the vitamin D/Uric Acid relationship with UA as the exposure variable (Table 2.6) did not change the relationships identified in the studies by Peng et al and Alemzadeh et al. Peng and colleagues defined elevated serum UA as greater than or equal to the 90th percentile for that group, 5.3mg/dL in pre-menopausal and 6.0mg/dL in post-menopausal women. There was no statistically significant difference in 25(OH)D levels in pre-menopausal women (40(33-51)ng/mL vs 41(36-54)ng/mL, median (interquartile range), p-value 0.364) but in post-menopausal women vitamin D levels were lower in women with elevated UA (40(32-58)ng/mL vs 35(28-57)ng/mL, p-value 0.006) (Peng, 2012). Alemzadeh and colleagues also defined elevated uric acid as ≥6.0mg/dL. They found that subjects with elevated uric acid levels had statistically significantly lower levels of 25(OH)D (19.2 ± 8ng/mL vs 23.2 ± 8.4ng/mL, p-value 0.0027). (Alemzadeh, 2016) Contrary to the findings of Peng and Alezadeh's studies, Hernández and colleagues did not find an association between UA and vitamin D. They conducted a cohort study of men over 50 (n=868) to look at uric acid and bone density. While this was a cohort study, for the purposes of this review it is a cross-sectional study because they only examined the 25(OH)D and UA levels in participants at baseline, so we only have a snap-shot association between UA and vitamin D levels. In that measurement, 25(OH)D levels in men did not differ (23.0 ± 8.1ng/mL verses 22.9 ± 8.1ng/mL, p-value 0.96) by UA level above or below the median (6.0mg/dL)for the cohort (Hernández, 2015). In contrast to the analysis where vitamin D was the exposure variable, the UA levels used between studies is the same in all but one case, with 6.0mg/dL as the cutoff between normal and elevated. Although this common factor makes the results much easier to compare, the relationship between higher UA levels and 25(OH)D levels remains unclear.

Table 2.6: Observational studies of impact of difference in UA on 25(OH)D.

First Author	Year	Country	Population	Serum Uric Acid, mg/dL	25(OH)D Levels, ng/mL	Stat. Sig.
Cross-sectional Studies						
Alemzadeh	2016	United States	Adolescents, 13-18yrs, obese n=152	Normal, <6.0	23.2 ± 8.4	0.0027
				Elevated, ≥6.0	19.2 ± 8	
Hernández	2015	Spain	Men, >50yrs n=868	below median, <6.0	23.0±8.1	0.96
				above median, >6.0	22.9±8.1	
Peng	2012	China	Women, <55yrs n=858	Normal	40(33, 51) M(IQ)	0.364
				Elevated, ≤5.3, <90th %tile	41(36, 54)	
			Women, >55yrs n=868	Normal	40(32, 58)	0.006
				Elevated, ≤6.0, <90th %tile	35(28, 57)	

2.4.2 1,25-Dihydroxyvitamin D and 1-α hydroxylase

Fewer studies have examined the relationship between 1,25(OH)D and UA, but they have consistently found an association (Table 2.7). Two animal studies explored the hypothesis that high uric acid levels interfere with vitamin D by inhibiting the enzyme, 1-α hydroxylase, responsible for the final step in converting vitamin D to its active form, calcitriol ($1,25(OH)_2D$). Hsu and colleagues (1991) conducted an animal study to determine whether purine metabolites, including UA, would impact calcitriol metabolism. They found that infusion of male rats (5 per group) with 20 mL of 50mg/dL sodium urate solution led to a statistically significant decrease in calcitriol concentration compared to saline-infused controls (control: 229.58±12.22 vs treatment: 147.68±10.66pmol/L). Similarly, Chen and colleagues (2014) examined 25 male Sprague–Dawley rats divided into 4 groups. One group was treated with a substance that elevated uric acid levels by inhibiting uricase, one group was treated with a substance that reduced uric acid by inhibiting xanthine oxidase, one group was treated with both substances and one group served as an untreated control. All rats were killed 24 hours after treatment,

calcitriol levels and the ratio of 25(OH)D to 1,25(OH)D was measured and kidneys were examined. The hyperuricemic rats were found to have lower serum calcitriol (control: 836±56 vs hyperuricemic: 708±73 pmol/L, p-value 0.0055), lower 1,25(OH)D:25(OH)D ratios in serum (p-value 0.021) and lower levels of 1-α hydroxylase in their kidneys as measured by immunofluorescence compared to controls (5.7 vs 11.4 in controls, arbitrary units, p-value 0.015). Their levels of 25(OH)D were also lower but this difference was not statistically significant (p-value 0.095) (Chen, 2014).

Chen and colleagues also preformed an in vitro cell culture study of human kidney tubules treated with different doses of uric acid and examined them after 24 hours. The UA-treated tubule cells had 50% less 1-α hydroxylase mRNA compared to untreated controls (p-value-0.01). While all treated cells showed reduced mRNA, only the cells treated with the highest dose, 10mg/dL of UA, had significantly lower levels of 1-α hydroxylase protein in the 24 hour time period (p-value- 0.02). Cell changes were also examined with 10 mg/dL of uric acid over different lengths of time, up to 24 hours. Expression of mRNA decreased over 6 hours and then remained steady at 62% of control level (p-value-0.049). Protein expression reached its lowest point after 16 hours at 23% of control and rebounded slightly to 36% after 24 hours (p-value 0.02). Chen et al suspected that the mechanism involved was that uric acid activated nuclear factor κ-B which in turn suppressed 1-α hydroxylase, based on previous studies. To test this, they pre-treated cell cultures with a nuclear factor κ-B inhibitor and found that the impact of uric acid on 1-α hydroxylase was eliminated.

Table 2.7: Animal and cellular studies of impact of UA on 1,25-Dihydroxyvitamin D and 1-α hydroxylase.

First Author	Year	Country	Population	1,25(OH)D pmol/L	Serum Uric Acid, mg/dL	1-α hydroxylase, arbitrary units	Stat. Sig.
Animal Studies							
Hsu	1991	U.S.	Rats 5 per group	229.58±12.22	1.1±0.1		<0.001
				147.68±10.66	4.2±0.3		
Chen	2014	U.S.	Rats n=25	836 ± 56	1.9 ± 0.1		0.0055
				708 ± 73	3.3 ± 1.4		
			Rats n=25		1.9 ± 0.1	11.4	0.015
					3.3 ± 1.4	5.7	
Cellular Studies							
Chen	2014	U.S.	Human Kidney Tubules		Control	8.8	0.002
					10	3 .3	

2.4.3 Parathyroid Hormone (PTH)

Studying the relationship between UA and 1-α hydroxylase in humans presents a challenge because 1,25(OH)D is difficult to measure in humans due to its short half life. It is also only present when called for by the presence of parathyroid hormone. However, if 1,25(OH)D production does not increase in response to PTH, PTH levels will continue to rise, thus making elevated PTH levels a proxy measurement for low 1,25(OH)D levels, either because not enough precursor, 25(OH)D, is present or because 1-α hydroxylase has been inhibited (Chen, 2014). Table 2.8 summarizes the results of studies that looked at the association between PTH and UA. Chen et al performed a cross-sectional analysis on the 9005 participants in NHANES 2003-2006 over the age of 18 without kidney disease and with complete data including serum PTH and uric acid. Many potential confounders were included in the analysis, including age, sex, race and ethnicity, diabetes, hypertension, BMI, and serum 25(OH)D concentration. The odds ratio for elevated PTH (>65pg/mL) for every 1 mg/dL increase in uric acid was 1.15 (95% CI: 1.08-1.22; $P < 0.0001$), adjusted for confounders. This relationship was independent of 25(OH)D levels. These

results suggest an association between elevated UA levels and inhibition of 1-α hydroxylase, leading to low 1,25(OH)D and then to elevated PTH.

Some of the cross-sectional studies discussed in earlier sections of this review examined PTH and UA levels. This relationship was the focus of the study conducted by Alemzadeh et al (2016). They found that adolescents with elevated UA (≥6.0mg/dL) had higher mean PTH levels than those with normal UA (50.5±12.3 vs 35.7±14.7pg/mL, p-value <0.0001). They also found those with elevated UA had a higher PTH:25(OH)D ratio(1.35±0.9 vs 0.75±0.5, p-value <0.0001) suggesting that they were making less 1,25(OH)D from the same amount of 25(OH)D. Hernández and colleagues (2015) also found marginally statistically significant higher PTH levels (52.8 (42.1–65.0)pg/mL vs 50.9 (39.3–63.2)pg/mL, median(interquartile range), p-value-0.05) for those above versus below the median UA level of 6.0mg/dL.

An earlier cross-sectional study of PTH and UA, conducted by Hui et all (2012), found the odds ratio for elevated UA levels (>7mg/dL in men, >5.7mg/dL in women) to increase by quintile of PTH (OR 1.0, 1.07, 1.22, 1.36, 1.39, p-value 0.03 for trend) in the fully adjusted model, which accounted for age, sex, race, BMI, use of diuretics, allopurinol and uricosuric agents, hypertension, serum levels of calcium, alkaline phosphatase, phosphorus, vitamin D; and glomerular filtration rate as well as total daily intake of alcohol, energy, protein, sugar, and caffeine. Chin and colleagues (2015) conducted a similar cross-sectional study in Malaysian men (n=320) and found that elevated PTH (> 65 pg/mL) levels were positively associated with hyperuricemia(>7.0mg/dL), but the effect size was very small and may not be clinically important (OR 1.045 (1.017-1.075) p-value 0.002). Their model was adjusted for age, body mass index, ethnicity, serum 25(OH)D, blood urea nitrogen, and creatinine level. A study by Paik and

Table 2.8: Observational studies of association between PTH and UA.

First Author	Year	Country	Population	PTH Levels pg/mL	Serum Uric Acid, mg/dL	OR	Stat. Sig.
Cross-Sectional Studies							
Alemzadeh	2016	U.S.	Adolescents, 13-18yrs, obese n=152	35.7 ± 14.7	Normal, <6.0		<0.0001
				50.5 ± 12.3	Elevated, ≥6.0		
Chen	2014	U.S.	Adults n=9773	≤65	5.2± 1.4	1.21 (1.14, 1.28) per 1 mg/dL (59.48 µmol/L) UA	< 0.0001
				> 65	5.9 ±1.6		
Chin	2015	Malaysia	Men, >20yrs, n=380	Normal PTH, 10-65	Normal, ≤7.0	1.05 (1.02, 1.08)	0.002
				Increased PTH, > 65	Hyperurice mic, >7.0		
Hernández	2015	Spain	Men, <50yrs n=868	50.9 (39.3–63.2)	below median, <6.0		0.05
				52.8 (42.1–65.0)	above median, >6.0		
Hui	2012	U.S.	Adults, >18yrs n= 8,316	Quantile 1, 6-27	Normal vs Elevated, >7.0 in men, >5.7 in women	1, referent	0.03 for trend
				Q 2, 28-36		1.07 (0.77, 1.49)	
				Q 3, 37-44		1.22 (0.81, 1.82)	
				Q 4, 45-58		1.36 (1.03, 1.80)	
				Q5 59-491		1.39 (1.03, 1.88)	
Paik	2012	U.S.	Adults, White, n=4026	Increase of 4.9(2.7-7.0) in Q4 vs Q1	Quartile1: <4.3		<0.01 for trend
			Adults, Black, n=1792	Increase of 5.5(3.0-8.1) in Q4 vs Q1	Quartile 4: ≥6.1		<0.01 for trend
			Adults, Mexican-American, n=1834	Increase of 4.8(1.5-8.1) in Q4 vs Q1			0.02 for trend

colleagues (2012) investigating the impact of various factors on PTH levels found that PTH levels differed by race. They calculated the increase in PTH by quartiles of UA and found that, in the fourth compared to the first quartile, non-Hispanic Whites had an increase of 4.9pg/mL (95% CI: 2.7-7.0pg/mL) (p-value for trend <0.01), Blacks had an increase of 5.5pg/mL (95% CI: 3.0-8.1pg/mL) (p-value for the trend <0.01), and Mexican-Americans had an increase in PTH of 4.8pg/mL (95% CI: 1.5-8.1pg/mL) (p-value for the trend 0.02). Despite their small differences, all groups showed an association between PTH and UA. The results of these three studies support the hypothesis that UA raises PTH levels via inhibition of 1-α hydroxylase, although caution must be used in interpreting the results of cross-sectional studies.

2.5 Discussion

A positive association between SSB intake and elevated BP and HTN is supported by the literature, but the association is not very strong in most studies (Barrio-Lopez, 2013; Bremer, 2009; Cohen, 2012; Duffey, 2010; Ejtahed, 2015; Jayalath, 2015; Mirmiran, 2015; Nguyen, 2009; Sayon-Orea; 2015; Wang, 2013). Marked heterogeneity, as evidenced by wide 95% confidence intervals (Barrio-Lopez, 2013; Kim, 2012; Mirmiran, 2015), may have contributed to this and suggests that while the impact of SSB intake on BP may be small at the population level, for some people it may play a much larger role. UA production differs between men and women in response to SSB intake (Bobridge, 2013; Choi, 2008; Gao, 2007), which provides one possible mechanism for that heterogeneity. Another possible difference being explored in this review is whether vitamin D status interacts with SSB intake or the UA produced in response to high SSB intake.

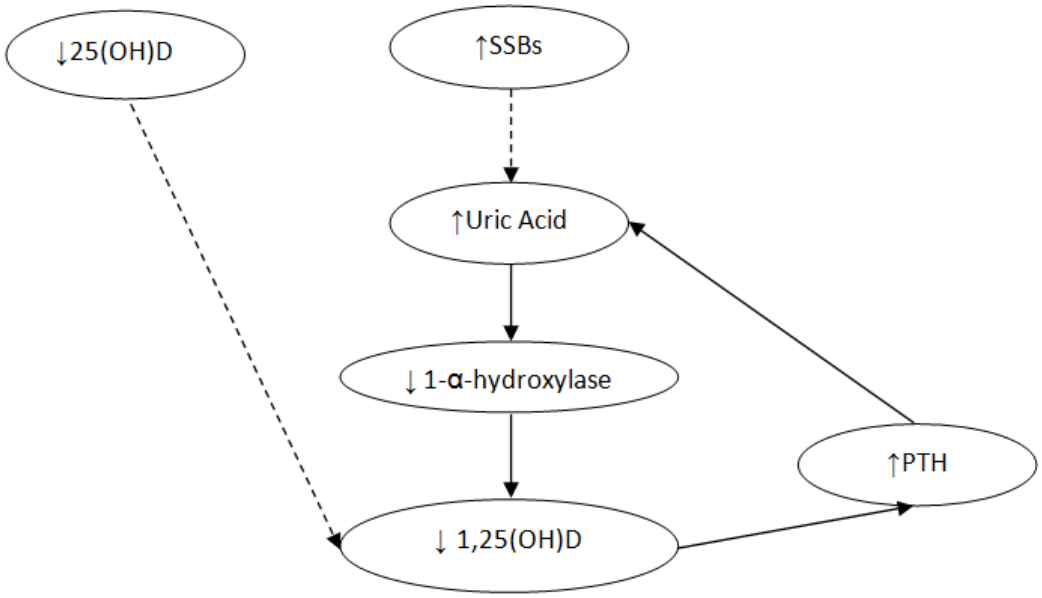
Of studies examining a possible relationship between vitamin D and uric acid, those looking at 25(OH)D are the most numerous and the most inconclusive (Alemzadeh, 2016; Al-Gauhari, 1974; Brazier, 2005; Hernández, 2015; Peng, 2012). Looking at analyses with either 25(OH)D or UA as the outcome variable, associations were found in post-menopausal Chinese women (Peng, 2012) and a mixed gender group of American adolescents (Alemzadeh, 2016). No association was found in pre-menopausal Chinese women (Peng, 2012), Spanish men over age 50 (Hernández, 2015), or French women over age 65 taking part in a supplement trial (Brazier, 2005). An animal study examining 25(OH)D and UA also found no association (Al-Gauhari, 1974). The only unifying feature seems to be that studies conducted specifically to look at the relationship between 25(OH)D and uric acid, or the related topic of PTH and UA, found an association (Alemzadeh, 2016; Peng, 2012) and those that looked at the two factors incidentally did not (Brazier, 2005; Hernández, 2015). One possible cause of this could be publication bias. Studies with null findings are less likely to be published, so it may have been easier for studies that did not find an association to be published if this null result was not their primary outcome measurement. While two of the studies examining metabolic changes in general did have overall null findings, their primary objective was to examine the safety of supplements, so observing no change in metabolic parameters can be regarded as a success in these cases. Therefore, there may be more data showing no association that were not available for this review, which would further weaken the case that there is a true association between 25(OH)D and uric acid.

Few studies have investigated the relationship between 1,25(OH)D, 1-α hydroxylase or PTH and UA, but these studies have consistently found an association (Alemzadeh, 2016; Chen, 2014; Chin, 2015; Hernández, 2015; Hsu, 1991; Hui, 2012; Paik, 2012). On the other hand, 1,25(OH)D and 1-α hydroxylase studies have only been conducted in animal models and in vitro (Chen, 2014; Hsu, 1991). The studies looking at PTH are all cross-sectional (Alemzadeh, 2016;

Chen, 2014; Chin, 2015; Hernández, 2015; Hui, 2012; Paik, 2012), so it is impossible to assess temporality in the relationship. Chen and colleagues made the case that elevated PTH levels represented inhibition of 1-α hydroxylase by high UA levels (Chen, 2014). However, Hui and Chin and colleagues had a different interpretation. They saw the association of PTH and UA as evidence that elevated PTH levels inhibit the urinary excretion of UA, and therefore hyperparathyroidism can lead to hyperuricemia (Chin, 2015; Hui, 2012;). They based this hypothesis on other studies showing the UA-lowering effects of either treating primary hyperparathyroidism with parathyroidectomy (Hisatome, 1992) or cessation of synthetic PTH used to treat bone disease (Miller, 2007). This hypothesis opens up the possibility of a positive feedback loop where low 25(OH)D levels cause an increase in PTH, which then blocks the excretion of UA, which inhibits the production of 1,25(OH)D and further increases PTH. On the other hand, most of the studies that looked at the PTH/UA relationship controlled for 25(OH)D levels (Alemzadeh, 2016; Chen, 2014; Chin, 2015; Hui, 2012; Paik, 2012), which suggests that there may have been some other factor in the initial rise in UA (Figure 3). Further study will be needed to determine whether that other factor might be sugar-sweetened beverage intake. The practical implications of this proposed pathway is that both lowering SSB intake as well as vitamin D repletion may be needed to short-circuit this pathway and rule it out as a contributor to elevated blood pressure.

The purpose of this review was to determine whether available evidence supports a possible interaction between vitamin D status and sugar-sweetened beverage intake in their impact on blood pressure and hypertension risk. Could good vitamin D status protect against the impact of high SSB intake on HTN? Based on the results of the review, this association seems unlikely (Al-Gauhari, 1974; Brazier, 2005; Hernández, 2015; Peng, 2012). If high SSB intake can

Figure 2.3: Proposed pathway.



Either low 25(OH)D levels or high sugar-sweetened beverage intake could be the first step in the proposed circular pathway where elevated uric acid leads to suppressed 1-α-hydroxylase, low 1,25(OH)D levels, and thus to elevated PTH levels, which further elevates uric acid.

suppress 1-α hydroxylase by raising UA, vitamin D would still be unable to fulfill its role in BP regulation, even if high levels of 25(OH)D were present. Could high SSB intake nullify the positive effect of vitamin D repletion? This scenario seems more likely. If subjects with high SSB intake where unable to make use of the 25(OH)D, it would be unable to lower their blood pressure. Even if other subjects did experience a BP-lowering effect, that relationship would be obscured by the within-group variability introduced by not stratifying the results according to SSB intake. However, this is merely conjecture, as no published research looks at all the steps in this pathway. The proposed study will examine all of these factors - SSBs, UA, vitamin D, PTH and HTN - in the same subjects to test the hypothesis that an interaction between SSB and vitamin D influences the association of each variable with HTN. Understanding these relationships will bring us one step closer to understanding the etiology of primary hypertension.

CHAPTER 3

HYPOTHESES AND SPECIFIC AIMS

1. Sugar-sweetened beverage intake will be positively associated with SBP in men and women aged 20-74 years from NHANES 2003-2006. Uric acid will play a mechanistic role in that association.

    Specific Aim 1: SSB intake will be positively associated with elevated systolic blood pressure when assessed with linear and logistic regression adjusted for age, race/ethnicity, BMI, leisure time physical activity, smoking and alcohol use.

    Specific Aim 2: SSB intake will be positively associated with serum uric acid (mg/dL).

    Specific Aim 3: Serum uric acid (mg/dL) concentration will be positively associated with blood pressure and/or HTN.

2. Vitamin D (25(OH)D, nmol/L) levels will be inversely associated with SBP. High serum uric acid (mg/dL) will attenuate this association; therefore the protective effect of good vitamin D status on blood pressure and/or HTN will be less significant in subjects who drank more SSBs.

    Specific Aim 1: 25(OH)D (nmol/L) concentration will be negatively associated with elevated systolic blood pressure when assessed with linear and logistic regression, when adjusted as above.

    Specific Aim 2: Serum PTH (pg/mL) concentration will be positively associated with blood pressure and/or HTN when controlling for 25(OH)D (nmol/L).

    Specific Aim 3: Serum UA (mg/dL) will be positively associated with PTH (pg/mL).

    Specific Aim 4: Stratified analysis by SSB intake quartile will show that the protective effect of higher 25(OH)D (nmol/L) concentration on blood pressure and/or HTN is less significant in subjects in the highest SSB intake quartile.

CHAPTER 4

METHODS

4.1 Study Population

This study examined participants in the 2003-2004 and 2005-2006 National Health and Nutrition Survey (NHANES), the only two data releases that include serum PTH measurements. NHANES collects cross-sectional data from a representative sample of the United States population of non-institutionalized civilians in order to assess health and lifestyle trends. The survey is conducted in three phases consisting of a household screener to determine eligibility; an interview, which includes gathering demographic, health and dietary information, and a physical examination at which blood and urine samples were collected. The survey subjects included in this analysis are men and women aged 20-74 who did not report a diagnosis of hypertension, weak or failing kidneys, diagnosed diabetes or the use of uric-acid-lowering agents and who completed the physical examination (n=2,875). Previous research on uric acid suggests that testosterone may play a role in the uric acid formation and that estrogen may have a protective effect against the development of hyperuricemia (Gao, 2007; Choi, 2008). Therefore, this study included subgroup analysis by gender and, if any difference between the genders was found, further subgroup analysis by pre- and post-menopausal women.

Prevalent cases of hypertension, defined as being told by a doctor that you have elevated blood pressure at least two times or being prescribed antihypertensive medication, were excluded to prevent medication or other blood pressure lowering therapies from impacting the outcome variable. Subjects taking allopurinol and other uricosic agents were excluded for the same reason. Kidney failure impacts the production of 1,25(OH)D; therefore, subjects who answered yes to the question “Have you ever been told you have weak or failing

kidneys" were also excluded. The age range for inclusion begins at 20 because data gathered from the alcohol use questionnaire, an important confounder for HTN, are only included in the public release for subjects 20 years or above. Subjects over the age of 75 were also excluded, since increasing age is strongly correlated with HTN. Statistics from the American Heart Association show that 76.4% of men and 79.9% of women above age 75 have HTN (Mozaffarian, 2015). Age may be such an important predictor of HTN that it has the potential to overwhelm the impact of other factors, like SSB use and vitamin D status.

4.2 Measurements

Blood pressure was measured during the physical examination at the Mobile Examination Center. Participants sat quietly for five minutes after which three or four blood pressure measurements were taken with a calibrated sphygmomanometer (Calibrated® V-Lok® cuff, Latex Inflation Bulb, Air-Flo® Control Valve, Baumanometer® calibrated mercury true gravity wall model pressure gauge) by a trained examiner (CDC, 2003). Details on the examination procedure can be found in the National Health and Nutrition Examination Survey Physicians Examination Procedures Manuel (CDC, 2003).

Serum 25(OH)D was assessed using the Diasorin (formerly Incstar) (Stillwater, MN) 25-hydroxyvitamin D antibody assay. An Elecsys 1010 analyzer (Roche Diagnostics, Indianapolis, IN) was used to measure serum PTH by electrochemiluminescent immunoassay. Serum uric acid was measured with a Beckman Synchron LX20 analyzer (Brea, CA). Details on the collection, storage and analysis procedure can be found in the National Health and Nutrition Examination Survey Laboratory Procedures Manuel (CDC, 2004).

BMI was calculated as kg/m^2 from height and weight measurements taken during the physical examination. All other covariate data were collected by survey. SSB intake was collected

by food frequency questionnaire mailed to participants and filled out by them. Examples of all questionnaires used can be found at https://www.cdc.gov/nchs/nhanes/nhanes2003-2004/questionnaires03_04.htm.

4.3 Statistical Analysis

All statistical analyses were conducted using STATA/IC version 14.2 statistical software (STATA Corporation, College Station, TX, USA). Means, standard deviations and ranges were used to define characteristics of the study population for continuous variables and number and percent were used for categorical variables. T-tests and chi-square tests were used to determine if there was a statistically significant difference in means and proportions for covariates between subjects with elevated (≥120mmHg) or normal (<120mmHg) systolic blood pressure.

All linear and logistic regressions used the same three models. Model 1 controlled for age and gender. Model 2 also included race/ethnicity and BMI and Model 3 had smoking, alcohol use, and physical activity added. In addition to these three models, subpopulation analysis by gender was also performed using Model 3. Both linear and logistic regression were used to assess the associations between SSB intake and BP, SSB intake and UA, UA and BP, vitamin D status and BP, PTH and BP, and PTH and UA, using all three models. The regressions examining PTH with BP and UA were also run controlling for vitamin D status. Education level, household income, poverty to income ratio, serum vitamin C and waist circumference were all also evaluated for inclusion in the final models. They were evaluated to see if their univariate association with SBP had a p-value less than 0.20. If so, the fully adjusted model, Model 3, was run with and without each of the covariates and those whose addition resulted in a greater than 10% change in the beta coefficients for SBP measurements would have been included in the final model. None of these additional variables met that standard.

Blood pressure was assessed both as a continuous variable and also categorically, as defined above. Subjects whose SBP was more than two standard deviations away from the mean for the population were excluded from this study (n=164). Vitamin D was categorized by serum 25(OH)D concentration as less than 50nmol/L, 50-75nmol/L, and greater than 75nmol/L. SSBs categories were determined by quartiles of intake. Physical activity was categorized by metabolic activity of task (MET) scores divided into quartiles with a fifth group, the reference group, being those who reported no leisure time physical activity. Smoking was categorized as current smokers or non-smokers. Alcohol use was divided by the average number of drinks on days when alcohol was drunk into categories of non-drinker, one to two drinks, three to four drinks or five or more. The two continuous variables used were UA and PTH. UA was acceptably normally distributed, but PTH was skewed, so a log transformed variable of PTH was used in the regressions. Where PTH was the outcome variable, the anti-log was presented.

In order to answer the question of whether high SSB intake modifies the impact of low vitamin D levels on blood pressure or vise versa, a possible interaction between SSB and 25(OH)D status on blood pressure or hypertension was investigated by means of linear regression and logistic regression. Both variables were included in the same model with SBP as the outcome variable to see if controlling for both modified the associations. Evidence of an interaction was also investigated using a multiplicative term of SSB intake and vitamin D as continuous variables. Stratified analyses by vitamin D category and SSB intake quartile were also conducted using Model 3.

CHAPTER 5

RESULTS

Table 5.1 shows the unadjusted study population characteristics divided by systolic blood pressure above or below the elevated range. Those with elevated SBP were older, heavier, had lower mean serum 25(OH)D but higher UA and PTH, and were more likely to be men and African American. There was a difference of borderline significance in physical activity levels and no difference in mean SSB intake, smoking or alcohol use.

Linear and logistic regression models exploring the association between SSB and SBP are shown in Table 5.2. In linear regression of the total population, SSB intake was positively associated with blood pressure. In Model 3, there was an increase of 1.40mmHg (95% CI: 0.22-2.57, p-value 0.02) in the fourth quartile of SSB intake relative to the first, after adjustment for age, gender, race, BMI, physical activity, alcohol use and smoking. In logistic regression, increased SSB intake was associated with a significant 40% increase in elevated SBP risk, but this association was attenuated in the fully adjusted model and was no longer significant. Subgroup analysis by gender of the fully adjusted model revealed a striking difference between men and women. Men showed no statistically significant association in any quartile in either linear or logistic regression. However, in women there was a statistically significant increase of 1.55mmHG (95% CI: 0.05-3.05, p-value 0.042) in the third quartile and 1.78mmHG (95% CI: 0.18-3.37, p-value 0.029) in the fourth quartile. In logistic regression, risk of elevated SBP was 68% higher among subjects in the fourth SSB intake quartile compared to those in the first quartile (OR: 1.68, 95% CI: 1.12-2.50, p-value 0.011).

Table 5.1: Subject characteristics by normal versus elevated SBP (n=3,287).

Systolic Blood Pressure	**≤120mmHg (n=1,875)**	**>120mmHg (n=1,000)**	**p-value**
Mean (SD)			
Age	36.6 (12.8)	45.4 (14.8)	**0.000**
BMI	26.9 (5.5)	28.6 (5.5)	**0.000**
SSB Intake (servings/day)	1.07 (1.7)	1.07 (1.6)	0.964
Serum 25(OH)D (nmol/L)	62.8 (23.8)	58.2 (22.0)	**0.000**
Serum Uric Acid (mg/dL) (n=2,862)	4.8 (1.3)	5.5 (1.3)	**0.000**
Serum PTH (pg/mL) (n=2,872)	38.6 (17.9)	44.6 (19.9)	**0.000**
Count (row %)			
Gender			**0.000**
Male	711 (53.7)	614 (46.3)	
Female	1,164 (75.1)	386 (24.9)	
BMI			**0.000**
Normal (Under 25kg/m^2)	784 (74.2)	272 (25.8)	
Overweight (25-29.9kg/m^2)	640 (63.1)	374 (36.9)	
Obese (Over 30 kg/m^2)	451 (56.0)	354 (34.98)	
Race			**0.016**
White	1,008 (65.2)	537 (34.8)	
Black	311 (60.0)	207 (40.0)	
Hispanic	469 (69.0)	211 (31.0)	
Other	87 (65.9)	45 (34.1)	
Physical Activity [1]			0.056
Inactive	1,275 (66.7)	637 (33.3)	
MET Quartile 1	145 (60.7)	94 (39.3)	
MET Quartile 2	158 (67.5)	76 (32.5)	
MET Quartile 3	147 (61.3)	93 (38.8)	
MET Quartile 4	150 (60.0)	100 (40.0)	
Smoking			0.778
Current smoker	471 (65.8)	256 (35.2)	
Non-smoker	1,404 (65.4)	744 (34.6)	
Alcohol Use[2]			0.062
Non-drinker	441 (68.3)	205 (31.7)	
1 to 2 drinks/ day	855 (64.6)	468 (35.4)	
3-4 drinks/ day	362 (66.4)	183 (33.6)	
5 or more drinks/day	217 (60.1)	144 (39.9)	

1 Physical activity: Active=has engaged in moderate or vigorous leisure-time physical activity in the last 30 days, Inactive=has not engaged in leisure-time physical activity in last 30 days
2 Alcohol Use, n=1,313: Average number of drinks on days when alcohol was consumed

Table 5.2: Linear and logistic regression analysis of the association of SBP with SSB intake.

SSB Intake Servings per day	**Q1 0-0.08**	**Q2 0.09-0.42**	**Q3 0.43-1.28**	**Q4 1.29-14**
Whole Population (n=2,875)				
Model 1	Reference			
Beta (95% CI)	0	1.15 (0.01, 2.28)	1.66 (0.51, 2.82)	2.31 (1.13, 3.49)
p-value		**0.048**	**0.005**	**0.000**
OR (95% CI)	1.00	1.30 (1.02, 1.64)	1.25 (0.98, 1.59)	1.40 (1.09, 1.78)
p-value		**0.031**	0.066	**0.008**
Model 2				
Beta (95% CI)	0	0.75 (-0.37, 1.86)	1.17 (0.03, 2.30)	1.54 (0.37, 2.70)
p-value		0.188	**0.045**	**0.010**
OR (95% CI)	1.00	1.23 (0.97, 1.57)	1.18 (0.93, 1.51)	1.28 (0.99, 1.64)
p-value		0.086	0.178	0.057
Model 3				
Beta (95% CI)	0	0.73 (-0.38, 1.84)	1.06 (-0.08, 2.20)	1.40 (0.22, 2.57)
p-value		0.199	0.069	**0.020**
OR (95% CI)	1.00	1.23 (0.97, 1.56)	1.17 (0.91, 1.50)	1.26 (0.98, 1.63)
p-value		0.092	0.225	0.074
Men (n=1,325)				
Model 3				
Beta (95% CI)	0	1.10 (-0.70, 2.91)	0.86 (-0.91, 2.62)	0.99 (-0.80, 2.77)
p-value		0.231	0.340	0.277
OR (95% CI)	1.00	1.24 (0.87, 1.75)	1.06 (0.76, 1.49)	1.03 (0.73, 1.45)
p-value		0.232	0.737	0.858
Women (n=1,550)				
Model 3				
Beta (95% CI)	0	0.69 (-0.71, 2.09)	1.55 (0.05, 3.05)	1.78 (0.18, 3.37)
p-value		0.336	**0.042**	**0.029**
OR (95% CI)	1.00	1.27 (0.90, 1.81)	1.39 (0.95, 2.05)	1.68 (1.12, 2.50)
p-value		0.175	0.088	**0.011**

Model 1: Adjusted for age and gender
Model 2: Adjusted for Model 1 plus race and BMI category
Model 3: Adjusted for Model 2 plus leisure time physical activity, smoking status, and alcohol

Further subgroup analysis was performed with women divided into pre- and post-menopausal groups based on self-report of menopause or hysterectomy on the questionnaire portion of NHANES. No statistically significant association between SSB intake and SBP was found in post-menopausal women (Table 5.3). However, in pre-menopausal women, the

Table 5.3: Linear and logistic regression analysis of the association of SBP with SSB intake in women.

SSB Intake Servings per day	**Q1 0-0.08**	**Q2 0.09-0.42**	**Q3 0.43-1.28**	**Q4 1.29-14**
Women, controlling for menopause status (n=1,548)				
Model 3				
Beta (95% CI)	0	0.60 (-0.80, 2.00)	1.48 (-0.02, 2.98)	1.64 (0.05, 3.24)
p-value		0.402	0.053	**0.044**
OR (95% CI)	1.00	1.27 (0.89, 1.78)	1.39 (0.95, 2.04)	1.68 (1.13, 2.51)
p-value		0.182	0.090	**0.011**
Post-menopause (n=374)				
Model 3				
Beta (95% CI)		-2.08 (-5.05, 0.90)	0.05 (-3.61, 3.71)	1.34 (-2.67, 5.34)
p-value		0.171	0.977	0.512
OR (95% CI)		0.73 (0.42, 1.27)	1.35 (0.69, 2.65)	1.76 (0.83, 3.73)
p-value		0.265	0.376	0.138
Pre-menopause (n=1,174)				
Model 3				
Beta (95% CI)	0	1.73 (0.15, 3.30)	2.02 (0.39, 3.65)	2.30 (0.58, 4.02)
p-value		**0.032**	**0.015**	**0.009**
OR (95% CI)	1.00	1.87 (1.17, 2.98)	1.58 (0.96, 2.59)	2.05 (1.24, 3.41)
p-value		**0.008**	0.071	**0.005**

Model 3: Adjusted for age, gender, race, BMI category, leisure time physical activity, smoking status, and alcohol

positive association between SSB intake and SBP was much stronger than in women as a whole, even after controlling for menopause status. In linear regression, pre-menopausal women showed a statistically significant increase in SBP in all three quartiles relative to the lowest quartile; 1.73mmHG in quartile 2, 2.02mmHg in quartile 3 and 2.30mmHg in quartile 4. In quartile 4, pre-menopausal women also showed a statistically significant 105% increase in risk of elevated SBP (OR: 2.05, 95% CI: 1.24-3.41, p-value 0.005).

Serum UA was found to be associated with SBP, with a 0.83mmHg increase in SBP per mg/dL increase in UA concentration in the fully adjusted model (Beta: 0.83mmHg, 95% CI: 0.44-1.21, p-value 0.000). This association is statistically significant but the effect size is very small, raising doubts about its biological significance. However, logistic regression analysis revealed a 14% increase in risk of elevated SBP in the third model (OR: 1.14, 95% CI: 1.05-1.24, p-value 0.001), which may be clinically relevant. As with the association between SSBs and SBP, the

analysis is more interesting when separated by gender. No significant association between serum UA and SBP was observed in men (OR: 0.99, 95% CI: 0.9-1.11, p-value: 0.992), but risk of elevated SBP was 32% higher per mg/dL increase in UA in women (OR: 1.32, 95% CI: 1.14-1.52, p-value: 0.000). Pre-menopausal women in particular showed an association, with a 1.35mmHg increase in SBP and a 47% increased risk of elevated SBP per mg/dL increase in UA (Beta: 1.35, 95% CI 0.73-1.98, p-value 0.000; OR 1.47, 95% CI 1.23-1.76, p-value 0.000), whereas post-menopausal women showed no statistically significant association in linear or logistic regression. No association was found between SSB intake and serum UA in any intake quartile in any of the three models, either in the whole study population or in men and women separately.

Vitamin D in the form of 25(OH)D concentration was inversely associated with blood pressure in the fully-adjusted model (Table 5.4). SBP was 1.74mmHg lower in those with levels over 75nmol/L compared to those with 50nmol/L or less (Beta: -1.74, 95% CI: -2.87- -0.61, p-value 0.003). In logistic regression models, the risk of elevated SBP was 30% lower in those in the highest 25(OH)D category versus those in the lowest category (OR: 0.70, 95% CI: 0.55-0.90, p-value: 0.005). The relationship between 25(OH)D concentration and SBP was largely unchanged with subgroup analysis by gender, however, the association was no longer statistically significant. Further subgroup analysis again revealed that pre-menopausal women had a much stronger association than post-menopausal women, with a decrease of 1.66mmHg in the middle 25(OH)D group (Beta: -1.66, 95% CI: -3.12, -0.19, p-value 0.026) and 1.80mmHg in the highest group relative to the lowest group (Beta: -1.80, 95% CI: -3.41, -0.19, p-value 0.029). In logistic regression, pre-menopausal women in the highest 25(OH)D group also showed a borderline significant 38% decrease in risk of elevated SBP (OR: 0.62%, 95% CI: 0.38-1.01, p-value 0.053).

Table 5.4: Linear and logistic regression analysis of the association of SBP with Vitamin D.

25(OH)D (nmol/L)	<50nmol/L	50-75nmol/L	>75nmol/L
Whole Population (n=2,875)			
Model 1	Reference		
Beta (95% CI)	0	-1.95 (-2.89, -1.01)	-3.19 (-4.22, -2.16)
p-value		**0.000**	**0.000**
OR (95% CI)	1.00	0.75 (0.62, 0.91)	0.58 (0.47, 0.73)
p-value		**0.003**	**0.000**
Model 2			
Beta (95% CI)	0	-0.82 (-1.79, 0.16)	-1.66 (-2.79, -0.54)
p-value		0.100	**0.004**
OR (95% CI)	1.00	0.88 (0.71, 1.08)	0.72 (0.57, 0.93)
p-value		0.206	**0.010**
Model 3			
Beta (95% CI)	0	-0.84 (-1.82, 0.14)	-1.74 (-2.87, -0.61)
p-value		0.093	**0.003**
OR (95% CI)	1.00	0.86 (0.70, 1.06)	0.70 (0.55, 0.90)
p-value		0.166	**0.005**
Men (n=1,325)			
Beta (95% CI)		-0.21 (-1.65, 1.23)	-1.47 (-3.22, 0.30)
p-value		0.774	0.103
OR (95% CI)		0.90 (0.68, 1.19)	0.77 (0.55, 1.08)
p-value		0.459	0.127
Women (n=1,550)			
Beta (95% CI)		-1.33 (-2.66, -0.003)	-1.52 (-3.00, -0.04)
p-value		**0.049**	**0.044**
OR (95% CI)		0.82 (0.60, 1.14)	0.71 (0.49, 1.03)
p-value		0.240	0.074

Model 1: Adjusted for Age
Model 2: Adjusted for Model 1 plus Race and BMI category
Model 3: Adjusted for Model 2 plus Leisure time physical activity, smoking status, and alcohol

Examining the relationship between 25(OH)D concentration and SBP with 25(OH)D dichotomized as deficient or not deficient (below or above 37.5nmol/L) also revealed greater differences between the genders. The analysis of the total population was within 0.1mmHg of the results of the linear regression analysis and one percentage point of the results of the logistic regression analysis above. However, analysis separated by gender revealed no significant association in men but a 1.64mmHg reduction in SBP (Beta: -1.63, 95% CI: -3.15, -0.11, p-value 0.035) and a 33% reduction in risk of elevated SBP for women who were not vitamin D deficient compared to those who were deficient (OR: 0.67, 95% CI: 0.47-0.95, p-value 0.025). Post-

menopausal women showed no association in either linear or logistic regression but non-deficient pre-menopausal women had a 2.47mmHg reduction in SBP compared to those who were deficient (Beta: -2.47, 95% CI: -4.13, -0.82, p-value 0.003) and a 45% reduction in risk of elevated SBP (OR 0.55, 95% CI: 0.35-0.85, p-value 0.007).

The association between SBP and PTH, as an index of 1,25(OH)D availability, was also examined (Table 5.5). SBP, as a continuous variable, was significantly positively associated with

Table 5.5: Linear and logistic regression analysis of the association of SBP with PTH (pg/mL in linear; elevated PTH (>65pg/mL) in logistic).

PTH Only		**Controlling for 25(OH)D**
Whole Population (n=2,872)		
Model 1		
Beta (95% CI)	3.21 (2.29, 4.13)	2.59 (1.63, 3.55)
p-value	**0.000**	**0.000**
OR (95% CI)	1.95 (1.50, 2.55)	1.79 (1.37, 2.35)
p-value	**0.000**	**0.000**
Model 2		
Beta (95% CI)	2.53 (1.61, 3.46)	2.35 (1.40, 3.29)
p-value	**0.000**	**0.000**
OR (95% CI)	1.79 (1.37, 2.35)	176 (1.32, 2.28)
p-value	**0.000**	**0.000**
Model 3		
Beta (95% CI)	2.58 (1.65, 3.51)	2.37 (1.40, 3.33)
p-value	**0.000**	**0.000**
OR (95% CI)	1.70 (1.30-2.23)	1.64 (1.25-2.16)
p-value	**0.000**	**0.000**
Men (n=1,323)		
Model 3		
Beta (95% CI)	3.20 (1.71, 4.68)	3.08 (1.57, 4.60)
p-value	**0.000**	**0.000**
OR (95% CI)	1.83 (1.25-2.70)	1.79 (1.22-2.65)
p-value	**0.002**	**0.003**
Women (n=1,549)		
Model 3		
Beta (95% CI)	1.83 (0.63, 3.03)	1.61 (0.36, 2.85)
p-value	**0.003**	**0.012**
OR (95% CI)	1.50 (1.00-2.25)	1.43 (0.95-1.18)
p-value	**0.050**	0.090

Model 1: Adjusted for Age and gender
Model 2: Adjusted for Model 1 plus Race and BMI category
Model 3: Adjusted for Model 2 plus Leisure time physical activity, smoking status, and alcohol

PTH, which remained largely unchanged in all models with and without controlling for 25(OH)D concentration. For each additional pg/mL of serum PTH, subjects showed a 2.37mmHg higher SBP in the fully adjusted model in the linear regression (Beta: 2.37mmHg, 95% CI: 1.40-3.33, p-value 0.000). In logistic regression, the risk of elevated SBP of was 64% higher in subjects with elevated serum PTH (OR: 1.64, 95% CI: 1.25-2.16, p-value 0.000 for fully adjusted model). The findings were markedly different in men and women. When SBP was examined as a continuous variable, men showed a 3.08mmHg increase in SBP per pg/mL increase in PTH (Beta: 3.08mmHg, 95% CI: 1.57-4.60, p-value 0.000), while women only had a 1.61mmHg increase (Beta: 1.61, 95% CI: 0.36-2.85, p-value 0.012). In logistic regression, men with elevated PTH had a statistically significant 79% increased risk of elevated SBP (OR: 1.79, 95% CI: 1.22-2.65, p-value 0.003) and women with elevated PTH had a much smaller and non-significant 43% increase in risk (OR: 1.43, 95% CI 0.95-1.18, p-value 0.090 for women). In logistic regression, post-menopausal women showed no statistically significant association between PTH and SBP, but pre-menopausal women with elevated PTH showed an 83% increased risk of elevated SBP (OR: 1.83, 95% CI: 1.09-3.09, p-value 0.023.)

To investigate the hypothesis that high UA concentration would lead to decreased 1,25(OH)D production and subsequently lead to a buildup of PTH, the association between PTH and UA was also examined. Each mg/dL increase in UA was associated with a statistically significant increase of 1.05pg/mL of PTH (Beta: 1.05, 95% CI: 1.04-1.07, p-value 0.000 in fully adjusted model). This corresponds with a 23% increased risk of elevated PTH (>65pg/mL) in the fully adjusted model controlled for 25(OH)D concentration (OR: 1.23, 95% CI: 1.09-1.38, p-value 0.001). There was very little difference between men and women in subgroup analysis by gender.

Including both SSB intake and 25(OH)D status in the same model did not substantially modify either association with SBP or risk of elevated SBP in the fully adjusted model (Table 5.6), which held true for subgroup analysis by gender. Including a multiplicative term to assess interaction between vitamin D and SSB intake did not result in a statistically significant association or modification of the main effects. Stratified analysis by SSB intake quartile revealed no statistically significant associations between 25(OH)D concentration and SBP.

Table 5.6: Linear and logistic regression analysis of the association of SBP with SSB intake and Vitamin D.

Whole Population (n=2,875)				
SSB Intake	**Q1**	**Q2**	**Q3**	**Q4**
Model 3				
Beta (95% CI)	0.00	0.70 (-0.41, 1.82)	1.01 (-0.13, 2.15)	1.28 (0.10, 2.46)
p-value		0.214	0.083	**0.034**
OR (95% CI)	1.00	1.22 (0.96, 1.56)	1.16 (0.90, 1.48)	1.23 (0.96, 1.59)
p-value		0.103	0.251	0.107
Vitamin D	**<50nmol/L**	**50-75nmol/L**	**>75nmol/L**	
Model 3				
Beta (95% CI)	0.00	-0.79 (-1.77, 0.19)	-1.66 (-2.79, -0.53)	
p-value		0.116	**0.004**	
OR (95% CI)	1.00	0.87 (0.71, 1.07)	0.71 (0.56, 0.91)	
p-value		0.191	**0.007**	

Model 3: Adjusted for age, gender, race, BMI category, leisure time physical activity, smoking status, and alcohol

Analyzing the data stratified by Vitamin D status, there was a statistically significant finding in one group; for subjects with 25(OH)D concentrations below 50nmol/L, being in the highest SSB intake quartile resulted in a 66% increase in risk of HTN (OR: 1.66, 95% CI: 1.05-2.61, p-value 0.029). All interaction and stratified models were adjusted for age, gender, race, BMI category, leisure time physical activity, smoking status, and alcohol.

CHAPTER 6

DISCUSSION

6.1 Sugar-sweetened Beverages and Hypertension

We found a positive association between SSB intake (in quartiles) and systolic blood pressure, whether blood pressure was modeled as a continuous or a categorical variable. This observation is consistent with previous research on SSB intake in adults (Barrio-Lopez, 2013; Cohen, 2012; Ejtahed, 2015; Jayalath,2015; Sayon-Orea, 2015). For the continuous analysis, this association was statistically significant only in the fourth quartile for the fully adjusted model, with an increase in SBP of 1.40mmHg relative to the lowest intake quartile. In logistic regression, the risk of elevated SBP was also 26% higher in the fourth quartile, but this association was not statistically significant. Earlier studies reported associations of a similar magnitude: a 1.8mmHg increase in SBP (Ejtahed, 2015) and ORs for HTN of 1.27 (Ejtahed, 2015), 1.13 (Cohen, 2012) and 1.12 (Jayalath,2015).

Subgroup analysis by gender revealed no statistically significant association in men between SSB intake and increased SBP in any quartile. Among women, on the other hand, a statistically significant association was observed in both the third (1.55mmHg increase) and fourth (1.78mmHG increase) SSB quartiles in the continuous SBP analysis, as well as a 68% increase in risk of elevated SBP in the 4^{th} quartile in the logistic analysis. This difference between genders was observed in some earlier studies as well. Cohen et al found a statistically significant association between SSB intake and HTN in their analysis of the Nurses' Health Study (HR: 1.12, 95% CI: 1.08–1.17) and Nurses' Health Study II (HR: 1.17, 95% CI: 1.11–1.23) participants, which are female cohorts, but not in Health Professionals Study (HR: 1.06, 95% CI: 0.99–1.14) participants, which is a male cohort (Cohen, 2012). Sayon-Orea also found a stronger,

statistically significant association in women but none in men (women: HR: 1.55, 95% CI: 1.11–2.15, men: HR: 1.20, 95% CI: 0.91–1.57) (Sayon-Orea, 2015). These findings indicate that the association between SSBs and SBP depends on gender and that high SSB intake may only be a risk factor for elevated SBP in women. To elucidate a possible cause for that difference, further subgroup analysis was performed on women divided into pre- and post-menopausal groups. That analysis showed no statistically significant association between SSB intake and SBP in post-menopausal women, but in pre-menopausal women showed a 2.30mmHg increase in SBP in quartile 4 in linear regression as well as a statistically significant 105% increase in risk of elevated SBP in logistic regression. This sharp difference between pre- and post-menopausal women in the impact of SSB intake on SBP suggests that estrogen may have a mechanistic role in that relationship, since a drop in estrogen levels is one of the primary changes of menopause.

Our study confirms earlier reports of an association between serum UA and BP or HTN (Alper, 2005; Bobridge, 2013; Nguyen, 2009; Sundstrom, 2005). In the fully adjusted linear regression model, each mg/dL increase in UA concentration was associated with a statistically significant 0.83mmHg increase in SBP and a 14% increase in risk of elevated SBP per mg/dL increase in UA was found in logistic regression. Alper and colleagues (2005) previously found that the association was stronger in men than in women, and we also found a similar result in our study. No significant association between serum UA and SBP was observed in men but in women the risk of elevated SBP was 32% higher per mg/dL increase in UA. Pre-menopausal women showed a 1.35mmHg increase in SBP and a 47% increased risk of elevated SBP per mg/dL increase in UA while no relationship was found in post-menopausal women. As with the analysis of SSB and SBP, the difference in findings between men and women and pre- and post-menopausal women suggests a possible role for estrogen in the mechanism linking UA and SBP.

The similar pattern of the association between SSB intake with SBP and UA with SBP, with an association found in women but not men and the strongest association in pre-menopausal women, seemed promising for the hypothesis that increased UA was integral to the relationship between SSBs and SBP. Increased uric acid production has been widely proposed as a possible mechanistic link between SSB intake and increased BP (Brown, 2011; Ejtahed, 2015; Jayalath, 2015; Kim, 2012; Nguyen, 2009), and some earlier studies that examined the impact of SSB intake on serum UA concentration did find a statistically significant association, although the effect size was very small (Choi, 2008; Gao, 2007). However, we found no association between SSB intake and serum UA in any quartile. No association was observed in either gender in subgroup analysis, despite earlier studies suggesting that men were most likely to form UA in response to SSB intake (Choi, 2008; Gao, 2007). Additional metabolic studies may be needed to determine the mechanism underlying the gender differences observed in earlier studies of SSBs and UA. Finding no association between SSBs and UA also leaves unanswered the question of mechanisms in the associations we observed in pre-menopausal women between SSBs and SBP and UA and SBP in other parts of this study.

6.2 Vitamin D and Hypertension

We found a negative association between 25(OH)D status and blood pressure. In adjusted linear regression, being in the highest 25(OH)D category was associated with a 1.74mmHg decrease in SBP relative to the lowest category. In addition, a 30% reduction in risk of elevated SBP was observed in subjects with serum 25(OH)D concentrations greater than 75nmol/L compared to those with under 50nmol/L. This finding is generally consistent with a 2011 meta-analysis that found a 27% decrease in risk of HTN in subjects with the highest 25(OH)D levels (Burgaz, 2011). When we analyzed by gender, the relationship between vitamin

D and SBP was similar but was no longer statistically significant, perhaps as the result of the smaller sample size. That would suggest that gender does not play a role in the relationship between 25(OH)D and SBP. However, further subgroup analysis again revealed that pre-menopausal women had a much stronger association than post-menopausal women, with a 1.80mmHg decrease in SBP and a borderline significant 38% decrease in risk of elevated SBP in the highest group relative to the lowest. Analysis with 25(OH)D as a dichotomous variable (deficient or not deficient) resulted in larger differences between the genders. Here there was no significant association in men but a 1.64mmHg reduction in SBP and a 33% reduction in risk of elevated SBP for women who were not vitamin D deficient compared to those who were deficient. Further subgroup analysis for women revealed that post-menopausal women had no association in either linear or logistic regression but non-deficient pre-menopausal women had a 2.47mmHg reduction in SBP compared to those who were deficient and a 45% reduction in risk of elevated SBP. As with the analysis looking at the impact of SSBs and UA on SBP, the difference between pre-menopausal women and men or post-menopausal women suggests that estrogen may be involved in the relationship between 25(OH)D and SBP.

Serum PTH has been proposed as an index of the body's ability to convert 25(OH)D to 1,25(OH)D (Chen, 2014). As such, the possibility of an association between serum PTH and SBP was examined to determine if inability to activate vitamin D was more important to BP outcomes than simply the availability of sufficient precursor in the form of 25(OH)D. A statistically significant positive association was found between PTH and SBP. For each additional pg/mL of serum PTH, subjects showed a 2.37mmHg higher SBP in the fully adjusted model in the linear regression and in logistic regression the risk of elevated SBP was 64% higher in subjects with elevated serum PTH.

The findings were markedly different in men and women, but in this case the association was nearly twice as strong in men in both linear and logistic regression. In logistic regression, post-menopausal women showed no statistically significant association between PTH and SBP, but pre-menopausal women with elevated PTH showed an 83% increased risk of elevated SBP. Finding a similar relationship between pre-menopausal women relative to post-menopausal women and between men relative to women suggests that the sex hormone impacting the association between PTH and SBP may be testosterone, rather than estrogen. Testosterone levels drop in women after menopause; so men and pre-menopausal women are similar in that they have higher testosterone levels whereas men and post-menopausal women are similar in that they have lower estrogen levels. While the relationships between SBP and SSBs, 25(OH)D and PTH all appear to be impacted by sex hormones, the specific androgen involved may be different.

Serum UA was positively associated with PTH, with a statistically significant increase of 1.05pg/mL of PTH per mg/dL increase in UA and a 23% increased risk of elevated PTH (>65pg/mL) in the fully adjusted model controlled for 25(OH)D concentration. These findings are consistent with earlier studies of UA and PTH, many of which used the same 2003-2006 NHANES data, as these are the only years for which PTH data are available (Alemzadeh, 2016; Chen, 2014; Chin, 2015; Hernández, 2015; Hsu, 1991; Hui, 2012; Paik, 2012). Controlling for 25(OH)D did not substantially modify the relationship in any of the models. These findings suggest that UA may play a role in the elevation of PTH and therefore SBP. However, the fact that the association between UA and SBP was found to be more significant in women and the association between PTH and SBP was found to be more significant in men raises doubts about connecting the two pathways.

6.3 Interaction Between SSB Intake and Vitamin D

One of the main objectives of this study was to assess possible effect modification by vitamin D of the relationship between SSB intake and HTN. Evidence supporting the proposed mechanism for that interaction—high SSB intake increases serum UA, which decreases 25(OH) D activation to 1,25(OH)D as indexed by serum PTH—was not found in the study population, with no association observed between SSB intake and UA. The study has also found no evidence of effect modification. Including both SSB intake and 25(OH)D status in regression models did not substantially modify either association with SBP or risk of elevated SBP in the fully adjusted model, either in the whole population or in subgroup analysis by gender. Using a multiplicative term of 25(OH)D and SSB intake also did not produce a statistically significant result or modify either individual association to a meaningful degree. Stratified analysis both by SSB intake quartile also provided no evidence of an interaction.

Stratification by 25(OH)D status showed an interaction between vitamin D and SSB intake in subjects in the highest SSB intake quartile. For subjects with 25(OH)D concentrations below 50nmol/L, being in the highest SSB intake quartile was associated with a borderline-significant 2.04mmHg increase in SBP and a statistically significant 66% increase in risk of elevated SBP. While these findings provide some evidence of effect modification, a multiplicative interaction term inserted into the final model was not significant. We suggest that the increased risk of elevated SBP in people who have both low 25(OH)D status and high SSB intake is simply the result of having two risk factors for high SBP, but that there is no evidence that the effect is more than additive.

6.4 Limitations

In assessing the relationship between SSB intake and serum UA, an attempt was made to understand the impact of difference in testosterone and estrogen levels by using subgroup analysis by gender and between pre- and post-menopausal women. Although controlling for these variables directly in our analysis may have been more informative, testosterone and estradiol were only measured in a subset of men during the 2003-2004 NHANES survey. After excluding subjects who did not complete the food frequency questionnaire, only a very small sample (n=134) remained, so these data were not analyzed as part of the current study.

Another limitation in the assessment of the association between UA and SBP was the cross-sectional nature of the study. Some earlier animal research suggested that the impact of UA on HTN may be delayed; structural changes to the kidneys caused by UA may not increase BP immediately but will result in HTN in the presence of a high-sodium diet in the future (Watanabe, 2002). If that is the case, a cross-sectional study is less likely to find an association between UA and HTN since UA levels may not remain high at the time elevated BP is detected.

6.5 Future Directions

The findings of this study do not provide evidence of an interaction between SSBs or 25(OH)D and SBP. Therefore, future studies examining the effect of SSB intake on BP will not need to include PTH, whose inclusion in our study limited our available sample size to years for which PTH measurements were available. Removing these requirements opens up the possibility of conducting a much larger study of this effect while still using NHANES data. The FFQ was used only in 2003-2006, so including data from other years would involve calculating SSB intake from the 24-hour recall data. This would be more time consuming and complicated but would have the added benefit of allowing for the inclusion in the model of other dietary

factors which were not included in the FFQ, such as sodium intake. If the effect remained significant in cross-sectional analysis of a larger population, more complex study designs, such as cohort studies, could be used to further investigate the connection. Such studies have been conducted in the past, but few have directly investigated the mechanism involved. The most unexpected finding of this study was a null finding; no association was found between SSB intake and serum UA. Further studies will be needed to firmly conclude that uric acid is not the mechanism by which high SSB intake increases BP, and ideally these studies will include measurements of testosterone and estrogen. Other possible mechanisms could also be investigated, such as insulin resistance. Cross-sectional and cohort study data could also be used to investigate this possibility, if fasting insulin and glucose level data were available.

6.6 Conclusion

Consistent with earlier studies, we found that SSB intake was positively associated with SBP and 25(OH)D concentration was negatively associated with SBP. For pre-menopausal women, reducing the number of SSBs consumed may have a beneficial SBP-lowering effect, and both men and women may benefit from having a higher serum 25(OH)D concentrations. We did not find an association between SSB intake and UA or evidence of an interaction between SSBs and vitamin D in their impact on SBP. Further studies will be needed to elucidate the mechanisms by which low serum 25(OH)D concentration and high SSB intake increase SBP.

BIBLIOGRAPHY

Alemzadeh, R., & Kichler, J. (2016). Uric acid-induced inflammation is mediated by the parathyroid hormone: 25-hydroxyvitamin D ratio in obese adolescents. *Metabolic Syndrome and Related Disorders, 14*(3), 167-174.

Al-Gauhari, A., Al-Nagdy, S., El-Sabbagh, M., & Eisa, E. (1974). Biochemical studies on some aspects of protein metabolism in vitamin-D deficient rats. *Comparative Biochemistry and Physiology Part A: Physiology, 47*(3), 845-854.

Alper, A. B.,Jr, Chen, W., Yau, L., Srinivasan, S. R., Berenson, G. S., & Hamm, L. L. (2005). Childhood uric acid predicts adult blood pressure: The bogalusa heart study. *Hypertension (Dallas, Tex.: 1979), 45*(1), 34-38.

Ambrosini, G. L., Oddy, W. H., Huang, R. C., Mori, T. A., Beilin, L. J., & Jebb, S. A. (2013). Prospective associations between sugar-sweetened beverage intakes and cardiometabolic risk factors in adolescents. *The American Journal of Clinical Nutrition, 98*(2), 327-334.

Asghar, Z. A., Thompson, A., Chi, M., Cusumano, A., Scheaffer, S., Al-Hammadi, N., et al. (2016). Maternal fructose drives placental uric acid production leading to adverse fetal outcomes. *Scientific Reports, 6*, 25091.

Barrio-Lopez, M. T., Martinez-Gonzalez, M. A., Fernandez-Montero, A., Beunza, J. J., Zazpe, I., & Bes-Rastrollo, M. (2013). Prospective study of changes in sugar-sweetened beverage consumption and the incidence of the metabolic syndrome and its components: The SUN cohort. *British Journal of Nutrition, 110*(09), 1722-1731.

Beveridge, L. A., Struthers, A. D., Khan, F., Jorde, R., Scragg, R., Macdonald, H. M., et al. (2015). Effect of vitamin D supplementation on blood pressure: A systematic review and meta-analysis incorporating individual patient data. *JAMA Internal Medicine, 175*(5), 745-754.

Bobridge, K., Haines, G., Mori, T. A., Beilin, L. J., Oddy, W. H., Sherriff, J., et al. (2013). Dietary fructose in relation to blood pressure and serum uric acid in adolescent boys and girls. *Journal of Human Hypertension, 27*(4), 217-224.

Brazier, M., Grados, F., Kamel, S., Mathieu, M., Morel, A., Maamer, M., et al. (2005). Clinical and laboratory safety of one year's use of a combination calcium vitamin D tablet in ambulatory elderly women with vitamin D insufficiency: Results of a mlticenter, rndomized, double-blind, placebo-controlled study. *Clinical Therapeutics, 27*(12), 1885-1893.

Bremer, A. A., Auinger, P., & Byrd, R. S. (2009). Relationship between insulin resistance–associated metabolic parameters and anthropometric measurements with sugar-sweetened beverage intake and physical activity levels in US adolescents: Findings from the 1999-2004 national health and nutrition examination survey. *Archives of Pediatrics & Adolescent Medicine, 163*(4), 328-335.

Brown, I. J., Stamler, J., Van Horn, L., Robertson, C. E., Chan, Q., Dyer, A. R., et al. (2011). Sugar-sweetened beverage, sugar intake of individuals, and their blood pressure: International study of macro/micronutrients and blood pressure. *Hypertension, 57*(4), 695-701. Centers for Disease Control and Prevention (CDC). National Center for Health Statistics (NCHS). National Health and Nutrition Examination Survey Laboratory Procedures Manuel. Hyattsville, MD: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, 2004, https://www.cdc.gov/nchs/data/nhanes/nhanes_03_04/lab.pdf.

Centers for Disease Control and Prevention (CDC). National Center for Health Statistics (NCHS). National Health and Nutrition Examination Survey Physician Examination Procedures Manuel. Hyattsville, MD: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, 2003 https://www.cdc.gov/nchs/data/nhanes/nhanes_03_04/PE.pdf.

Centers for Disease Control and Prevention (CDC). National Center for Health Statistics (NCHS). National Health and Nutrition Examination Survey Questionnaire. Hyattsville, MD: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, 2003-2004, https://www.cdc.gov/nchs/nhanes/nhanes2003-2004/questionnaires03_04.htm.

Chen, W., Roncal-Jimenez, C., Lanaspa, M., Gerard, S., Chonchol, M., Johnson, R. J., et al. (2014). Uric acid suppresses 1 alpha hydroxylase in vitro and in vivo. *Metabolism, 63*(1), 150-160.

Chen, L., Caballero, B., Mitchell, D. C., Loria, C., Lin, P. H., Champagne, C. M., et al. (2010). Reducing consumption of sugar-sweetened beverages is associated with reduced blood pressure: A prospective study among United States adults. *Circulation, 121*(22), 2398-2406.

Chin, K., Nirwana, S. I., & Ngah, W. Z. W. (2015). Significant association between parathyroid hormone and uric acid level in men. *Clinical Interventions in Aging, 10*, 1377.

Cohen, L., Curhan, G., & Forman, J. (2012). Association of sweetened beverage intake with incident hypertension. *Journal of General Internal Medicine, 27*(9), 1127-1134.

Dhingra, R., Sullivan, L., Jacques, P. F., Wang, T. J., Fox, C. S., Meigs, J. B., et al. (2007). Soft drink consumption and risk of developing cardiometabolic risk factors and the metabolic syndrome in middle-aged adults in the community. *Circulation, 116*(5), 480-488.

Duffey, K. J., Gordon-Larsen, P., Steffen, L. M., Jacobs, D. R., & Popkin, B. M. (2010). Drinking caloric beverages increases the risk of adverse cardiometabolic outcomes in the coronary artery risk development in young adults (CARDIA) study. *The American Journal of Clinical Nutrition, 92*(4), 954-959.

Ejtahed, H., Bahadoran, Z., Mirmiran, P., & Azizi, F. (2015). Sugar-sweetened beverage consumption is associated with metabolic syndrome in Iranian adults: Tehran lipid and glucose study. *Endocrinology and Metabolism, 30*(3), 334-342.

Feig, D. I., Soletsky, B., & Johnson, R. J. (2008). Effect of allopurinol on blood pressure of adolescents with newly diagnosed essential hypertension: A randomized trial. *Jama, 300*(8), 924-932.

Forman, J. P., Curhan, G. C., & Taylor, E. N. (2008). Plasma 25-hydroxyvitamin D levels and risk of incident hypertension among young women. *Hypertension (Dallas, Tex.: 1979), 52*(5), 828-832.

Gao, X., Qi, L., Qiao, N., Choi, H. K., Curhan, G., Tucker, K. L., et al. (2007). Intake of added sugar and sugar-sweetened drink and serum uric acid concentration in US men and women. *Hypertension (Dallas, Tex.: 1979), 50*(2), 306-312.

Granger, J. P., Alexander, B. T., & Llinas, M. (2002). Mechanisms of pressure natriuresis. *Current Hypertension Reports, 4*(2), 152-159.

Ha, V., Sievenpiper, J. L., de Souza, R. J., Chiavaroli, L., Wang, D. D., Cozma, A. I., et al. (2012). Effect of fructose on blood pressure: A systematic review and meta-analysis of controlled feeding trials. *Hypertension (Dallas, Tex.: 1979), 59*(4), 787-795.

Hernández, J., Nan, D., Martínez, J., Pariente, E., Sierra, I., González-Macías, J., et al. (2015). Serum uric acid is associated with quantitative ultrasound parameters in men: Data from the camargo cohort. *Osteoporosis International, 26*(7), 1989-1995.

Hisatome, I., Ishimura, M., Sasaki, N., Yamakawa, M., Kosaka, H., Tanaka, Y., et al. (1992). Renal handling of urate in two patients with hyperuricemia and primary hyperparathyroidism. *Internal Medicine, 31*(6), 807-811.

Hsu, C. H., Patel, S. R., Young, E. W., & Vanholder, R. (1991). Effects of purine derivatives on calcitriol metabolism in rats. *The American Journal of Physiology, 260*(4 Pt 2), F596-601.

Hui, J. Y., Choi, J. W. J., Mount, D. B., Zhu, Y., Zhang, Y., & Choi, H. K. (2012). The independent association between parathyroid hormone levels and hyperuricemia: A national population study. *Arthritis Research & Therapy, 14*(2), 1.

Ivy, J. R., & Bailey, M. A. (2014). Pressure natriuresis and the renal control of arterial blood pressure. *The Journal of Physiology, 592*(18), 3955-3967.

Jayalath, V. H., de Souza, R. J., Ha, V., Mirrahimi, A., Blanco-Mejia, S., Di Buono, M., et al. (2015). Sugar-sweetened beverage consumption and incident hypertension: A systematic review and meta-analysis of prospective cohorts. *The American Journal of Clinical Nutrition, 102*(4), 914-921.

Joyner, M. J., & Limberg, J. K. (2014). Blood pressure regulation: Every adaptation is an integration? *European Journal of Applied Physiology, 114*(3), 445-450.

Khosla, U. M., Zharikov, S., Finch, J. L., Nakagawa, T., Roncal, C., Mu, W., et al. (2005). Hyperuricemia induces endothelial dysfunction. *Kidney International, 67*(5), 1739-1742.

Kim, Y. H., Abris, G. P., Sung, M., & Lee, J. E. (2012). Consumption of sugar-sweetened beverages and blood pressure in the United States: The national health and nutrition examination survey 2003-2006. *Clinical Nutrition Research, 1*(1), 85-93.

Lind, L., Wengle, B., Lithell, H., & Ljunghall, S. (1991). No major metabolic alterations accompany the hypotensive effect of active vitamin D: Results from three double-blind, placebo-controlled studies. *Upsala Journal of Medical Sciences, 96*(3), 199-204.

Loh, D., Moy, F., Zaharan, N., Jalaludin, M., & Mohamed, Z. (2016). Sugar-sweetened beverage intake and its associations with cardiometabolic risks among adolescents. *Pediatric Obesity,*

Maersk, M., Belza, A., Stodkilde-Jorgensen, H., Ringgaard, S., Chabanova, E., Thomsen, H., et al. (2012). Sucrose-sweetened beverages increase fat storage in the liver, muscle, and visceral fat depot: A 6-mo randomized intervention study. *The American Journal of Clinical Nutrition, 95*(2), 283-289.

Mandal, A. K., & Mount, D. B. (2015). The molecular physiology of uric acid homeostasis. *Annual Review of Physiology, 77*, 323-345.

Mazzali, M., Hughes, J., Kim, Y. G., Jefferson, J. A., Kang, D. H., Gordon, K. L., et al. (2001). Elevated uric acid increases blood pressure in the rat by a novel crystal-independent mechanism. *Hypertension (Dallas, Tex.: 1979), 38*(5), 1101-1106.

Miller, P., Schwartz, E., Chen, P., Misurski, D., & Krege, J. (2007). Teriparatide in postmenopausal women with osteoporosis and mild or moderate renal impairment. *Osteoporosis International, 18*(1), 59-68.

Min, B. (2013). Effects of vitamin D on blood pressure and endothelial function. *The Korean Journal of Physiology & Pharmacology, 17*(5), 385-392.

Mirmiran, P., Yuzbashian, E., Asghari, G., Hosseinpour-Niazi, S., & Azizi, F. (2015). Consumption of sugar sweetened beverage is associated with incidence of metabolic syndrome in Tehranian children and adolescents. *Nutrition & Metabolism, 12*(1), 1.

Mironova, E., Boiko, N., Bugaj, V., Kucher, V., & Stockand, J. (2015). Regulation of Na excretion and arterial blood pressure by purinergic signalling intrinsic to the distal nephron: Consequences and mechanisms. *Acta Physiologica, 213*(1), 213-221.

Mozaffarian, D., Benjamin, E. J., Go, A. S., Arnett, D. K., Blaha, M. J., Cushman, M., et al. (2015). American Heart Association statistics committee and stroke statistics subcommittee. *Heart Disease and Stroke statistics–2015 Update: A Report from the American Heart Association.Circulation, 131*(4), e29-e322.

National Center for Chronic Disease Prevention and Health Promotion, Division for Heart Disease and Stroke Prevention. (2016). *High blood pressure facts.* Retrieved 12/19/2016, from http://www.cdc.gov/bloodpressure/facts.htm

Nguyen, S., Choi, H. K., Lustig, R. H., & Hsu, C. (2009). Sugar-sweetened beverages, serum uric acid, and blood pressure in adolescents. *The Journal of Pediatrics, 154*(6), 807-813.

Nishi, E. E., Bergamaschi, C. T., & Campos, R. R. (2015). The crosstalk between the kidney and the central nervous system: The role of renal nerves in blood pressure regulation. *Experimental Physiology, 100*(5), 479-484.

Paik, J., Farwell, W., & Taylor, E. (2012). Demographic, dietary, and serum factors and parathyroid hormone in the National Health and Nutrition Examination Survey. *Osteoporosis International, 23*(6), 1727-1736.

Peng, H., Li, H., Li, C., Chao, X., Zhang, Q., & Zhang, Y. (2013). Association between vitamin D insufficiency and elevated serum uric acid among middle-aged and elderly Chinese Han women. *PloS One, 8*(4), e61159.

Perez-Pozo, S., Schold, J., Nakagawa, T., Sanchez-Lozada, L., Johnson, R., & Lillo, J. L. (2010). Excessive fructose intake induces the features of metabolic syndrome in healthy adult men: Role of uric acid in the hypertensive response. *International Journal of Obesity, 34*(3), 454-461.

Sanchez-Lozada, L. G., Tapia, E., Jimenez, A., Bautista, P., Cristobal, M., Nepomuceno, T., et al. (2007). Fructose-induced metabolic syndrome is associated with glomerular hypertension and renal microvascular damage in rats. *American Journal of Physiology.Renal Physiology, 292*(1), F423-9.

Sayon-Orea, C., Martinez-Gonzalez, M. A., Gea, A., Alonso, A., Pimenta, A. M., & Bes-Rastrollo, M. (2015). Baseline consumption and changes in sugar-sweetened beverage consumption and the incidence of hypertension: The SUN project. *Clinical Nutrition, 34*(6), 1133-1140.

Stipanuk, M. H., & Caudill, M. A. (2013). *Biochemical, physiological, and molecular aspects of human nutrition* Elsevier health sciences.

Sundstrom, J., Sullivan, L., D'Agostino, R. B., Levy, D., Kannel, W. B., & Vasan, R. S. (2005). Relations of serum uric acid to longitudinal blood pressure tracking and hypertension incidence. *Hypertension (Dallas, Tex.: 1979), 45*(1), 28-33.

Wadei, H. M., & Textor, S. C. (2012). The role of the kidney in regulating arterial blood pressure. *Nature Reviews Nephrology, 8*(10), 602-609.

Wang, J., Mark, S., Henderson, M., O'Loughlin, J., Tremblay, A., Wortman, J., et al. (2013). Adiposity and glucose intolerance exacerbate components of metabolic syndrome in children consuming sugar-sweetened beverages: QUALITY cohort study. *Pediatric Obesity, 8*(4), 284-293.

Wang, D. D., Sievenpiper, J. L., de Souza, R. J., Chiavaroli, L., Ha, V., Cozma, A. I., et al. (2012). The effects of fructose intake on serum uric acid vary among controlled dietary trials. *The Journal of Nutrition, 142*(5), 916-923.