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Exploring the relationship between genetic variation in taste receptor genes

and salt taste perception among people with hypertension

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A Dissertation
Submitted to the Faculty of
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in Partial Fulfillment of the Requirements
for the Degree of Doctor of Philosophy
in Nutrition
in the Department of Food Science, Nutrition and Health Promotion

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Different taste preferences and genetic variations may lead to particular food patterns that contribute to nutrient-related health outcomes such as hypertension. The objective of this study was to investigate single polymorphism of taste genes and salt taste perception in order to determine whether single nucleotide polymorphisms (SNPs) in the salt taste receptor genes (*SCNN1B*, *TRPV1*) affect salt taste perception in hypertensive participants. A cross-sectional study of 253 adults age 20-82 from each group, hypertensive (49%) and normotensive (51%), were enrolled. Salt taste recognition threshold, food preference score, and salt taste receptor genotype were determined. The hypertensive group had a higher salt taste recognition threshold than the normotensive group. However, there was no correlation between salt taste recognition threshold and salty food preference. Results also provide evidence that the polymorphism TRPV1, rs4790522 with AA genotype is associated with a lower sensitivity threshold of salt taste.

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Pradtana Tapanee

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CHAPTER I

INTRODUCTION

Hypertension is a non-communicable chronic disease that affects an estimated 75 million people or 1 in 3 adults in the United States, and only fifty-four percent of people with high blood pressure have their condition under control (Fryar et al. 2017). Untreated hypertension leads to many degenerative diseases, including heart disease and stroke, which are the leading causes of death in the United States (Heron 2019). Some of hypertension's risk factors can be modified such as smoking, sedentary activity, consumption of high sodium food, and alcohol consumption. Dietary factors are a potent modifiable element for regulation of blood pressure and prevention of its complications, such as cardiovascular disease, stroke, and renal disease (Mayo Clinic 2016). A randomized controlled Trial of a Non Pharmacological Intervention of sodium reduction and weight intervention in the Elderly (TONE) was conducted over 10-15 years (The Trials of Hypertension Prevention, Phase I and II). It was reported that there was a 25% reduction in the risk of cardiovascular diseases in the intervention group in comparison to the control group (Cook et al. 2007). However, the average American consumes more than 3,400 milligrams of sodium per day (American Heart Association 2016), and few adults (10%) maintain their sodium intake at the recommended level of 1,500 milligrams of sodium per day (DeNoon 2016). Salt taste perception, or salt sensitivity, impacts food preference and sodium consumption. Two genes, epithelial sodium channel (*ENaC*) and the transient receptor potential cation subfamily V member 1 (*TRPV1*), have been identified as salt taste receptors with different

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genetic variants that affect taste sensitivity (Dias et al. 2013). However, no studies could be located that have evaluated the association between salt taste perception and taste receptors in hypertensive patients. Therefore, the main objective of this research was to investigate single polymorphism of taste genes and salt taste perception in order to determine whether single nucleotide polymorphisms (SNPs) in the salt taste receptor genes (*SCNN1B, TRPV1*) affect salt taste perception in hypertensive participants. Thus, the specific objectives of this study were to:

1. Determine differences of salt sensitivity threshold between hypertensive and normotensive participants.

2. Investigate the allele frequencies of SNPs of taste receptor genes (*SCNN1B, TRPV1*) in hypertensive and normotensive participants.

3. Determine whether SNPs in participants' taste receptor genes (*SCNN1B*, *TRPV1*) affect the perception of saltiness.

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CHAPTER II

LITERATURE REVIEW

Hypertension

Definition and classification of hypertension

Blood pressure is the force in the blood vessel pushing against the arterial walls. There are two arterial blood pressure values, systolic blood pressure (SBP) and diastolic blood pressure (DBP), which are measured in millimeters of mercury (mmHg, e.g. SBP/DBP; 129/78 mmHg). Hypertension or high blood pressure is a non-communicable chronic disease. Blood pressure in adults age 18 years or older can be classified into four categories: normal (<120/80 mmHg), prehypertension (120-139/80-89 mmHg), stage 1 hypertension (140-159/90-99 mmHg) and stage 2 hypertension (≥160/100 mmHg) (Table 2.1) (American Heart Association 2017). Hypertension is a modifiable risk factor for vascular disease, myocardial infarction, stroke, and renal failure (James et al. 2014).

Hypertension affects 77.9 million or 1 in 3 adults in the United States (Hernandez-Vila 2015). Among adults that are 20 years or older, African Americans have the highest prevalence of hypertension, followed by Non-Hispanic Whites and Hispanics (African Americans 43.3%, Non-Hispanic Whites 29.1%, and Hispanics 28.2%, respectively) (Fryar et al. 2017). Moreover, the annual United States health system's cost of hypertension is approximately $300 billion per year, which is higher than diabetes ($82 billion) and arthritis ($30 billion) (Felder et al. 2013). Only fifty-four percent of people with high blood pressure have their condition under control

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(Fryar et al. 2017). Untreated hypertension leads to degenerative diseases, including impaired kidney function and heart disease and stroke, which are the leading causes of death in the United States (Heron 2019). Hypertension is also a global concern. Worldwide, an estimated 1.13 billion people have hypertension with most hypertensive individuals living in low or middle income countries (World Health Organization 2019).

Table 2.1 Hypertension classification

Category	Systolic blood pressure		Diastolic blood pressure
Normal	<120	and	<80
Pre -hypertension	120-139	or	80-89
Stage 1 Hypertension	140-159	or	90-99
Stage 2 Hypertension	≥160	or	≥100

Classification based on the Joint National Committee (JNC 8) guidelines

Sodium

Sodium is a major cation in extracellular fluid and plays a pivotal role in the body by maintaining cell membrane potential, nerve impulse transmission, smooth muscle contraction and fluid and electrolyte balance (Jones 2004). In order to maintain all of these functions in a balanced state, sodium concentration is tightly controlled within 135-145 mEq/L in the body's circulation. Sodium is filtered through the renal glomeruli in circulation, and then excreted in the urine. Prior to excretion through urine, the filtered sodium is reabsorbed at the renal tubules for maintenance of daily sodium turnover. Under a normal balanced conditions, daily sodium output (in urine, sweat, saliva and feces) is equal to daily sodium intake. Sodium has an important role in the body, but the human body needs a limited amount of it. The minimum intake level of sodium is estimated at 200–500 mg/day for proper bodily function (World Health Organization 2014). Sodium is naturally in foods and in processed foods, salt (sodium chloride) and several

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condiments such as fish sauce, soy sauce, oyster sauce, monosodium glutamate (MSG), etc. The major source of sodium chloride is 80% from sodium-containing condiments (Mozaffarian et al. 2014).

Hypertension and sodium consumption

The recommendation of sodium intkae is less than 1,500 mg of sodium, or 3.75 grams of salt per day for an adult (Allgood et al. 2018)(Allgood et al. 2018)(National Academy of Sciences 2019) while the American Heart Association recommends daily sodium intake slightly higher at 2,300 mg per day (Crouch et al. 2018). Excessive salt intake can lead to either short-term or long-term health consequences. In the short-term, excessive salt intake can cause fluid retention and increased blood pressure. In the long-term, high salt intake can lead to long-term health problems, including hypertension, cardiovascular disease, and kidney disease (Cogswell et al. 2016).

Meta-analyses of randomized sodium reduction trials have confirmed positive effects of sodium reduction on blood pressure and decreased incidence of stroke and mortality due to coronary heart disease (Karppanen and Mervaala 2006; Pickle et al. 1997). A worldwide epidemiological and observational study (INTERSALT) of 10,079 men and women aged 20-59 years from 32 countries was conducted to evaluate the relationship between salt intake and blood pressure. This was done by evaluating 24-hour urinary sodium excretion and blood pressure. A significant positive linear relationship existed between 24-hour sodium excretion and systolic blood pressure (Stamler 1997). This study demonstrated that high sodium intake increased systolic blood pressure over 24 hours by 2.08 mmHg per gram of sodium increase in people with hypertension (Mente et al. 2016). The 2009–2012 National Health and Nutrition Examination Survey (NHANES) indicated that the average American consumed more than 3,400 milligrams

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of sodium per day and 89% of adults and more than 90% of children consumed more dietary sodium than the daily recommendation (Jackson et al. 2016).

Salt sensitivity

Salt sensitivity is a change in blood pressure of ≥ 5 mmHg in response to reduced sodium intake (D'Elia 2018; Felder et al. 2013). Salt sensitivity is more common in African Americans, the elderly, the obese and people with type 2 diabetes mellitus (Campese et al. 1982). Previous studies indicated that salt sensitivity is associated with cardiovascular and metabolic syndrome diseases (D'Elia 2018). Moreover, hypertensive patients with salt sensitivity have a 3-fold higher incidence of cardiovascular disease than hypertensive patients without salt sensitivity (Rodriguez-Iturbe and Vaziri 2007). A cohort study was conducted with 430 normal subjects and 278 hypertensive subjects to determine the association between salt sensitivity and mortality. Results indicated that salt sensitivity in both groups was associated with an increased risk of cardiovascular disease events and mortality (Weinberger et al. 2001).

Multiple methods are available to determine salt sensitivity. The most referenced method that is reliable and accepted is the measurement of the blood pressure response to a change in dietary salt intake over 7 days of 3 periods of sodium intake including normal (109 mmol/day), low (10 mmol/day), and high (250 mmol/day) sensitivity (He et al. 2009; Sanada, Jones, and Jose 2011). Mean arterial pressure (MAP) is commonly used to classify salt sensitivity. Weinberger classified salt sensitivity and salt resistance by measuring blood pressure after an intravenous infusion of two liters of saline and then measured blood pressure again after the subject consumed a low sodium diet (10 mmol/day) and 40 g of furosemide. Participants who had a decreased MAP of more than 10 mmHg were considered salt sensitive, while participants with salt resistance showed a decrease of less than 5 mmHg MAP (Weinberger et al. 1986).

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Based on Weinberger's criteria, fifty-one percent of 43,186,000 hypertensive people in the United States are salt sensitive (Kanbay et al. 2011). However, salt sensitivity is difficult to assess using blood pressure due to the lack of universal consensus on definition, low compliance, high expense, and the lack of reimbursement from healthcare insurers. Therefore, genetic methods have been used to determine salt sensitivity (Felder et al. 2013). The Genetic Epidemiology Network of Salt Sensitivity study (GenSalt) revealed that the single nucleotide polymorphism (SNP) of the GNAI2 gene (rs10510755) is positively associated with salt sensitivity (Zhang et al. 2018). In addition, NEDD4L (rs2288774), a regulator of the amiloride-sensitive epithelial sodium channel (ENaC), was also associated with salt sensitivity (Dahlberg et al. 2007). This genetic information may be a biomarker for salt sensitivity.

Salt taste perception

Salt taste and its influence on food selection

Taste is stimulated when a substance activates specialized protein receptor cells that are located on taste buds in the oral cavity as well as the upper gastrointestinal and respiratory tracts. Most of the taste buds are located on the back and front of the tongue. Others are found on the soft palate, the back of the roof of the mouth and pharynx, and each taste bud contains 50 to 100 taste receptor cells (Breslin 2013). Humans can sense five established basic tastes including sweet, sour, salty, bitter, and umami. Human taste perception develops in children and has been emphasized as a driver of food preference, which are the best predictors of food acceptance in children (Ventura and Worobey 2013). The aging process has a significant effect on salt taste intensity. At 50 years or older, the salt perception decreases, which may result in higher salt consumption (Suchecka et al. 2016). Moreover, taste is important for the identification of toxicity and nutrients, and also contributes to food liking and consumption (Puputti et al. 2018).

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Individual differences in the five taste perceptions may affect dietary habits, nutritional status, and nutrition-related chronic disease risk (Garcia-Bailo et al. 2009).

In general, taste preference is influenced by environment and genetic factors. However, the environment may have a more important role than genetic factors (Chamoun et al. 2016). Different taste preferences may lead to particular food patterns that contribute to nutrient-related health outcomes since salt preference has been correlated with shorter stature and higher percentage of body fat in children between 5-10 years of age (Ahrens 2015). This may be because most high-salt foods are also energy dense foods, which can affect adiposity (Mennella et al. 2014).

Determination of salt taste perception

Thresholds are the concentrations of an ingredient in which a stimulus can be detected. Thresholds are classified into four categories: detection threshold, recognition threshold, difference threshold, and terminal threshold (Civille and Carr 2015). The detection threshold or absolute threshold is the lowest concentration of the stimulus that can be detected. The recognition threshold is the level at which a stimulus can be detected and recognized. It is normally higher than the detection threshold. The difference threshold is the level at which an increase in stimulus produces a noticeable difference while the terminal threshold is the level in which an increase in stimulus concentration no longer causes a noticeable difference in the sensory response (Civille and Carr 2015). The most common threshold used in salt taste perception research is salt taste recognition thresholds. Previous studies have provided several methods to determine recognition threshold. However, the basic concept of the procedure is conducted with a range of salt solutions that were offered to panelists one at a time (Liem 2017).

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A modified Harris-Kalmus procedure has commonly been used to determine the recognition threshold for salty taste (Wise and Breslin 2013). A modified Harris-Kalmus procedure was developed from the Harris and Kalmus technique for differentiation of threshold stimuli for tasting phenylthiourea (PTC) in 1949 (Galindo-Cuspinera et al. 2009). In the modified Harris-Kalmus procedure, salt solutions are introduced one by one in ascending order based on salt concentration for panelists to identify the lowest salt concentration in which a salty taste can be perceived by the panelist. Three cups of the salt solution at the concentration that was reported as recognized by the panelist, and 3 cups of water were then offered randomly and the participant was asked to sort them correctly (Chen et al. 2009; Wise and Breslin 2013).

Genetics of salt taste

Salt taste results from the interaction of the alkali metal groups, especially sodium with ENaC formed by the gene products of *SCNN1A, SCNN1B, SCNN1G*, and *SCNN1D* and the transient receptor potential cation subfamily V member 1 (TRPV1: transient receptor potential cation channel subfamily V member 1, encoded by the *TRPV1* gene) channel that are present in human taste buds on the tongue (Dias et al. 2013; Liem 2017). A sodium channel or ENaC consists of four subunits (α, β, γ, δ) which are located in the taste cell wall. Sodium cations can enter the cell through the ENaC by depolarizing the taste receptor cells (TRCs). Taste cells open voltage-dependent calcium channels, and the positive calcium ions trigger neurotransmitter release (Bigiani and Cuoghi 2007). Neurotransmitters are conveyed to the brain, and the brain interprets the taste (Liem 2017). Another ion channel that is involved in salt taste perception is the TRPV1 channel which is amiloride insensitive (Dias et al. 2013). Polymorphism in *ENaC* and *TRPV1* sodium channel genes are related to salt taste preference (Chamoun et al. 2018; Dias et al. 2013; Robino, Decorti, and Gasparini 2014). In the *SCNN1B* gene, a subunit of *ENaC*, Dias

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et al., (2013) reported that the polymorphisms of rs239345 (A>T) and rs3785368 (C>T) were associated with taste sensitivity. The homozygous genotype of the A and the T alleles of the rs239345 and rs3785368 polymorphisms were more sensitive to salt solutions than carriers of the T or C alleles. Moreover, the polymorphism of rs4790522 (A>C) of the *TRPV1* gene was associated with a greater preference with carriers of the C allele having higher salt preference in comparison to the A allele in children (Chamoun et al. 2017). The T allele of rs8065080 (C>T) polymorphism of the *TRPV1* gene were also more sensitive to salt solutions than the homozygous expression of the C allele (Dias et al. 2013).

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CHAPTER III

MATERIALS AND METHODS

Study design and IRB approval

This study is a cross-sectional design. Participants were divided into normotensive and hypertensive groups. Each group was asked to complete a questionnaire, provide saliva samples, and perform the salt taste threshold test. The study was approved by the Institutional Review Board (IRB) at Mississippi State University (IRB-18-510) and all procedures followed were in accordance with the ethical standards of the IRB and the Helsinki Declaration of 1975, as revised in 2000. The reserach protocol is presented in Figure 3.1.

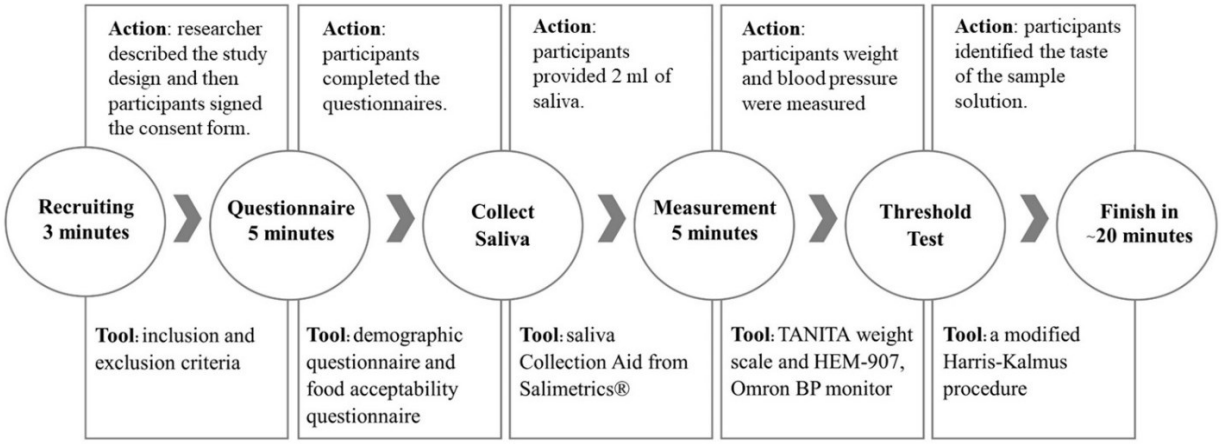

Figure 3.1 Research flow diagram

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Participants

The sample size was estimated according to test for the difference between two independent means by assuming a 95% confidence level at 80% power, with the common standard deviation of outcome variable in hypertensive and normotensive groups (σ) and the difference in mean between 2 groups (Δ) (Piovesana, Sampaio, and Gallani 2013), respectively.

Sample size equation:

$$\text{n/group} = 2\left[\frac{(Z\alpha/2+Z\beta)\sigma}{\Delta}\right]^2 \quad (4.1)$$

$Z_{\alpha/2} = 1.96$ $Z_{\beta} = 0.842$

$\sigma = 0.031$ $\Delta = 0.011$

It was calculated that a total of 125 participants were needed for each group, hypertension and normotensive groups. A total of 260 adults age 20-82 from hypertensive and normotensive groups participated in this study. All individuals provided informed consent prior to participation.

Recruitment of study participants

Data from 125 participants from the hypertensive group and 128 participants from the normotensive groups, were included in the study since 7 participants were ineligible (Figure 3.2). The normotensive and hypertensive volunteers were recruited from the community at health-related events, conferences, community centers or churches in Mississippi. Posters and emails were distributed that announced the research project at community events and academic departments at Mississippi State University. After participants completed the study, they received nutrition education materials that consisted of pamphlets about dietary management and hypertension.

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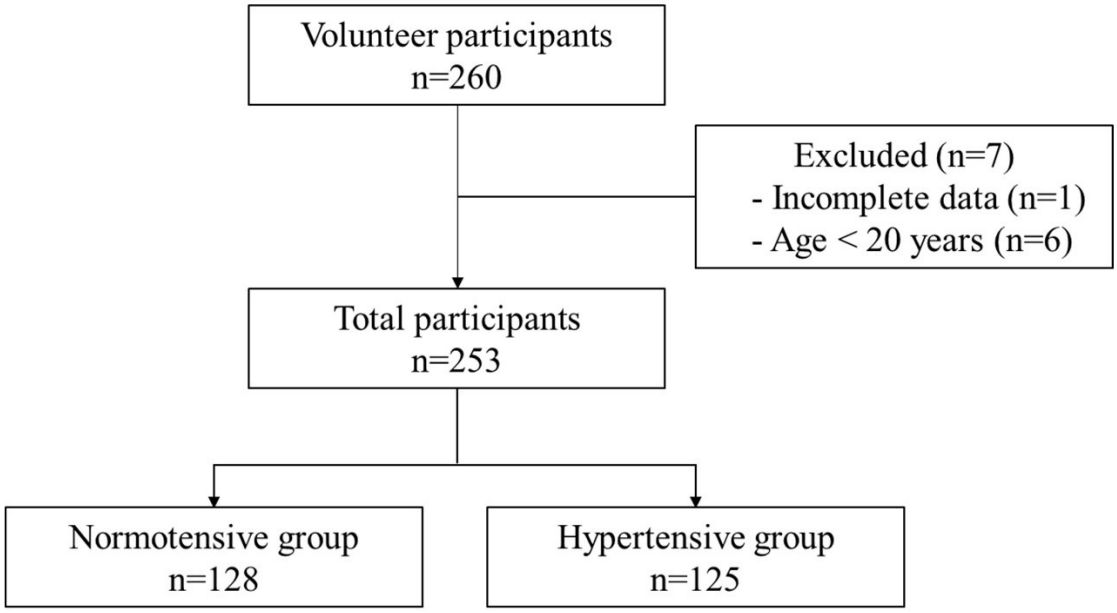

Figure 3.2 Participant recruitment

Inclusion criteria

Hypertensive group

- Age 20 years of age or older

- Has a clinical diagnosis of primary hypertension.

-Taking hypertensive medication.

-Able and willing to complete the informed consent process.

-Agree to provide a saliva sample and complete the questionnaire.

Normotensive group

- Age 20 years of age or older

- Never been diagnosed with hypertension.

- Able and willing to complete the informed consent process.

- Agree to provide a saliva sample and complete the questionnaire.

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Exclusion criteria

Hypertensive/ Normotensive groups

- Have an alteration in taste
- Experience xerostomia (dry mouth)
- Regularly take medication affecting taste or saliva production
- Self reported pregnancy or breastfeeding

Demographic and food acceptability information

Demographic and food liking information was obtained through questionnaires (Appendix B). The questionnaire consisted of three parts: 1) general information, 2) health information, and 3) food acceptability information.

Part 1: Personal information included age, gender, income, education level and occupation.

Part 2: Health information included chronic health conditions, nutritional behavior and physical activity.

Part 3: Food acceptability information included food lists in 6 categories; participants rated each of the food and beverage items in terms of how intensely he or she liked or disliked them.

The questionnaire contained checklist questions and short answer questions and was over 5 pages long. The modified questionnaire (Stein, Cowart, and Beauchamp 2012) was used to evaluate food acceptability.

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Blood pressure measurement

Blood pressure was measured by using an automatic monitor (HEM-907, Omron, Healthcare Co., Kyoto, Japan). This upper arm digital blood pressure monitor was easy to use and automatically adjusts to the person's arm size. All participants were in the sitting position for measurement after 5 min of rest.

Food preference scores

Participants completed two pages of general Labeled Magnitude Scale (gLMS) questions by rating the intensity of all possible experiences they can like or dislike, not just foods (Appendix B). The first page was an orientation to the gLMS by asking participants to rate the intensity of strongest sensation, e.g. pain and smell. The scales that corresponded to liking were +100: most intense pleasure of any kind ever experienced, 0: neutral, and -100: most intense displeasure of any kind ever experienced. On the second page, participants rated 24 food items in terms of how intensely he or she likes or dislikes them by using the gLMS. Twenty-four food items consisted of high salt foods, low salt foods, high sweet foods, and low sweet foods from food lists described by Stein et al. (2012). In addition, foods were assigned to categories on the basis of perceptual ratings of an adult panel (Stein et al. 2012). The classifications of liking/disliking based on categories from the gLMS of 0=neutral, ±6 weakly, ±17 moderately, ±35 strongly, ±53 very strongly and ±100 strongest of any kind applied to liking/disliking.

Saliva collection and genotyping

Participants were asked to provide 2 ml of saliva using the Passive Drool collection method with the Saliva Collection Aid from Salimetrics®. Saliva samples were blotted on filter paper (Fisher Scientific, Pittsburgh, PA) and DNA was extracted using TaqMan Sample-to-

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SNPTM Kit (Applied Biosystems, Foster City, CA). Four SNPs, SCNN1B gene: rs239345, rs3785368, and TRPV1 gene: rs8065080, rs4790522, were analyzed using TaqMan allelic discrimination assays and the QuantStudio5 real-time PCR system. Four SNPs, *SCNN1B* gene: rs239345, rs3785368, and *TRPV1* gene: rs8065080, rs4790522 were analyzed with justification provided in Table 3.1. The SNPs were analyzed using TaqMan allelic discrimination assays and the QuantStudio5 real-time PCR system. All alleles were reported in the forward orientation. The probe sequence from sequence 5'-3'for the genotyped SNPs were as follows:

rs239345-FAM probe

AGCTGTTCTTCCTTGCTTTGCTTCC**T**CAGCTCTTGTGGTAAAGCTGTTTGC

rs239345-VIC probe

AGCTGTTCTTCCTTGCTTTGCTTCC**A**CAGCTCTTGTGGTAAAGCTGTTTGC

rs3785368-FAM probe

AGATAGACAGATAGATAGCTAAAGAA**A**GAGTTTATTAAATATTAACTCACAT

rs3785368-VIC probe

AGATAGACAGATAGATAGCTAAAGA**G**GAGTTTATTAAATATTAACTCACAT

rs8065080-FAM probe

GTGGAAAACCCGAACAAGAAGACGA**T**GTAGACAAACATGAAACGGCACAGG

rs8065080-VIC probe

GTGGAAAACCCGAACAAGAAGACGA**C**GTAGACAAACATGAAACGGCACAGG

rs4790522-FAM probe

TGTCCCAGTAGAGACTGACCATCCA**C**ACTGTTTAGTAAAGTGAGTAAAAAC

rs4790522-VIC probe

TGTCCCAGTAGAGACTGACCATCCAA**A**ACTGTTTAGTAAAGTGAGTAAAAAC

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Table 3.1 Justification for selection of SNPs genotyping

Gene	SNPs number	Health impact	Reference
SCNN1B	rs239345	The A allele carriers perceived salt solutions less intensely than carriers of the T allele	Dias et al., 2013
	rs3785368	The T allele carriers perceived salt solutions less intensely than carriers of the C allele. (the reverse orientation)	Dias et al., 2013
TRPV1	rs8065080	Carriers of the T allele were significantly more sensitive to salt solutions than the CC genotype	Dias et al., 2013
	rs4790522	Carriers of the C allele had higher salt preference than A allele in children	Chamoun et al., 2017

Salt taste perception

Salt taste perceptions were determined using a modified Harris-Kalmus procedure (Galindo-Cuspinera et al. 2009). The lowest concentration that participants could recognize a salty taste in the salt solution series was defined as the recognition threshold. Participants tasted the solutions of sodium chloride (NaCl) (Morton Salt, non-iodized salt, Chicago, Illinois, USA) in ascending order of concentration (0.000, 0.001, 0.002, 0.004, 0.008, 0.016, 0.032, 0.064, 0.128, and 0.256 mol/L) and identified which sample was the first one to have a salty taste. One sample at the concentration which they recognized were sorted into ‘tastes” and “water”, with two blanks. Failure to sort the solutions correctly into two groups led to panelists receiving increased concentrations until the recognition threshold was obtained.

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Data analysis

All data were analyzed using the statistical analysis software, Statistical Package for Social Sciences (SPSS) version 24.0 (SPSS Inc., Chicago, Illinois, USA). Frequencies and percentages described participants' general characteristics and genotype variation between groups. The chi-squared test and Cramer's V test were used to analyze the differences in demographic variables between groups. The genotype frequencies of 4 SNPs were tested for consistency with the Hardy–Weinberg Equilibrium (HWE) using the chi-square tests. Mean differences in characteristic data and salt taste thresholds between and within hypertension and normotensive participants were performed using independent t-tests and analysis of variance (ANOVA). Mann-Whitney tests were tested for mean rank differences in salt intake behaviors and salt taste thresholds between 2 groups. The Spearman correlation tests were applied to evaluate the correlation between salt taste threshold and food acceptability score. Allele frequencies in different groups of participants were compared using the chi-square test. Correlations were used to assess the effect of genotype on salt taste threshold. Mean differences in thresholds across genotypes were assessed using ANOVA. Continous variables were presented as means ± standard deviations (SD). The criterion of significance was p value < 0.05.

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CHAPTER IV

RESULTS AND DISCUSSIONS

General characteristics and health information

The descriptive characteristics of 128 normotensives and 125 hypertensives are reported in Table 4.1. The mean age of normotensive group was 30.5±11.0 years with ages between 20-68 years comprising of 80 Caucasians (62%), 35 African Americans (27.1%), 11 Asians (8.5%), and 3 Hispanic/Latinos (2.3%). The hypertensive group had ages between 22-82 years with a mean age of 52.5±12.9 years. The majority of hypertensives were African Americans (n=68, 73.9%), followed by Caucasians (n=22, 23.9%), and Asians (n=2, 2.2%). Female participants represented 74% of normotensives and 70% of hypertensives. There was a significant difference in age, race, and education levels between normotensive and hypertensive groups (p value< 0.001). According to the hypertension prevalence among adults in the United States during 2015–2016, it was shown that the prevalence of hypertension was higher in the age group 60 and over (63.1%) than 40-59 (33.2%) and 20-29 (7.5%) (Fryar et al. 2017). However, the prevalence of hypertension in this study was high in the age group 40-59 (52%), followed by 60 and over (32.8%) and 20-30 (15.2%) (Figure 4.1). Results also indicated that African Americans (75.2%) had the highest prevalence of hypertension, followed by Caucasians (21.6%). This result showed similar trends as the hypertension prevalence among adults in the United States during 2015–2016 (Figure 4.2). In addition, the majority of normotensive participants had bachelor degrees

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(29.7%) and some college (27.3%) compared to hypertensive participants, with 22.6% having some college and (21.8%) with high school degrees (Table 4.1).

Table 4.1 General characteristics of participants by hypertension status

Variables	Normotensive (N=128)	Hypertensive (N=125)	p value
Mean age (years)	30.5±11.0	52.5±12.9	<0.001§
Gender (N, %)			0.414¥
- Female	95 (74.2)	87 (69.6)	
- Male	33 (25.8)	38 (30.4)	
Weight (kg)	78.8±23.8	94.5±23.7	
Height (cm)	167.2±8.7	168.4±9.6	
Race (N, %)			<0.001‡
- White/Caucasian	80 (62.5)	27 (21.6)	
- Black/African American	34 (26.6)	94 (75.2)	
- Asian	11 (8.6)	3 (2.4)	
- Hispanic/Latino	3 (2.3)	1 (0.8)	
Highest educational graduation (N, %)			<0.001‡
- Less than high school degree	1 (0.8)	15 (12.1)	
- High school degree or equivalent	15 (11.7)	27 (21.8)	
- Some college but no degree	35 (27.3)	28 (22.6)	
- Associate degree	14 (10.9)	11 (8.9)	
- Bachelor degree	38 (29.7)	23 (18.5)	
- Graduate degree	25 (19.5)	20 (16.1)	

Data are presented as mean ± SD and n (%). Statistical differences were determined using
§independent samples t-test with p value<0.001
¥Chi-squared test
‡Cramer's V test with p value<0.001

Health information

Participants' health information is reported in Table 4.2. The mean systolic and diastolic blood pressure values of the normotensive group were 125.6±15.9 and 78.4±11.0 mmHg, respectively, while mean systolic and diastolic blood pressure values were 145.2±21.2 and 89.0±12.9 mmHg in hypertensive group, respectively. Systolic and diastolic blood pressures of the hypertensive group were significantly higher than normotensive group (p value< 0.001).

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There were 36% of participants with hypertension who controlled their systolic blood pressure to less than 140 mmHg and diastolic blood pressure to less than 90 mmHg (Figure 4.3). The percentage of hypertensive participants who controlled their blood pressure was less than adults in the United States (48.3%) (Fryar et al. 2017) as shown in Figure 4.3. The Centers for Disease Control and Prevention (CDC) reported that the prevalence of controlled hypertension overall increased with age and was lower among those aged 18–39 (32.5%) years old than among those aged 40–59 (50.8%) and 60 and over (49.4%) (Fryar et al. 2017). However, the results of this study indicated that the 40-59 (43.1%) age group had the highest amount of controlled hypertension, followed by aged 18-39 (31.5%) and aged 60 and over (26.8%).

The study also indicated that 48.2% of all 253 participants were obese (BMI> 30 kg/m^2). There was a significant difference in BMI between the normotensives (28.1±7.7) and hypertensives (33.4±8.3) (p value< 0.001) (Table 4.2). Moreover, the prevalence of obesity in hypertensive participants (62.4%) was almost twofold greater than the obesity rate in normotensive participants (34.4%). The CDC reported that the prevalence of obesity in U.S. adults was 42.4% in 2017-2018 (Hales et al. 2017). According to NHANES III survey, a strong association was observed between BMI and hypertension among men and women in all race or ethnic groups and in most age groups (National Institutes of Health 1999). A previous study revealed that obesity in both childhood and adolescence was associated with a 2-fold for higher risk of adult hypertension, coronary heart disease, and stroke (Kodama et al. 2014).

Approximately 40% of normotensive participants reported moderate-intensity or 150 minutes/week of physical activity (Table 4.2). A majority of hypertensive participants reported their physical activity level at low-intensity or 75 minutes/week (34.7%) followed by no physical

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activity (31.4%). The results also showed that the physical activity level of normotensive participants was significantly higher than hypertensive participants (p value< 0.001).

Approximately 46% of the normotensive group reported eating modestly salty foods while 32% of hypertensive group reported eating foods that were a little salty as well as modestly salty food (Table 4.2). The majority of both groups reported that they seldom add salt or soy sauce to cooked dishes. Forty-six percent of hypertensive participants reported that they never add salt to food before tasting it.

Regarding the mean differences of blood pressure, BMI, and salt recognition thresholds between hypertensive and normotensive groups, our results indicated that blood pressure values were different in both groups, hypertensive/normotensive females and males. Salty test recognition and BMI were different between hypertensive and normotensive females (p value< 0.001) (Table 4.3). Normotensive and hypertensive males had differences in systolic and diastolic blood pressures (P value< 0.05) but BMI valus were similar for both groups of males. Table 4.4 shows BMI was different between ages 20-29 normotensive and hypertensive participants but not other age groups. BMI and salt threshold were different between African American hypertensives and not Caucasians with hypertension (Table 4.5). However, there were not enough Asian and Hispanic/Latino participants. Therefore, those data were not considered. Body mass index was different between males and females in both normotensive and hypertensive groups, and the difference was greater between males and females in normotensive than hypertensive groups as shown in Table 4.6.

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Table 4.2 Health information of participants by hypertension status

Variables	Normotensive	Hypertensive	p value
Systolic blood pressure	125.6±15.9	145.2±21.2	<0.001[§**]
Diastolic blood pressure	78.4±11.0	89.0±12.9	<0.001[§**]
BMI	28.1±7.7	33.4±8.3	<0.001[§**]
- Underweight	7 (5.5)	2 (1.6)	
- Normal	52 (40.6)	18 (14.4)	
- Overweight	25 (19.5)	27 (21.6)	
- Obese	44 (34.4)	78 (62.4)	
Physical Activity	2.7±1.1	2.1±1.0	<0.001[¥**]
- None	16 (12.7)	38 (31.4)	
- Low-intensity (75 minutes/week)	40 (31.7)	42 (34.7)	
- Moderate-intensity (150 minutes/week)	50 (39.7)	35 (28.9)	
- High-intensity (300 minutes/week)	20 (15.9)	6 (5.0)	
Typical salt content of food eaten	3.0±0.9	3.1±1.0	0.204[¥]
- Not salty	4 (3.1)	10 (8.0)	
- Little salt	31 (24.2)	40 (32.0)	
- Modestly salty	59 (46.1)	40 (32.0)	
- A little salty	30 (23.4)	29 (23.2)	
- Very salty	4 (3.1)	6 (4.8)	
Add salt or soy sauce to cooked dishes	3.3±1.1	3.4±1.3	0.394[¥]
- Never	12 (9.4)	24 (19.2)	
- Seldom	55 (43.0)	46 (36.8)	
- Usually	34 (26.6)	27 (21.6)	
- Frequently	18 (14.1)	12 (9.6)	
- Always	9 (7.0)	16 (12.8)	
Add salt to food before tasting	3.8±1.1	3.9±1.3	0.349[¥]
- Never	44 (34.4)	57 (45.6)	
- Seldom	47 (36.7)	29 (23.2)	
- Usually	18 (14.1)	20 (16.0)	
- Frequently	12 (9.4)	10 (8.0)	
- Always	7 (5.5)	9 (7.2)	

BMI categories using criteria of CDC (Centers for Disease Control and Prevention, 2014) for healthy adults. Data are presented as mean ± SD and n (%).

[§] Independent samples t-test

[¥] Independent samples Mann-Whitney U Test

[**] p value<0.001

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Table 4.3 Blood pressure, BMI, and salt recognition thresholds for normotensive and hypertensive groups across gender

Variables	Normotensive	Hypertensive	p value
Female	n=95	n=87	
Systolic blood pressure (mmHg)	122.46±15.3	144.2±21.3	<0.001**
Diastolic blood pressure (mmHg)	78.1±10.8	87.3±12.9	<0.001**
BMI (kg/m^2)	27.0±7.3	33.9±8.8	<0.001**
Salt recognition thresholds (mol/L)	0.022±0.021	0.036±0.034	<0.001**
Male	n=33	n=38	
Systolic blood pressure (mmHg)	134.6±14.5	147.6±20.9	0.004*
Diastolic blood pressure (mmHg)	79.1±11.8	86.2±13.1	0.021*
BMI (kg/m^2)	30.6±7.9	30.7±7.8	0.978
Salt recognition thresholds (mol/L)	0.025±0.020	0.032±0.023	0.184

Data are presented as mean ± SD. Statistical differences were determined using independent samples t-test. *p value< 0.05, **p value >0.001

In addition, BMI and salt recognition threshold differed in normotensive ages 20-39 which had a lower salt threshold and lower BMI than other age groups. However, in the hypertensive group, there were no differences in BMI and salt threshold across ages (Table 4.7). Our results also indicated that BMI differed between race in hypertensive group and but not in the normotensive group (Table 4.8). Therefore, the overall data of mean differences of blood pressure, BMI, and salt recognition thresholds between hypertensive and normotensive groups across gender, age, and race showed that gender, age, and race did not confound the salt recognition thresholds results. However, age and gender seemed to slightly confound the results.

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Table 4.4 Blood pressure, BMI, and salt recognition thresholds for normotensive and hypertensive groups across age groups

Variables	Normotensive	Hypertensive	p value
20-39 age	n=104	n=19	
Systolic blood pressure (mmHg)	124.5±15.5	141.8±18.8	<0.001**
Diastolic blood pressure (mmHg)	78.2±10.9	88.6±13.1	<0.001**
BMI (kg/m^2)	27.0±7.1	35.9±10.1	<0.001**
Salt recognition thresholds (mol/L)	0.020±0.16	0.027±0.028	0.117
40-59 age	n=21	n=65	
Systolic blood pressure (mmHg)	129.9±18.0	143.5±22.3	0.013*
Diastolic blood pressure (mmHg)	79.7±11.6	88.3±13.5	0.010*
BMI (kg/m^2)	31.3±8.1	33.3±8.6	0.349
Salt recognition thresholds (mol/L)	0.035±0034	0.034±0.036	0.877
60 and older age	n=3	n=41	
Systolic blood pressure (mmHg)	132.3±12.4	149.7±20.1	0.150
Diastolic blood pressure (mmHg)	77.0±16.4	84.1±11.7	0.327
BMI (kg/m^2)	35.8±10.0	31.0±7.6	0.312
Salt recognition thresholds (mol/L)	0.021±0.009	0.039±0.024	0.204

Data are presented as mean ± SD. Statistical differences were determined using One-way ANOVA, *p value< 0.05, **p value >0.001

Table 4.5 Blood pressure, BMI, and salt recognition thresholds for normotensive and hypertensive groups across races

Variables	Normotensive	Hypertensive	p value
White/Caucasian	n=80	n=27	
Systolic blood pressure (mmHg)	125.9±16.4	141.7±17.7	<0.001**
Diastolic blood pressure (mmHg)	78.6±12.1	84.9±9.6	0.015*
BMI (kg/m^2)	27.4±6.9	29.8±5.4	0.100
Salt recognition thresholds (mol/L)	0.023±0.019	0.028±0.023	0.294
Black/African American	n=34	n=94	
Systolic blood pressure (mmHg)	127.5±15.4	146.8±22.1	<0.001**
Diastolic blood pressure (mmHg)	79.7±8.4	88.0±13.6	<0.001**
BMI (kg/m^2)	29.7	34.0±9.2	0.018*
Salt recognition thresholds (mol/L)	0.022±0.024	0.037±0.033	0.017*

Data are presented as mean ± SD. Statistical differences were determined using independent samples t-test. *p value< 0.05, **p value >0.001

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Table 4.6 Blood pressure, BMI, and salt recognition thresholds for females and males across blood pressure status

Variables	Female	Male	p value
Normotensive	n=95	n=33	
Systolic blood pressure (mmHg)	122.46±15.3	134.6±14.5	<0.001**
Diastolic blood pressure (mmHg)	78.1±10.8	79.1±11.8	0.654
BMI (kg/m^2)	27.0±7.3	30.6±7.9	0.018*
Salt recognition thresholds (mol/L)	0.022±0.021	0.025±0.020	0.361
Hypertensive	n=87	n=38	
Systolic blood pressure (mmHg)	144.2±21.3	147.6±20.9	0.411
Diastolic blood pressure (mmHg)	87.3±12.9	86.2±13.1	0.667
BMI (kg/m^2)	33.9±8.8	30.7±7.8	0.050
Salt recognition thresholds (mol/L)	0.036±0.034	0.032±0.023	0.599

Data are presented as mean ± SD. Statistical differences were determined using independent samples t-test. *p value< 0.05, **p value >0.001

Table 4.7 Blood pressure, BMI, and salt recognition thresholds for age groups across blood pressure status

Variables	20-39 age	40-59 age	≥ 60 age	p value
Normotensive	n=104	n=21	n=3	
Systolic blood pressure (mmHg)	124.5±15.5	129.9±18.0	132.3±12.4	0.290
Diastolic blood pressure (mmHg)	78.2±10.9	79.7±11.6	77.0±16.4	0.837
BMI (kg/m^2)	27.0±7.1	31.3±8.1	35.8±10.0	0.011*
Salt recognition thresholds (mol/L)	0.020±0.16	0.035±0034	0.021±0.009	0.008*
Hypertensive	n=19	n=65	n=41	
Systolic blood pressure (mmHg)	141.8±18.8	143.5±22.3	149.7±20.1	0.255
Diastolic blood pressure (mmHg)	88.6±13.1	88.3±13.5	84.1±11.7	0.224
BMI (kg/m^2)	35.9±10.1	33.3±8.6	31.0±7.6	0.118
Salt recognition thresholds (mol/L)	0.027±0.028	0.034±0.036	0.039±0.024	0.366

Data are presented as mean ± SD. Statistical differences were determined using One-way ANOVA, *p value< 0.05

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Table 4.8 Blood pressure, BMI, and salt recognition thresholds for age groups across blood pressure status

Variables	White/Caucasian	Black/African American	p value
Normotensive	n=80	n=34	
Systolic blood pressure (mmHg)	125.9±16.4	127.5±15.4	0.624
Diastolic blood pressure (mmHg)	78.6±12.1	79.7±8.4	0.634
BMI (kg/m^2)	27.4±6.9	29.7	0.136
Salt recognition thresholds (mol/L)	0.023±0.019	0.022±0.024	0.844
Hypertensive	n=27	n=94	
Systolic blood pressure (mmHg)	141.7±17.7	146.8±22.1	0.272
Diastolic blood pressure (mmHg)	84.9±9.6	88.0±13.6	0.269
BMI (kg/m^2)	29.8±5.4	34.0±9.2	0.024*
Salt recognition thresholds (mol/L)	0.028±0.023	0.037±0.033	0.169

Data are presented as mean ± SD. Statistical differences were determined using independent samples t-test. *p value< 0.05

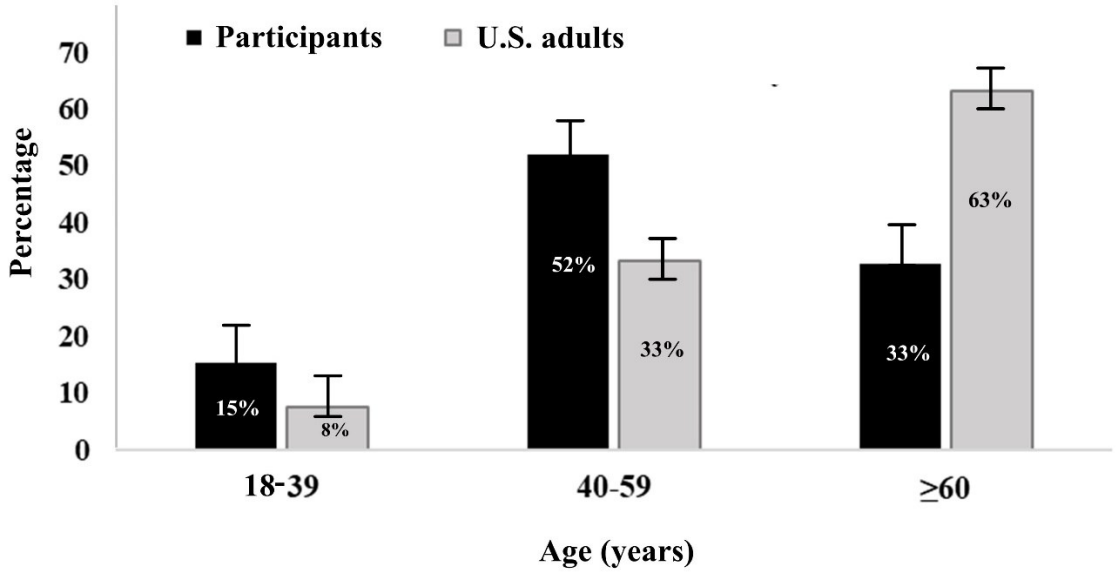

Figure 4.1 Comparison of the hypertension prevalence between participants and U.S. adults (Fryar et al. 2017) by age

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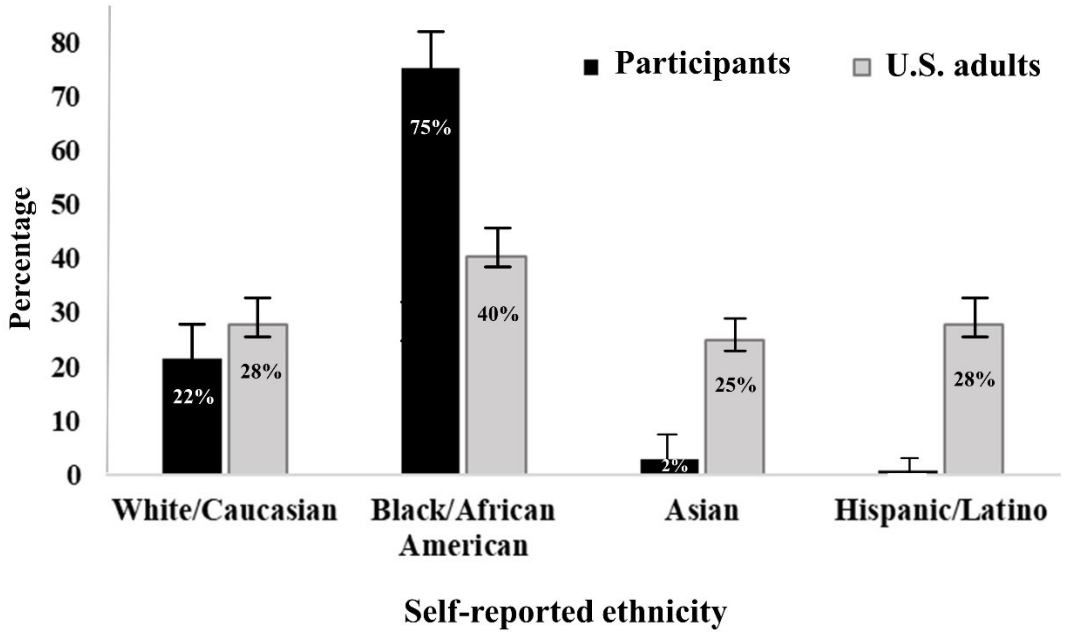

Figure 4.2 Comparison of the hypertension prevalence between participants and U.S. adults (Fryar et al. 2017) by self-reported ethnicity

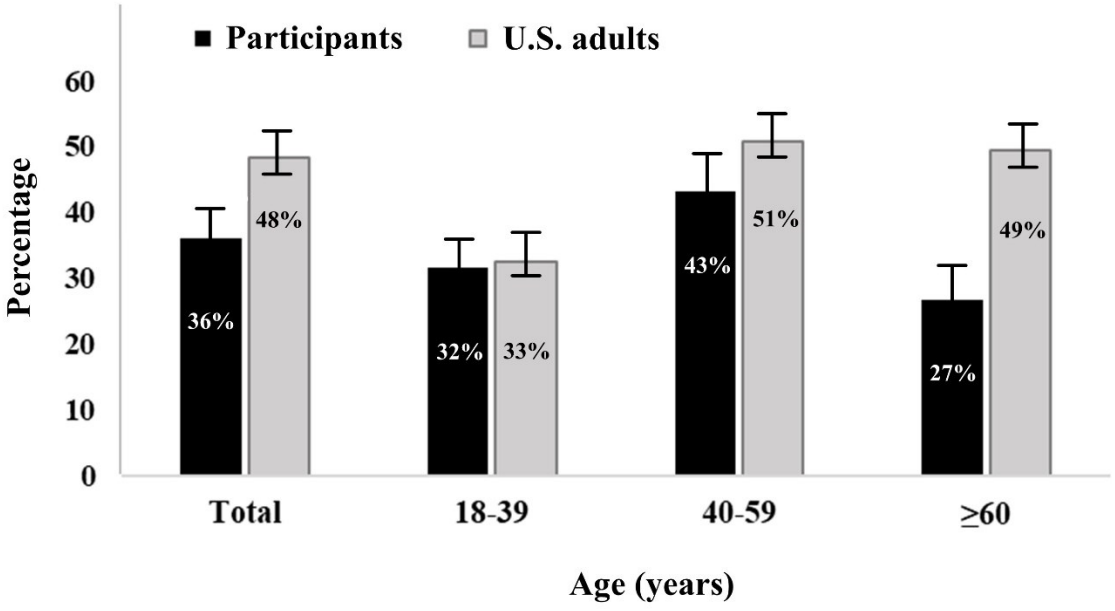

Figure 4.3 Comparison of controlled hypertension between participants and U.S. adults (Fryar et al. 2017) by age

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Differences in salt recognition threshold between hypertensive and normotensive participants

The mean recognition thresholds of normotensive and hypertensive groups were 0.022±0.020 mol/L and 0.035±0.031 mol/L, respectively. The greatest percentage of hypertensive participants (43%) recognized the salty taste at 0.032 mol/L while 37% of normotensive participants recognized it at 0.016 mol/L (Figure 4.4). Moreover, recognition threshold was significantly higher for the group of hypertensive participants (p value< 0.001) (Figure 4.5)

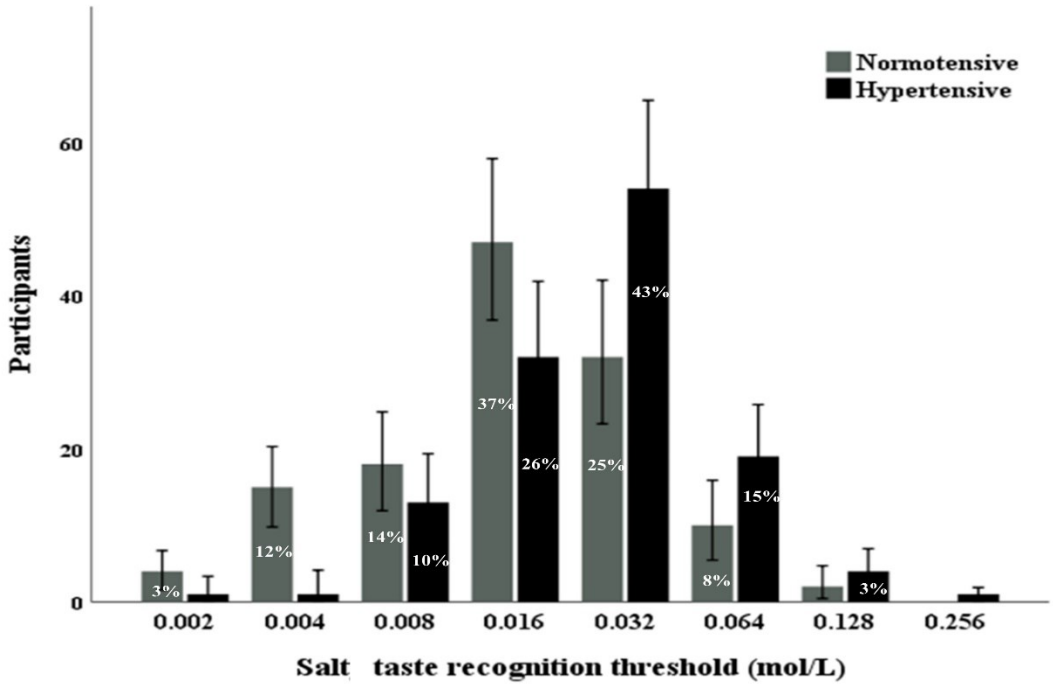

Figure 4.4 Distribution of recognition threshold by blood pressure status

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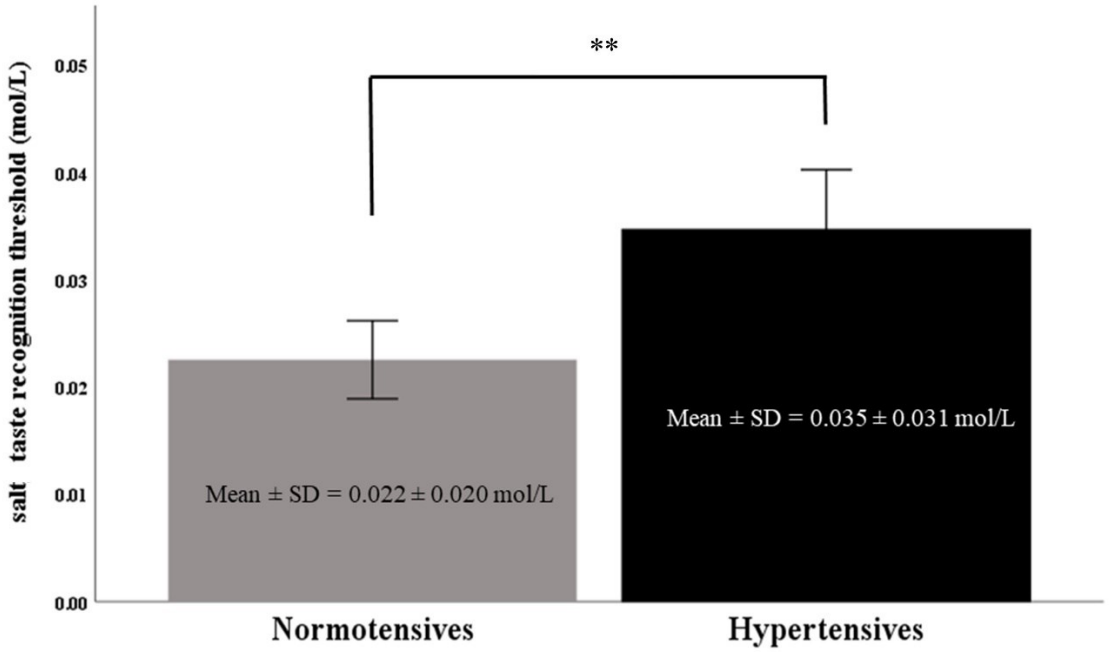

Figure 4.5 Salt taste recognition threshold and blood pressure status

Statistical differences were determined using Mann-Whitney U test, **p value<0.001

A study in Brazil revealed that the salt taste recognition threshold was 0.013±0.017 mol/L for the normotensive group and 0.027±0.016 mol/L for the hypertensive group which was significantly higher for the group of hypertensive participants (p value< 0.001) (Piovesana et al. 2013). These researchers also reported that the salt recognition threshold was positive and moderately correlated with total sodium intake for the whole group (Piovesana et al. 2013). A study with people in Turkey revealed that 55.5% of healthy participants recognized the salty taste at a level of 0.016 mol/L (Öner et al. 2016). These authors stated that nutritional behavior may be influenced by cultural factors, such as food that has been consumed since childhood. In addition, there was a study in chronic kidney disease patients which revealed that the salt taste recognition threshold was influenced by sodium intake and can be improved by sodium

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restriction (Kusaba et al. 2009). They reported that diuretics and antihypertensive medication increased the gustatory threshold. One possible reason was zinc deficiency due to on increased zinc concentration in urinary excretion due to the use of diuretics use (Kusaba et al. 2009). However, the mechanisms of zinc deficiency due to diuretic use is unclear.

The allele frequencies of SNPs (*SCNN1B, TRPV1*) of taste receptor genes in hypertensive and normotensive subjects

The genotype and allele frequencies of 4 SNPs (*SCNN1B*, rs239345, rs3785368; *TRPV1*, rs8065080, rs4790522) are presented in Table 4.9. All alleles were in Hardy Weinberg Equilibrium (HWE) with p values greater than 0.05. In the normotensive group, the minor alleles of SNPs rs239345, rs3785368, rs8065080, and rs4790522 had a frequency of 0.24 (allele A), 0.16 (allele A), 0.32 (allele C), and 0.43 (allele A), respectively, which were similar to the dbSNP database (NCBI 2020). The minor allele frequencies of rs239345 and rs4790522 in the hypertensive group were 0.35 (allele A) and 0.49 (allele A) which were similar to the dbSNP database (NCBI 2020) as well. However, the minor allele frequencies of rs3785368 (0.12; allele A) and rs8065080 (0.22; allele C) in the hypertensive group were less than what was reported from the NCBI reference data (p value = 0.027 and 0.032, respectively) (Figure4.6B and 4.7A).

Moreover, a risk for hypertension was observed in 2 polymorphisms, which were *SCNN1B*, rs239345 and *TRPV1*, rs8065080 (Table 4.10). The risk of having hypertension among the TT genotype of SNP rs8065080, TRPV1 gene was approximately 2 times greater than that of carriers of the C allele (CC and CT) with a 95% confidence interval of 1.14-3.13 (p value = 0.016). In addition, a risk for hypertension with an odds ratio of 0.55 (p value = 0.024) was

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observed between the TT genotype and carriers of the A allele in SNP rs239345, *SCNN1B* gene with a 95% confidence interval of 0.34-0.91.

A study of a white European population indicated that the variants of *SCNN1B*, rs239345, were associated with hypertension (Hannila-Handelberg et al. 2005). Upregulation of the protein expression of *SCNN1B* increases DNA methylation which may lead to increased blood pressure (Zhong et al. 2016). TRPV1 protein, a gene product of *TRPV1*, may play an important role in the regulation of salt and water homeostasis and blood pressure (Wang 2005; Wang and Sachs 2007). A study in rats revealed that the degeneration of TRPV1-positive sensory nerves reduced the salt sensitivity threshold of rat with a concomitant increase in blood pressure (Wang and Sachs 2007). This evidence suggests that *TRPV1* may play a counterbalancing role by inhibiting these prohypertensive systems (Wang 2008).

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Table 4.9 Genotypes and allele distribution in normotensive and hypertensive groups

Gene	Variant	Genotype	N (%)	MAF	HWE p-value§
Normotensive group					
SCNN1B	rs239345	AA	6 (4.7)	0.24	0.537
		AT	49 (38.3)		
		TT	73 (57.0)		
	rs3785368	AA	4 (3.1)	0.16	0.557
		AG	32 (25.0)		
		GG	92 (71.9)		
TRPV1	rs8065080	CC	17 (13.3)	0.32	0.153
		CT	49 (38.3)		
		TT	62 (48.4)		
	rs4790522	AA	23 (18.0)	0.43	0.819
		AC	64 (50.0)		
		CC	41 (32.0)		
Hypertensive group					
SCNN1B	rs239345	AA	15 (12.0)	0.35	0.957
		AT	57 (45.6)		
		TT	53 (42.4)		
	rs3785368	AA	3 (2.4)	0.12	0.250
		AG	23 (18.4)		
		GG	99 (79.2)		
TRPV1	rs8065080	CC	9 (7.2)	0.22	0.094
		CT	36 (28.8)		
		TT	80 (64.0)		
	rs4790522	AA	30 (24.0)	0.49	0.926
		AC	63 (50.4)		
		CC	32 (25.6)		

HWE; Hardy Weinberg Equilibrium, MAF; Minor Allele Frequency, §Chi-square test.

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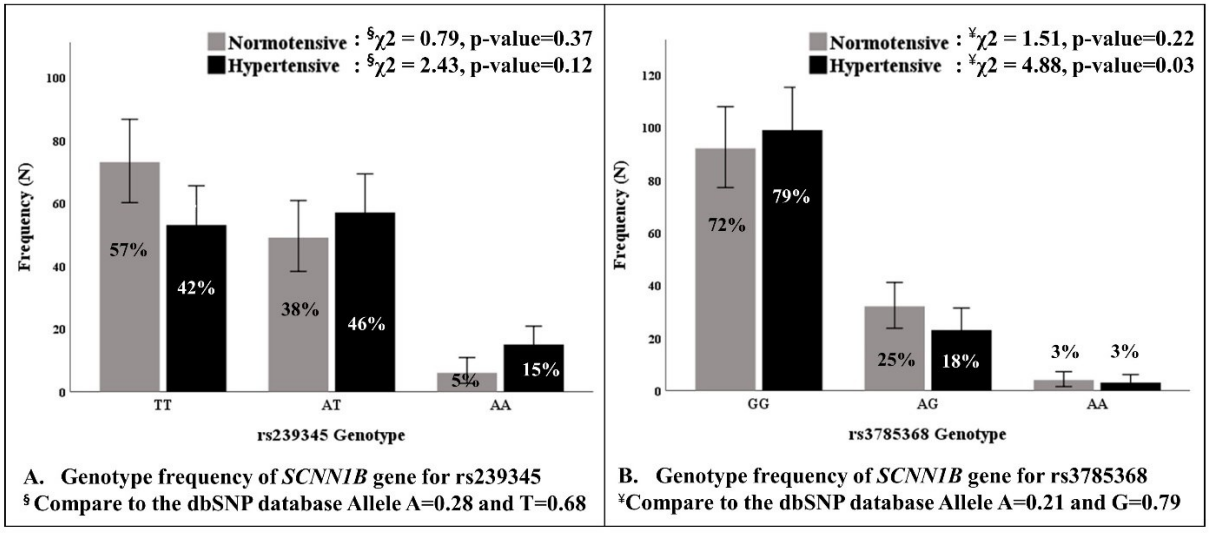

Figure 4.6 Genotype distribution of *SCNN1B* gene for rs239345 and rs3785368

χ2: Chi-square test was used to determine the difference between samples and the NCBI dbSNP database, p value< 0.05 considered significant.

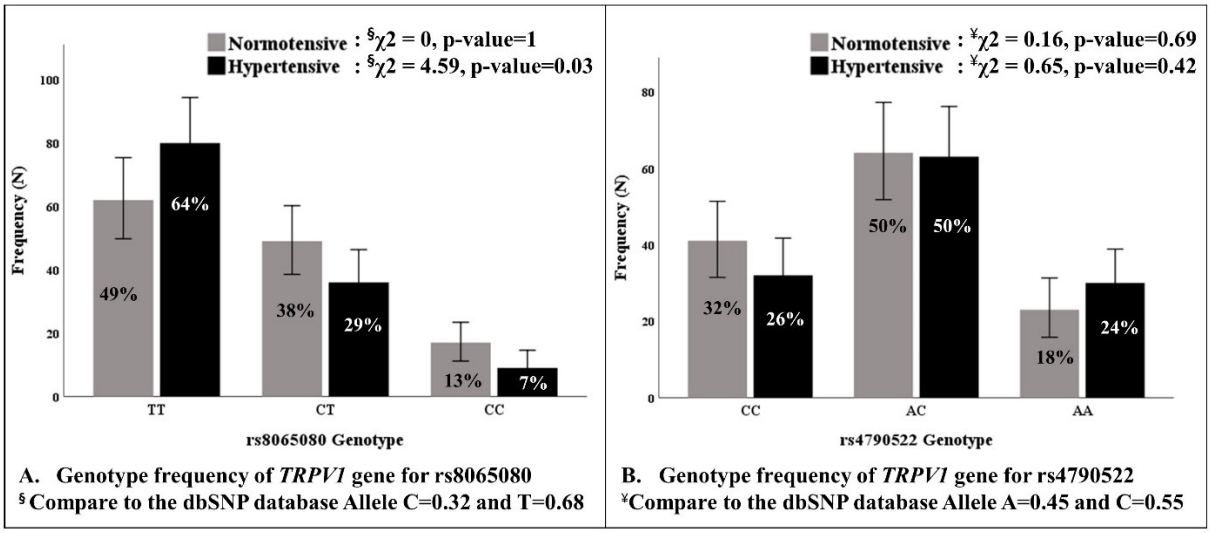

Figure 4.7 Genotype distribution of *TRPV1* gene for rs8065080 and rs4790522

χ2: Chi-square test was used to determine the difference between samples and the NCBI dbSNP database, p value< 0.05 considered significant.

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Table 4.10 The genotype distribution of *SCNN1B* and *TRPV1* variants and the risk of hypertension

Gene	Variant	Genotype	Hypertensive	Normotensive	Odd ratio (95%CI)	§p value
SCNN1B	rs239345	TT	53 (20.9)	73 (28.9)	0.55(0.34-0.91)	0.024*
		AA/AT	72 (28.5)	55 (21.7)		
	rs3785368	GG	99 (39.1)	92 (36.4)	1.49(0.83-2.66)	0.191
		AA/AG	26 (10.3)	36 (14.2)		
TRPV1	rs8065080	TT	80 (31.6)	62 (24.5)	1.89(1.14-3.13)	0.016*
		CC/CT	45 (17.8)	66 (26.1)		
	rs4790522	CC	32 (12.6)	41 (16.2)	0.73(0.82-1.26)	0.270
		AA/AC	93 (36.8)	87 (34.4)		

95%CI: 95% confidence interval, §Chi-square test, *p value< 0.05

Affect of single nucleotide polymorphisms (SNPs) in the taste receptor genes (*SCNN1B, TRPV1*) and the perception of saltiness

Genetic variations and food preferences

The results of food preferences are presented in Figures 4.8 and 4.9. The hypertensive group had higher food preference scores for hotdogs, a high salt food, than the normotensive group (p value< 0.05). No other differences existed (p value>0.05) between hypertensive and normotensive groups with respect to food preference scores for high salt foods. However, the hypertensive group had higher food preference scores for tuna, carrots, and soda compared to the normotensive group (p value< 0.05).

Food preference scores differed among *TRPV1* variant rs4790522 (p value< 0.05) which indicated that normotensive participants within the CC genotype preferred celery when compared to the AA genotype (p value = 0.013). In contrast, hypertensive participants with the AA genotype preferred celery more than the CC genotype (p value = 0.037). Moreover, hypertensive participants with the CC genotype preferred cookies and ice cream compared to the AA genotype (p value = 0.044 and 0.033, respectively). In addition, both groups had significant

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correlations between genotypes of 4 SNPs and food preference categories from the gLMS (0=neutral, ±6 weakly, ±17 moderately, ±35 strongly, ±53 very strongly, ±100 strongest of any kind applied to liking/disliking) of some food items such as cookies, fries, and olives (p value< 0.05). However, the correlations were weak ($r < \pm 0.25$) regarding the Colton's rule for interpreting the size of the correlations. This is in agreement with previous research, which reported that genetic taste measures were not associated with food preferences (Duffy and Bartoshuk 2000).

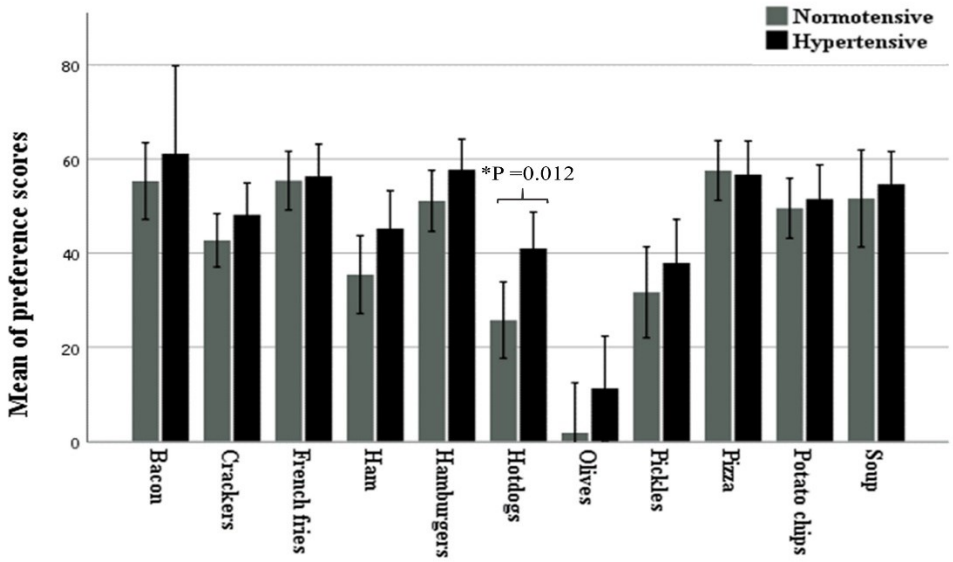

Figure 4.8 Comparison of food preference scores in high salt foods between normotensive and hypertensive participants.

Statistical differences were determined using independent t test, *$p < 0.05$

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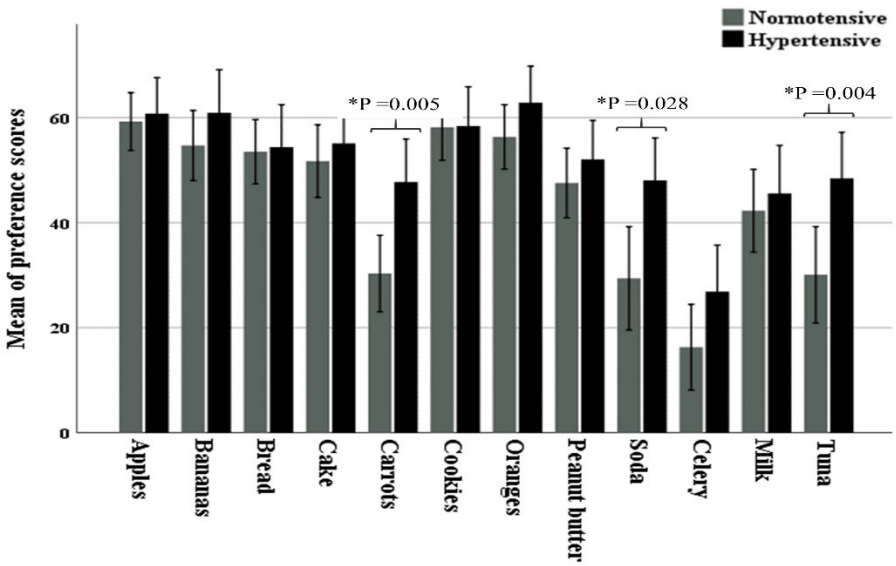

Figure 4.9 Comparison of food preference scores in high sweet and low salt foods between normotensive and hypertensive participants.

Statistical differences were determined using independent t test, *p < 0.05

Genetic variations and salt taste recognition thresholds

Results of salt taste recognition thresholds in normotensive and hypertensive groups across genotype are presented in Table 4.11. Salt taste recognition thresholds were not significantly different among genotypes of *SCNN1B* variants rs239345 (p value = 0.271), rs3785368 (p value = 0.835) and *TRPV1* variant rs8065080 (p value = 0.408) (Figures 4.10A, C, D). However, a significant difference was found among *TRPV1* variant rs4790522 in salt taste recognition threshold (p = 0.003) (Figure 4.10B). In addition, hypertensive participants with the AA genotype had a higher average salt taste recognition threshold than the CC genotype (p value = 0.022) (Figure 4.14B). In this study there was a significant difference in age between normotensive and hypertensive groups (p value< 0.001). Suchecka et al. (2016) reported that the aging process led to a decrease in salt perception in participants that were 50 years or older (Suchecka et al. 2016). However, there was no significant difference in salt taste recognition

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threshold between participamts that were between 20-50 years old and older within normotensive (p value = 0.355) and hypertensive (p value = 0.838) groups in the current study (data not shown).

Therefore, of 2 *SCNN1B* SNPs and 2 *TRPV1* SNPs examined, only the *TRPV1* variant rs4790522 with AA homozygotes had a greater salt taste recognition threshold than the CC homozygotes in both the whole group and also the hypertensive group. In contrast, the study by Dias et al. (2013) reported that carriers of the T allele perceived salt significantly higher than participants with the homozygous for the C allele in the *TRPV1* gene, rs8065080 (Dias et al. 2013). Participants that were homozygous for the A allele of the SCNN1B gene, rs239345 and the T allele of rs3785368 perceived the salt solution at significantly lower concentrations than carriers of the other respective alleles (Dias et al. 2013). The present study's finding is inconsistent with the results from the previous study in children, where carriers of the C allele of rs4790522 had higher salt recognition treshhold than carriers of the A allele (Chamoun et al. 2016). Therefore, age may have an effect on salt taste intensity or differnces in salt perception may be greater among children than among adults.

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Table 4.11 Salt recognition thresholds in normotensive and hypertensive groups across genotype

Gene	Variant	Genotype	Salt recognition thresholds (mol/L; mean±SD)	p value[¥]
Normotensive group				
SCNN1B	rs239345	AA	0.025±0.012	0.760
		AT	0.021±0.021	
		TT	0.023±0.021	
	rs3785368	AA	0.024±0.009	0.585
		AG	0.026±0.026	
		GG	0.021±0.019	
TRPV1	rs8065080	CC	0.023±0.031	0.785
		CT	0.021±0.015	
		TT	0.024±0.021	
	rs4790522	AA	0.028±0.029	0.390
		AC	0.022±0.020	
		CC	0.020±0.016	
Hypertensive group				
SCNN1B	rs239345	AA	0.043±0.028	0.531
		AT	0.032±0.035	
		TT	0.035±0.028	
	rs3785368	AA	0.027±0.009	0.873
		AG	0.037±0.028	
		GG	0.035±0.033	
TRPV1	rs8065080	CC	0.025±0.008	0.622
		CT	0.035±0.045	
		TT	0.036±0.025	
	rs4790522	AA	0.049±0.048	0.015*
		AC	0.032±0.025	
		CC	0.028±0.017	

[¥]Statistical differences were determined using One-way ANOVA, *p value< 0.05

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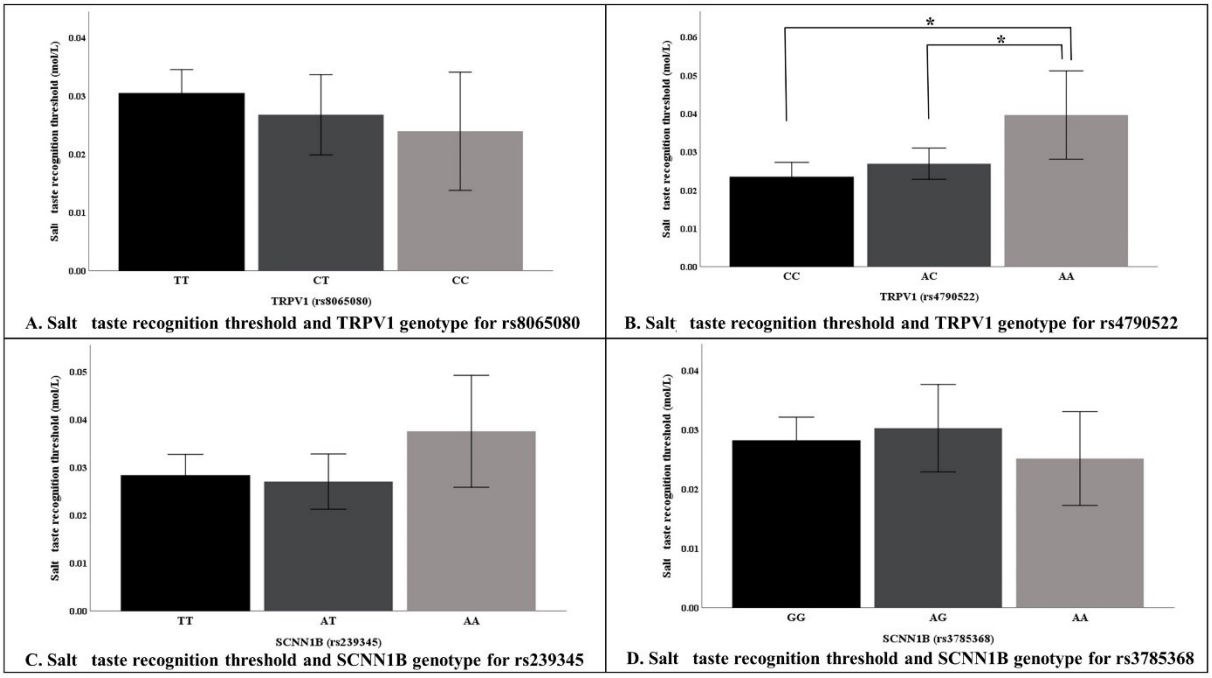

Figure 4.10 Salt taste recognition thresholds and *SCNN1B* and *TRPV1* genotypes

Statistical differences were determined using One-way ANOVA, *post-hoc test (p value< 0.05)

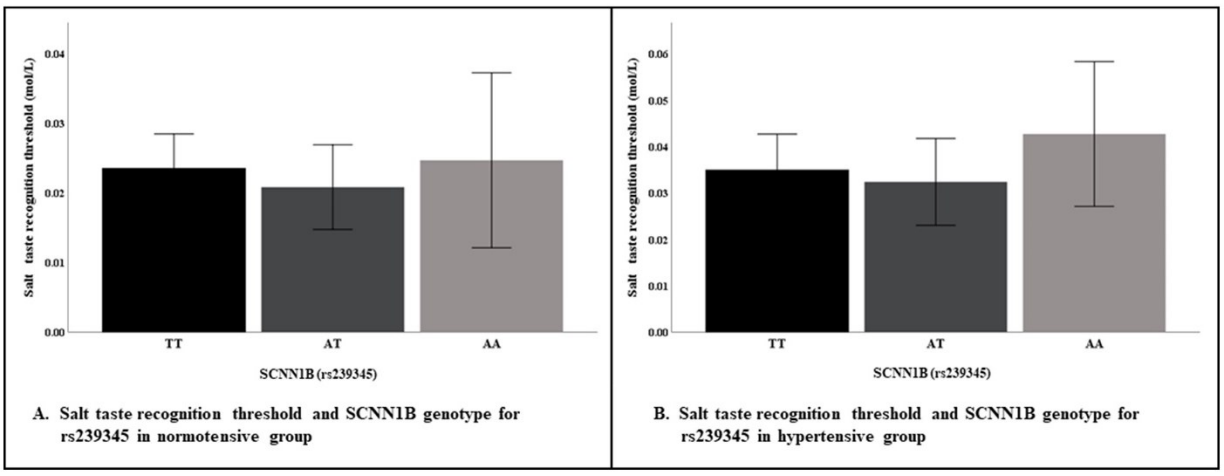

Figure 4.11 Salt taste recognition thresholds and *SCNN1B* genotypes for rs239345 by blood pressure status

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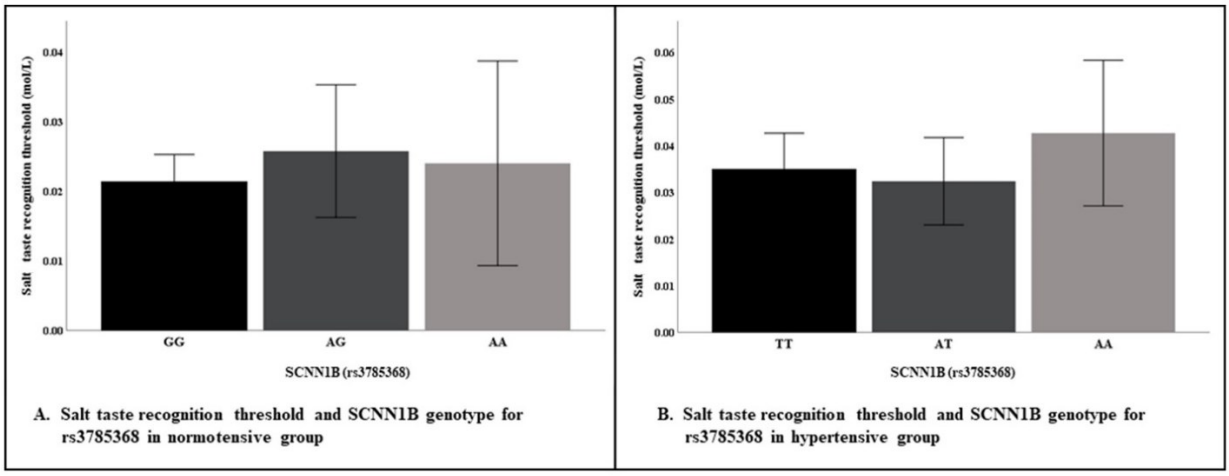

Figure 4.12 Salt taste recognition thresholds and *SCNN1B* genotypes for rs3785368 by blood pressure status

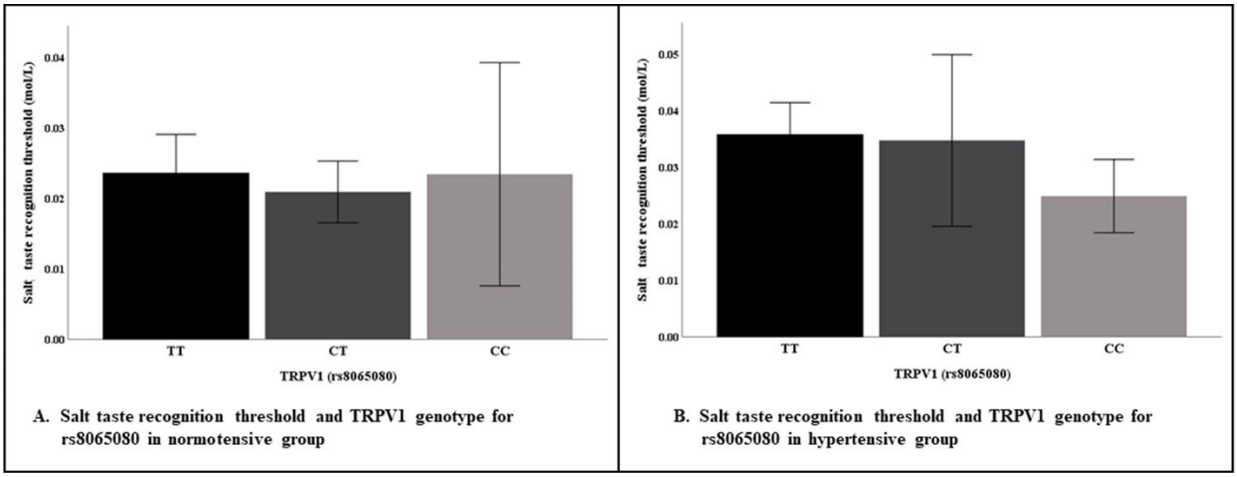

Figure 4.13 Salt taste recognition thresholds and *TRPV1* genotypes for rs8065080 by blood pressure status

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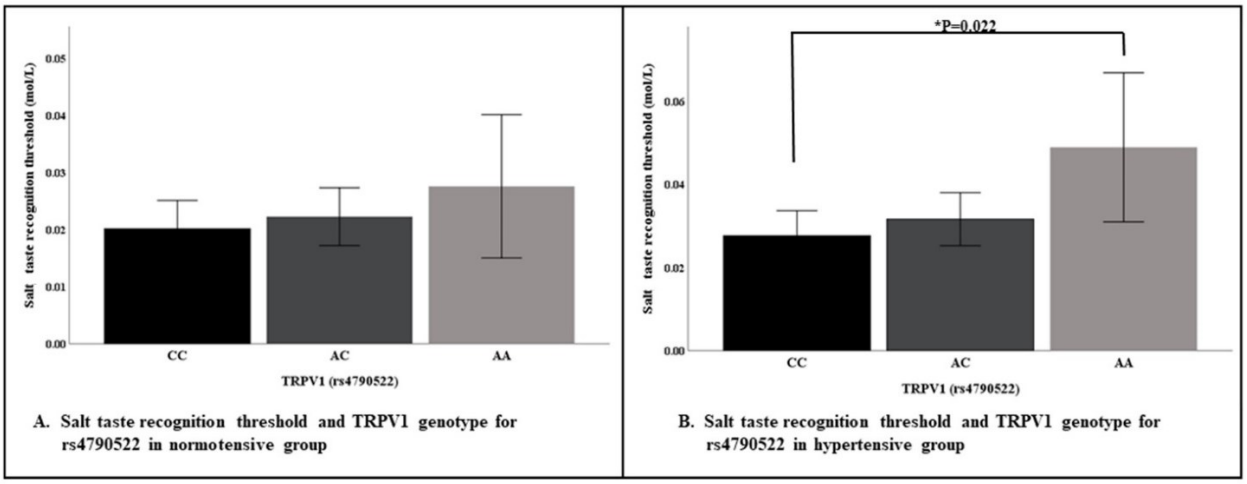

Figure 4.14 Salt taste recognition thresholds and *TRPV1* genotypes for rs4790522 by blood pressure status

Statistical differences were determined using One-way ANOVA, *post-hoc test (p value< 0.05)

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CHAPTER V

STRENGTHS, LIMITATIONS, AND CONCLUSIONS

Strengths and Limitations

Strengths of this study are a sufficient sample size with a 95% confidence level at 80% power, the use of a reliable procedure to determine recognition thresholds, and a thorough and standardized genetic analysis. Although we reached the number of required participants there were differences in age, BMI, ethnicity, and physical activity between normotensive and hypertensive groups. Nevertheless, there was no significant difference in salt taste recognition threshold between age 20-50 yesrs and older within normotensive and hypertensive groups in this study. Additional limitation of the study is the lack of information on confounding variables such as smoking status and obesity.

Conclusions

This study assessed genetic variations in *SCNN1B* and *TRPV1* genes and their link with the salt recognition threshold and hypertension risk. Results indicated that the hypertensive group had a higher salt taste recognition threshold than the normotensive group. However, there was not a significant correlation between salt taste recognition threshold and salty food preference. Results also provided evidence that the polymorphism *TRPV1*, rs4790522 with AA genotype was associated with a lower sensitivity threshold. Moreover, people with homozygous TT of SNP rs8065080, *TRPV1* gene had twice the risk of having hypertension than that of

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carriers of the C allele. In contrast, people with homozygous TT of SNP rs239345, *SCNN1B* gene were 45% less likely to have hypertension compared to carriers of the A allele.

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APPENDIX A

INSTITUTIONAL REVIEW BOARD (IRB) APPROVAL

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MSU IRB approval letter

From: nrs54@msstate.edu
Sent Date: Thursday, January 03, 2019 14:06:48 PM
To: ttm135@msstate.edu, dp127@msstate.edu, mws72@msstate.edu, pt419@msstate.edu, wc523@msstate.edu
Cc:
Bcc:
Subject: Approval Notice for Study # IRB-18-510, Exploring the relationship between genetic variation in taste receptor genes and salt taste perception among people with hypertension
Message:

Protocol ID: IRB-18-510
Principal Investigator: Terezie Mosby
Protocol Title: Exploring the relationship between genetic variation in taste receptor genes and salt taste perception among people with hypertension
Review Type: EXPEDITED
Approval Date: January 03, 2019
Expiration Date:December 15, 2020

The above referenced study has been approved. To access your approval documents, log into myProtocol and click on the protocol number to open the approved study. Your official approval letter can be found under the Event History section. For non-exempt approved studies, all stamped documents (e.g., consent, recruitment) can be found in the Attachment section and are labeled accordingly.

If you have any questions that the HRPP can assist you in answering, please do not hesitate to contact us at irb@research.msstate.edu or 662.325.3994.

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APPENDIX B

QUESTIONNAIRE

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Questionnaire

Example Project: The relationship between single nucleotide polymorphisms (SNPs) variation in taste receptor genes and salt taste perception in patients with hypertension.

Declaration: I am a Ph.D. student, concentration in nutrition at MSU. This questionnaire is intended to collect demographic and food acceptability information. The questionnaire consists of three parts: 1) general information; 2) health information; 3) food acceptability information. If you need assistance, please contact the co-investigator. Thank you.

Part 1: General information

Instructions: The questionnaire contains checklist questions and short answer questions. Please answer the most correct answer.

Q1: Gender ☐ Male ☐ Female ☐ Other, specify_____

Q2: Age_____ years

Q3: Race ☐ Non-Hispanic White ☐ Black/African American ☐ Asian

☐ Hispanic/Latino ☐ Native Hawaiian or other Pacific Islander

☐ American Indian or Alaskan Native ☐ Other, specify_____

Q4: Highest educational graduation

☐ Less than high school degree ☐ Associate degree

☐ High school degree or equivalent (e.g., GED) ☐ Bachelor degree

☐ Some college but no degree ☐ Graduate degree

Q5: Household income

☐ $0 to $9,999 ☐ $10,000 to $24,999 ☐ $25,000 to $49,999

☐ $50,000 to $74,999 ☐ $75,000 to $99,999 ☐ $100,000 to $124,999

☐ $125,000 to $149,999 ☐ $150,000 to $174,999 ☐ $175,000 to $199,999

☐ $200,000 and up

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Part 2: Health information

Instructions: The questionnaire contains checklist questions and short answer questions. Please answer the most correct answer.

Q6: Your weight_____lbs., height_____feet, inches

Q7: Do you have any of those chronic conditions?

☐ None ☐ Hypertension ☐ Diabetes ☐ Renal

☐ Heart disease ☐ Others, specify_____

Q8: Please chose the answer that applies to your physical activity intensity within the last three months.

☐ None

☐ Low-intensity [75 minutes (1 hour and 15 minutes) a week]

Please specify activity_____

☐ Moderate-intensity [150 minutes (2 hours and 30 minutes) a week]

Please specify activity_____

☐ High-intensity [300 minutes (5 hours) a week]

Please specify activity_____

Q9: Your typical salt content of food eaten

☐ Very salty ☐ A little salty ☐ Modestly salty ☐ Little salt ☐ Not salty

Q10: Do you add salt or soy sauce to cooked dishes

☐ Always ☐ Frequently ☐ Usually ☐ Seldom ☐ Never

Q11: Do you add salt to food before tasting

☐ Always ☐ Frequently ☐ Usually ☐ Seldom ☐ Never

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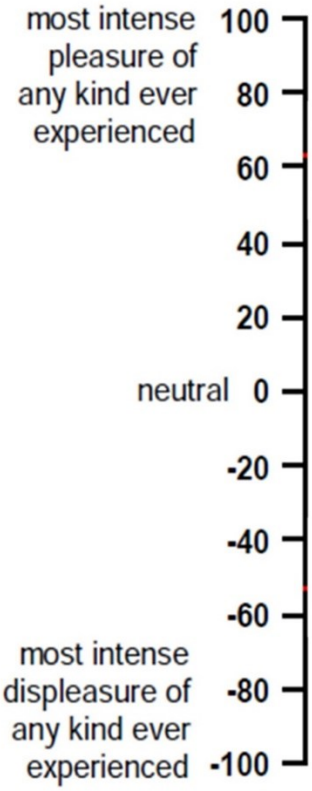
Part 3: Food acceptability information

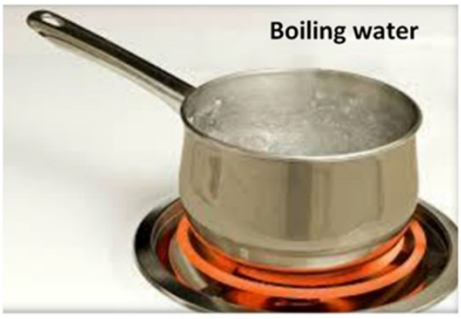
Instructions: The numbers on this scale range from -100 to 100 and refer to the intensity of all possible experiences you can like or dislike, not just foods.

- Below, draw a line to the location on the scale to represent the intensity of pain you would experience if you accidentally burned yourself with boiling water while cooking.

- Next, draw a line to the location on the scale that would represent the intensity of smell you would experience upon smelling rotting trash.



A. Generalized labeled magnitude scale (gLMS)



B. Boiling water

C. Smelling rotting trash

Figure B.1 A generalized labeled magnitude scale (gLMS)

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Instructions: Please rate each of following food and beverage items in terms of how intensely you like or dislike them then put your rating score in the table to the right of the item. If you have never tried a food or beverage listed here please write "N/A" for the score.

No.	Food item	Score
1.	Bread	
2.	Cookies	
3.	Crackers	
4.	Bacon	
5.	French fries	
6.	Ham	
7.	Hamburgers	
8.	Hot dogs	
9.	Pizza	
10.	Tuna	
11.	Olives	
12.	Soup	
13.	Apples	
14.	Bananas	
15.	Oranges	
16.	Carrots	
17.	Celery	
18.	Pickles	
19.	Cake	
20	Ice cream	
21.	Milk	
22.	Peanut butter	
23.	Soda	
24.	Potato chips	

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Salt taste perception test

Instructions:

1. The investigator will place 10 cups on the table. You will taste them in order. You will swish it around in your mouth (but do not swallow it) and spitting it out in the provided cup. You will then rinse once with water and spit it out. Please write down the number of the first cup that has a taste you can detect then write down the number of the first cup that has the salty taste.

Cup number _____ has a different taste than water.

Cup number _____ has the salty taste.

2. You will receive 3 samples. Please identify which cup is 'salty" or "water".

Cup number _____ is "salty".

Cup number _____ is "water".

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