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By

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The influence of single nucleotide polymorphisms in taste receptor gene TAS2R38 on eating behavior and body composition

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Taste impacts the palatability and intake of food, which is influenced by several factors such as cultural and genetic factors. Individual variations in taste perception may be important risk factors for poor eating habits and development of obesity. The differences in taste perception which impact dietary intake may lead to better understanding of obesity development and prevention of diet-related diseases. Obesity is one of the main causes for various health conditions in the United States as well as in the world. Genetic inheritance plays an important role in individual variations to taste and food choices. This study explored associations between two single nucleotide polymorphisms (SNPs, rs713598 and rs10246939) in the TAS2R38 bitter taste receptor gene, dietary intake, and body fat percentage. Five hundred presumably healthy students aged 18-25 years, including 86 (17%) males and 414 (83%) females from Mississippi State University participated in the study. Saliva was collected for genetic analysis, participants completed dietary history questionnaires and body composition was measured using bioelectrical impedance analysis. All statistical analysis of data was conducted using SPSS software to examine associations between SNPs, food intake, and

percentage of body fat. Our results did not show a significant association between the SNPs; rs713598 and rs10246939 in the TAS2R38 bitter taste receptor gene and dietary intake of vegetables and fruits as well as percentage of body fat in this group of participants. However, alcohol and caffeine intakes were significantly different between genotypes in rs713598; $p< 0.01$, $p< 0.05$, respectively.

Keywords: Taste; SNPs; genotypes; diet; body composition; obesity

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LIST OF ABBREVIATIONS

The following abbreviations are used in this dissertation:

AVI	Alanine. valine, and isoleucine
BF%	Body fat percentage
BIA	Bioelectrical impedance analysis
BMI	Body mass index
CI	Confidence interval
DXA	Dual energy X-ray absorptiometry
DHQ	Dietary history questionnaire
HWE	Hardy–Weinberg equilibrium
GPCR	G protein-coupled receptor
LBM	Lean body mass
MAF	Minor allele frequency
MF-BIA	Multiple-frequency bioelectrical impedance analysis
MPED	MyPyramid equivalents database
PA	Physical activity
PAV	Proline, alanine, and valine
PKD	Polycystic kidney disease
PROP	6-n-propylthiouracil
PTC	Phenylthiocarbamide

RT- PCR	Real time-polymerase chain reaction
SEM	Standard error of the mean
SPSS	Statistical Package for the Social Sciences
SF-BIA	Single-frequency bioelectrical impedance analysis
SNPs	Single nucleotide polymorphisms
TAS2R38	Taste receptor type 2 member 38
TBC	Taste bud cell
TRCs	Taste-receptor cells
USDA	United States Department of Agriculture
WHO	World Health Organization
χ^2	Chi-square

CHAPTER I

INTRODUCTION

Taste is one of the essential determinants of food consumption which can be influenced via a number of factors including nongenetic and genetic. Not much is known about the relationship between genetic variation of taste genes, body composition, and food intake. The genetic variation in taste receptors could influence food choices, and might impact nutritional and health status as well as the risk of chronic diseases. Human taste differences were first reported in the first half of the 20th century, but the main reasons for these variances have remained unclear. The term taste is used to mark the quality of food, which helps mammalians recognize if a food is beneficial or dangerous (toxic substances), and to accept or reject food. The taste system has five primary sensory qualities: bitter, salty, sour, sweet and umami (Tepper, Banni, Melis, Crnjar, & Barbarossa, 2014). The location of the sweet, umami, and bitter taste receptors is in a cluster on chromosome 1p36, whereas the bitter taste receptors are on chromosomes 12p13, 7q34, and 5p15 (Grimm & Steinle, 2011).

Perception of taste might differ between individuals depending on genetic variations in taste receptor genes and single nucleotie polymorphisms (SNPs) of the genes which code the various taste receptor cells (TRCs). Also, allelic variation can influence food perception and consumption (Bachmanov, 2005). Taste receptor cells are found in the mouth and organized into taste buds of 50-100 cells (Adler et al., 2000;

Kinnamon, Henzler, & Royer, 1993; Lindemann, 1996; Yarmolinsky, Zuker, & Ryba, 2009). There are three types of TRCs on the tongue: fungiform papillae, circumvallate papillae, and foliate papillae as well as four types of taste bud cells: type I, II, and III cells and basal cells. Taste receptor type 1 member 1 and taste receptor type 2 member 1 are proteins that in humans are encoded by the TAS1R1 and TAS2R1 genes, respectively. T1Rs and T2Rs are expressed in type II taste bud cells, but T2Rs do not overlap with T1Rs (Adler et al., 2000; B. Tepper et al., 2014; Yarmolinsky et al., 2009). Approximately 75% of taste buds are found on the tongue, and the others are distributed on the palate, pharynx, and larynx (Nosrat, Ebendal, & Olson, 1996).

According to *The State of Obesity 2017: Better Policies for a Healthier America,* released August 2017, Mississippi has the second highest adult obesity rate in the US with a rate of 37.3% (Molly, Stacy, & Alejandra, 2018). Obesity and overweight are defined as excessive fat accumulations in the body that might harm one's health which is measuerd by body fat percentage. Obesity is associated with increased risk of chronic diseases such as type 2 diabetes, hypertension, and cardiovascular diseases (Aljabri, Bokhari, & Akl, 2016; Dentali, Sharma, & Douketis, 2005).

Many factors influence body composition and food intake, but the strongest factors are gender as well as age (Yang, Smith, Keating, Allison, & Nagy, 2014). Females have a significantly higher amount of body fat and a significantly lower amount of lean body mass than males as determind by bioelectrical impedance analysis (BIA) (Yang et al., 2014). It is important that we understand how gene variation impacts taste perception and these changes in taste perception translate to differences in food consumption and possibly in body composition.

The purpose of this research was to assess SNPs of the TAS2R38 taste receptor gene and how this gene impacts food consumption and body composition. Food consumption patterns were determined by diet history questionnaires, body fat percentage (BF%) was measured by BIA, and Real-Time PCR System was used to detect variants of SNPs in the samples. Participants in the study included college students aged 18-25 years who resided in Mississippi.

CHAPTER II

LITERATURE REVIEW

Methods to Determine Body Composition

Body Mass Index

Body mass index (BMI) was one of the first ways that body weight was classified. It was first described in the 19th century by Adolphe Quetelet as "an index of height to weight" (Hall & Cole, 2006). In the 1950s, the weight to height index was reinvented by Ancel Keys, who called the measurement "the body mass index." BMI-adjusted weight for height could be used for different age groups. The National Institutes of Health (NIH, 1998) defines BMI as weight (kg)/height squared (m^2) and classifies individuals' BMI values according to Table 2.1.

Table 2.1 Classification of weight status by BMI

Classification	BMI (kg/m^2)
Underweight	< 18.5
Normal	18.5 – 24.9
Overweight	25.0 – 29.9
Obesity class I	30.0 – 34.9
Obesity class II	35.0 – 39.9
Obesity class III	≥ 40

In the past few decades, BMI has been used as a measure of a population's fitness and a signal of overall health. The government has collected data and has tracked trends showing increasing BMI throughout the population of the US. These data have alerted the US to an increased rate of obesity within the country. Studies like the one discussed in the article, "Prevalence of Obesity and Trends in the Distribution of Body Mass Index Among US Adults, 1999 – 2010," use BMI to demonstrate a change in health in the US population by showing changes in BMI trends (Flegal, Carroll, Kit, & Ogden, 2012). This study, NHANES (2009-2010 National Health and Nutrition Examination Survey) examined the anthropometric data for 5926 adult males and females (from a nationally representative sample of the US population in 2009-2010) and the anthropometric data for 22847 males and females (representative of the population of the US between 1999-2008). The results of the study showed that for the 2009-2010 years, the mean age-adjusted BMI for both males and females was 28.7 kg/m^2. The median BMI for men was 27.8 kg/m^2 and 27.3 kg/m^2 for women, and the prevalence of obesity was 35.5% in men, and 35.8% in women. In the 12-year period (1999-2010) there were significant increases in obesity rates within the non-Hispanic black female population (p=0.04) as well as the female Mexican-American population (p=0.046). In the male population that was tracked, there was a statistically significant result (p<0.001) in increased obesity for the entire time period (1999-2010).

Throughout the years, researchers have questioned whether BMI is the most appropriate way to look at weight and body composition in relation to health. Several interesting studies have become available within the past several decades that bring discrepancies with the use of BMI to light. One such study revealed that all Asian

populations showed a higher body fat percentage than Caucasian people at the same BMI (Deurenberg, Deurenberg-Yap, & Guricci, 2002). The study utilized dual energy X-ray absorptiometry (DXA) and anthropometric data, and found that for the same BMI, Asian populations had a BF% that was on average 3-5% higher than Caucasian BF%. This reduces the credibility of BMI as a method to review health status, as body composition clearly differs among ethnicities. For BMI to remain an indicator of health, separate BMI ranges might need to be established for different ethnic populations.

Obesity has been associated as a risk factor for many diseases such as cardiovascular disease, diabetes, and osteoarthritis as well as mortality. Studies have used BMI, calculated as weight in kilograms divided by height in meters squared, for a diagnosis of obesity. Studies have described that a U- shaped association between BMI (kg/m^2) and mortality which is correlated with relatively low and high BMI values, such as a BMI greater than 30 is associated with increased mortality from cardiovascular diseases, while a BMI less than 18.5 is associated with increased mortality from chronic wasting diseases. However, the relationship between mortality and BMI are still dependent on age and gender.

Bioelectrical Impedance Analysis

BIA is a method of determining the body composition of a person using electrical current, so that a researcher may be able to view a person's fat-free mass, BF% and total body water (Kyle et al., 2004). However, when BIA was first used in studies, the focus of BIA was mainly on water and electrolyte concentrations within the body, and how these variables interacted with bodily functions such as basal metabolic rate and blood flow within the body (Costello, 1997). It was not until later that BIA began to be used to determine body composition. The researcher Thomasset was one of the first scientists to utilize different frequencies to assist in determining the physiological breakdown of people (Bolot et al., 1977). There are some limitations with BIA, for example, physical activity, food consumption, hydration status, and metabolic disturbances can cause inaccuracies in BIA results. However, many researchers throughout the past decade have utilized BIA as a means to determine individuals' body composition. BIA is a recommended method for cross-sectional studies due to evaluation of many participants in a short time and excellent correlation (Langer et al., 2016). Also, the BIA method provides acceptable estimates for fat-free mass and BF% in participants with different characteristics such as age, gender, and ethnicity. Ramírez-Vélez et al. (2017) studied population of 1687 Colombian collegiate students and confirmed the validity of BIA for measuring BF%.

Waist Circumference

Waist circumference is an anthropometric measurement that may be used to assist in determining the health of a person. Even when utilizing data such as BMI, waist circumference is still useful because it can show fat distribution patterns (android vs gynoid obesity) which can have a large impact on a person's health (Sharma, 2002). Waist circumference data may actually be more useful in assessing health risk than BMI due to cardiovascular and metabolic risk factors associated with increased fat distribution around the abdominal area (Foucan, Hanley, Deloumeaux, & Suissa, 2002). The measurement can provide information on a person's level of health risk in addition to the information provided by BMI (Janssen, Katzmarzyk, & Ross, 2002). Waist circumference is also an easy and cost-effective way to assess a person (Levine et al., 2011). In 1998, NIH recommended that patients with a BMI higher than 25, waist circumference should also be measured (NIH, 1998). The NHANES III was conducted between the years of 1988 and 1994, and 1999 to 2000, and waist circumference was one of the measurements utilized in this study. The study was able to show that waist circumference within the male and female population increased significantly, which indicated an increase in abdominal obesity within the US population between the two time periods (Ford, Mokdad, & Giles, 2003).

BOD POD

The BOD POD became the first commercially air-displacement plethysmograph in the 1990s, and there is only one commercially available system, which is known as the trade name of BOD POD (Dempster & Aitkens, 1995). This type of system includes the BOD POD plethysmograph, computer, weighing scale, calibration weights, and cylinder with two chambers: a test chamber and a reference chamber. It is designed to measure body volume by air displacement to calculate body density (Fields, Goran, & McCrory, 2002). The BOD POD is a reliable and valid method for lung volume measurement. Also, it is accurate, quick, automated, noninvasive, and safe method evaluating body composition such as BF%, fat mass, and fat-free mass within a wide range of body types (eg, obese, children, elderly, and disabled) (Bentzur, Kravitz, & Lockner, 2008).

Skinfold Thickness

Skinfold thickness is measured by a caliper at several precise points on the body, which determines subcutaneous fat layer and BF%, and it is also called pinch test (Ojo & Adetola, 2017). Skinfold measurement is simple and an inexpensive technique available in many countries. Skinfolds (subcutaneous adipose tissue) and circumference measurements are used to determine the relationship between obesity and chronic diseases such as diabetes, cardiovascular disease, hypertension, and arthritis. Additionally, dietary history questionnaires with anthropometric measurements can provide useful information about the health of individuals, and skinfold thickness and BIA measurements can be used to predict body composition (Ramirez-Zea, Torun, Martorell, & Stein, 2006).

Dual Energy X-ray Absorptiometry

DXA is a gold standard measurement of bone mineral density for diagnosis of osteoporosis; moreover, DXA is able to provide information such as lean body mass (LBM) as well as BF%. DXA has been commercially available since 1987 (Carlson, Dugan, Buchbinder, Allegretto, & Schnakenberg, 1987). There are three major manufacturers of DXA instruments in the US; Hologic (Waltham, MA), Norland (Fort Atkinson, WI), and Lunar (Madison, WI). The first generation of DXA was limited to the measurement of bone mineral content and bone mineral density, but now DXA can measure both bone minerals and body fat. DXA has shown to be a valid and reliable instrument for measuring body composition (Blake & Fogelman, 2009). Obesity and osteoporosis are two challenges in clinical practices. Obesity is a condition of excessive body fat, and BMI is usually used as an indicator of obesity. Body composition such as excessive fat mass may affect the bones and add more stress on bone tissue (Agarwal & Uppin, 2016).

Summary of Determining Body Composition

Pasco et al. (2014) found that 17.3% of women and 31.6% of men were obese according to BMI but were misclassified according to BF% criteria. Also, the study suggested that BMI underestimates adiposity in young and elderly men (Pasco et al., 2014). Another study of 637 healthy women 18–40 years old observed that BIA and BMI (kg/m^2) methods similarly detected normal and obese women (27.67± 7.3) and (25.97± 4.7), respectively (Amani, 2007). Eisenkölbl, Kartasurya, and Widhalm (2001) observed that BIA measurements of BF% of obese participants were 10.6% lower than the DXA results, and BIA had a standard error of 10%. Chahar (2014) found in 30 men aged 26-49

years old that the mean and standard deviation values for BF% measurements by skinfold thickness, body mass index, and BIA were 19.95± 5.9, 19.67± 4.3, and 9.40± 4.1, respectively, which indicated that BIA tended to underestimate BF% comparison to other methods (Chahar, 2014). Furthermore, Aandstad, Holtberget, Hageberg, Holme, and Anderssen (2014) used several validated methods such as DXA, skinfold thickness, and BIA to predict BF% in 65 females and males. The results showed that BIA was the most reliable method in both genders, especially in females with 95% limits of agreement less than ±1% point (Aandstad et al., 2014)

Physical Activity

Physical activity (PA) is defined as any movement produced by skeletal muscles that requires energy expenditure while physical inactivity is lack of physical activity (Caspersen, Powell, & Christenson, 1985). PA plays an important role to gain muscle mass and increase muscle strength at any age and gender. Moreover, PA has a primary preventive impact on several chronic non-communicable diseases, such as heart diseases, hypertension, osteoporosis, and diabetes mellitus. Hu et al. (1999) reported that PA level was associated with a substantial reduction in risk of type 2 diabetes. The lack of PA is one of the major risk factors which can lead to overweight and obesity. High BMI or BF% may indicate poor physical fitness. Several studies observed an inverse relationship between PA and BMI; individuals who did more physical activity had lower BMI and BF% than less active people (Tiruneh, 2009). Also, it was reported that athletes had a lower BF% than non-athletes (Bernstein, Costanza, & Morabia, 2004).

The correlation between PA and BMI was weak in participants with normal BMI values. However, BMI was significantly associated with PA in all category groups of

obese individuals; sedentary (r=0.26, p=0.05), light PA (r=0.30, p=0.01), moderate PA (r=-0.35, p<0.01), vigorous PA (r=-0.39, p <0.001), activity counts/day (r=-0.50, p <0.001) and steps/day (r=-0.54, p<0.001) (Hemmingsson & Ekelund, 2007).

Paulo et al. (2015) found that higher education students (85 Italians, 94 Portuguese) who did supervised exercise had lower BMI, waist circumference, and higher respiratory function. São et al. (2016) observed that 58% of the students had low PA, 29% had moderate PA, and 13% had high PA. Eighty-six students participated in this study. Seventy-seven were women with average age of 21 years. All participants were students admitted to Nursing in the School of Health. University students are a unique group because most of them are young adults at a transitional time and learning to live independently. University students should adopt a healthy lifestyle that includes PA and a healthy diet.

Food Intake and Eating Behavior

Taste perception is one of the most critical determining factors of food preferences, dietary habits, and dietary consumption. Many factors can impact food consumption among individuals; genetic and non-genetic (diet, eating behavior, and PA) factors are engaged with the development of obesity (Fay, German, & Bruce German, 2008; French, Story, & Jeffery, 2001). Also, polymorphisms and genetic variation of the taste receptor genes have been associated with taste perception and food intake (Garcia-Bailo, Toguri, Eny, & El-Sohemy, 2009; Leterme, Brun, Dittmar, & Robin, 2008). Perception of each taste (bitter, sweet, salty, sour, and umami) is mediated by a different mechanism; G-protein coupled receptors bind bitter, sweet, and umami, ion channels bind salty taste, while sour is detected by a transient receptor-ion channel (Huang et al., 2006). The five types of taste receptors contain SNPs, which may affect taste perception, food intake, and consequently metabolic and health outcomes (Chamoun et al., 2018).

Eating behavior is a critical and complex process for the acquisition of energy substrates, which can be affected by biological and environmental factors. Taste refers to four oral perceptions which are sweet, bitter, sour, and salty in addition to umami (taste of monosodium glutamate). It is essential to determine and understand how taste and food intake can influence the risk of chronic disease. Few studies have shown how genetic variation modifies sweet and salt taste perception in humans, and its potential effect on food intake (Bachmanov & Beauchamp, 2007; Nasser, 2001). Sweet taste is linked with carbohydrate consumption and predicts the caloric content of food, which can be stimulated by several compounds such as artificial sweeteners, natural sugars, sweet proteins, and d-amino acids (Chandrashekar, Hoon, Ryba, & Zuker, 2006). The sweet

taste receptor (function expression) is a heteromeric protein structure comprised of T1R3 combined with T1R2 that responds to all classes of sweet tastants. Sweet taste is mediated by a small family of three G-protein-coupled receptors (GPCRs). T1R2 is unique to sweet taste sensation while the T1R3 protein is involved in umami taste sensation combined with T1R1 (Nelson et al., 2001). Dietary intakes of carbohydrates (eg. glucose, sucrose, and fructose) depend on the TAS1R2 gene (Habberstad, Drake, & Sonestedt, 2017). Therefore, the effect of genetic variation on sweet taste should focus on the TAS1R2 gene. Bitter taste is stimulated by several compounds. The influence of variation in TAS2R genes on perception of bitter taste has interested investigators but most research is focused on the TAS2R38 gene (Wooding et al., 2004).

TAS2R38 gene is a locus for bitter taste perception and bitter foods such as cruciferous vegetables (thiourea-containing compounds) and alcohol consumption. There are three variants, A49P (145G > C, rs713598), V262A (785T > C, rs1726866) and I296V (886A > G, rs10246939). This type of gene is associated with bitter taste sensitivity as tested with 6-n-propylthiouracil (PROP) and phenylthiocarbamide (PTC); PAV/PAV diplotype is sensitive to PTC/PROP taster while the AVI/AVI diplotype is sensitive to thiourea (N-C=S) moiety-containing chemicals non-taster (Choi et al., 2016).

Taste System and Anatomy

Taste is one of the essential determinants of food consumption which can be influenced via a number of factors including nongenetic and genetic (Figure 2.1). Humans consume food to survive, but some foods may contain toxic compounds. Therefore, taste helps mammalians determine if the food is beneficial or dangerous when consumed. This ability is available to mammals to choose safe foods. Umami and sweet are tastes that encourage consumption of food. However, bitter and sour are tastes that alert mammalians to possible toxins and help reject foods which have harmful substances. Finally, salt can be a taste which may be good or harmful depending on the type and concentration of salt (Yarmolinsky et al., 2009).

Taste receptor genes and proteins have different standard names. A corresponding gene symbol for mice and rats have Tas1r1, but humans have TAS1R1 name with uppercase letters and no italics for the corresponding proteins (Bachmanov & Beauchamp, 2007). The taste system has five primary sensory qualities: sweet, umami, bitter, sour, and salty (Tepper et al., 2014). Sweet taste allows people to know the food is energy-rich, salty taste selects the appropriate nutrient electrolyte balance, sour and bitter help prevent consumption of food toxins (Chandrashekar et al., 2006; Yarmolinsky et al., 2009). Umami is a savory taste induced by certain L-amino acids (Ikeda, 1909). Sweet, bitter, and umami tastes are mediated via GPCRs, but sour and salty tastes have specialized membrane channels for selective ion transport. The GPCR is the largest family of proteins in the mammalian genomes (Chaudhari, Landin, & Roper, 2000; Lindemann, 1996; Nelson et al., 2001). Taste cells occur singly or as clusters in taste buds. Taste receptor cells (TRCs) are found in the mouth and organized into taste buds.

Each taste bud has 50-100 cells (Adler et al., 2000; Yarmolinsky et al., 2009). Three types of taste buds are on the tongue: fungiform, circumvallate, and foliate papillae. Dozens of taste buds are found in the anterior two-thirds of the tongue and are the fungiform papillae, hundreds are located on the posterior one-thirds which are the circumvallate papillae, and hundreds of buds which are distributed on the lateral sides which are the foliate papillae as well as several taste buds are isolated on the soft palate (Tepper et al., 2014; Yarmolinsky et al., 2009). Taste buds have perigemmal fibers while taste cells have synaptic contact. Brain-derived neurotrophic factor is needed to maintain gustation papilla and taste buds during gustatory innervation whereas neurotrophin-3 is necessary for the tongue during somatosensory innervation.

Taste buds are recognized as three types of cells with each type having different morphological features and functions: type I, II, and III taste cells. Type I is termed "glial-like" because their primary purpose is to support other taste cells and transduction for salty taste. Type II is a type of cell thought to be the actual TRC for transduction of sweet, umami, and bitter tastes which express as GPCR. Type III taste cells respond directly to sour taste and carbonated solutions which are specialized chemical synapses (Perea-Martinez, Nagai, & Chaudhari, 2013).

Each year more than 200,000 people in the US are estimated to have taste disorders. However, they have not visited a physician for chemosensory assessment (Nosrat et al., 1996). People suffering from anosmia have a complete or partial loss of smell. Also, in older people, the sense of smell usually declines.

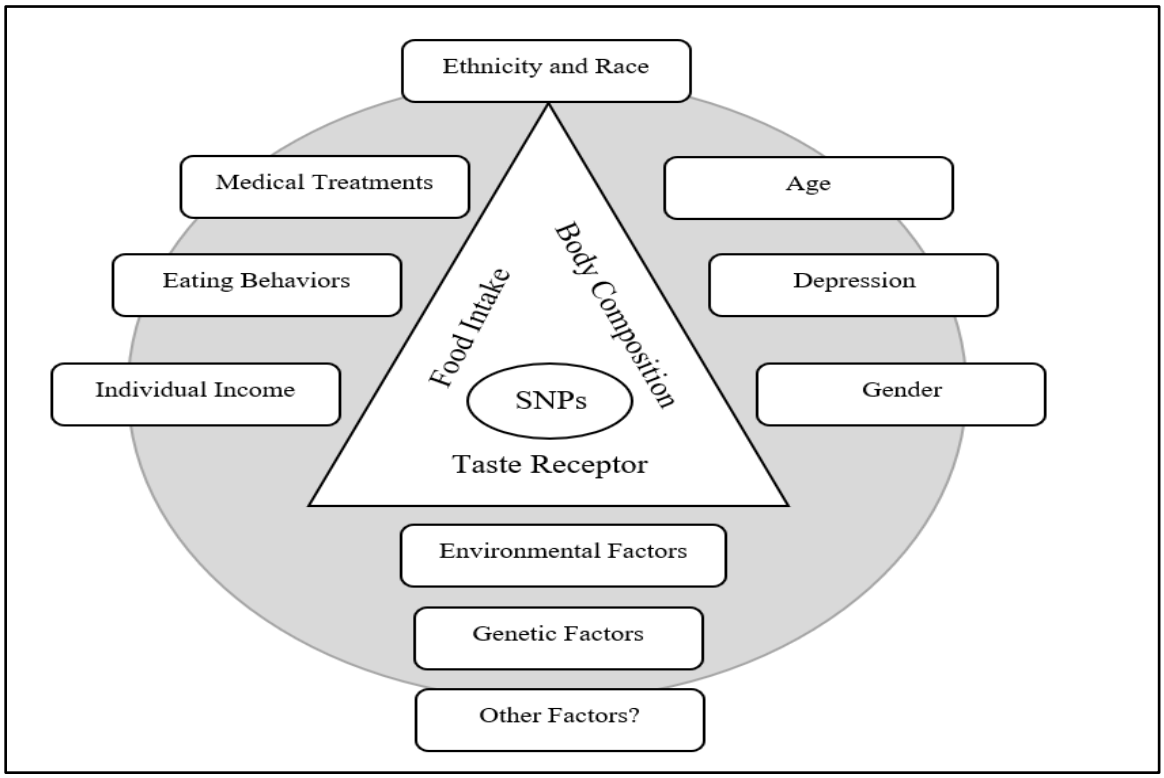


Figure 2.1 Graphic diagram showing factors which can influence the interactions between taste receptor genes, food intake, as well as body composition.

The Basic Tastes

Sweet Taste

Humans and mice have some conspicuous differences in their ability for tasting sweet substances (glucose, sucrose, fructose, and sugar alcohols) and artificial sweeteners (sucralose and aspartame) as well as some amino acids which have a sweet taste such as D-tryptophan, L-proline, L-glutamine, glycine, and D-phenylalanine. There are several contributing factors that influence total consumption of sugars such as race, age, gender, and genetics (Drewnowski, Mennella, Johnson, & Bellisle, 2012). This type of taste is mediated by a heterodimer of the taste receptors type 1 member 2 (TAS1R2) and member 3 (TAS1R3). The TAS1R2 is most relevant to transmit the sweet taste (Boughter & Bachmanov, 2007; Chandrashekar et al., 2006). Sweet and umami have a small family of three GPCRs — T1R1, T1R2, and T1R3 expressed in taste cells of the tongue and palate epithelium (Sainz, Korley, Battey, & Sullivan, 2001). T1Rs combine to produce at least two heteromeric receptors such as a T1R1 and T1R3 form for umami and T1R2 and T1R3 for the sweet taste. T1R2 is the subunit specific to sweet taste and T1R1 for umami taste perception (Damak et al., 2003; Li et al., 2002). The correlation between genetic variations in sweet taste receptor genes, SNPs, and consumption of sweet foods are important to ensure acquisition of macronutrients and micronutrients as well as to avoid toxic substances (Nelson et al., 2002; Zhao et al., 2003). A few studies have observed the relationship between variations in the TAS1R2 gene, BF%, and sugar consumption. Dias et al. (2015) observed that individuals with G allele (rs12033832) had a higher intake of carbohydrates (g/day) (GG/GA 277± 8 vs. AA 214± 23, p=0.03), total sugars (g/day) (GG/GA 130± 4 vs. AA 94± 13, p=0.009), and sucrose (g/day) (GG/GA 50± 2 vs. AA 36

± 6, p=0.008) compared with individuals who were AA homozygous, and there was a significant (p=0.003) interaction between TAS1R2 gene, SNPs, and BMI (Dias et al., 2015).

Bitter Taste

Bitter and sweet taste interact together to influence food intake and eating behavior. Many people avoid consuming vegetables and some fruits due to bitter taste, and consequently this may lead to consuming more fatty and sweet foods. This type of behavior has the potential to increase development of obesity (Goldstein, Daun, & Tepper, 2005). Bitter taste sensitivity is strongly related to food intake, eating behavior, health, and disease risk. Several studies have shown that bitter transduction in mammalian is mediated via GPCRs in taste receptor cell membranes involving natural G proteins (Wong, Gannon, & Margolskee, 1996). Humans and rodents have T2Rs which are genetically associated with taste perception. According to Fox (1932), the bitter compound, phenylthiocarbamide (PTC) is used to measure human taste sensitivity and categorize which individuals are tasters or non-tasters. Plants can use bitter compounds as protective agents from insects, infections, and oxidative stress. In vertebrates, the number of genes in different species varies extremely. For example, there are three TAS2R genes in chicken, forty-nine in frog, and 25 genes with 11 pseudogenes in human (Meyerhof, 2005).

TAS2R38 gene has been associated with bitter taste status, which has three common polymorphisms in three different positions in amino acid 49, 262, and 296 which are referred to as PAV (proline, alanine, and valine) or AVI (proline, alanine, and

valine) depending on amino acid position. Individuals exhibiting PAV are more sensitive to low concentrations of compounds than AVI individuals (Bufe et al., 2005). Also, a supertaster is a person who has a high number of fungiform papillae (Duffy, Davidson, et al., 2004). Homozygosity for the PAV amino acid haplotype is considered a marker for supertasters while individuals who are homozygous for the AVI amino acid haplotype are considered non-tasters (Chamoun et al., 2018).

Umami Taste

In humans, there are two amino compounds, monosodium glutamate (MSG), the sodium salt of glutamine, and aspartate that have unique savory sensations and are called the umami taste, which can be translated from the Japanese language as "delicious savory taste" (Roper, 2007). The word umami is used to describe the meaty and savory flavor in food. In the past, it was the five taste qualities of salty, sweet, bitter, sour, and hot. A hot sensation is dependent on the skin mechanical sensation. The first description of umami was published by Ikeda in 1909 and then translated into the English language in 2002 by Ikeda (Ikeda, 2002). Naturally, umami has three substances: MSG, disodium guanylate and disodium inosinate. The first description for glutamate receptor was metabotropic G-protein receptor taste-mGluR4, which has a truncated N-terminus and is expressed in rat taste buds that also elicits umami taste responses in humans and in rats stimulated by either MSG or L-2-amino- 4-phosphonobutyrate. Also, some suggest that mGluR4 could play an important role in umami taste receptor (Garcia-Bailo et al., 2009). T1R1 and T1R3 elicit the umami taste (heterodimer), which are highly selective for L-amino acids and not active for D-amino acids and others (Nelson et al., 2002).

Garcia-Bailo et al. (2009) reported that some individuals were unable to distinguish between MSG and sodium chloride (NaCl), which indicated that it was not easy to separate the umami taste from the salty taste component of MSG for some people. Also, the umami taste may be used as an indicator of purine-rich foods. High levels of purine, which breaks down into uric acid, is associated with kidney stones and gout. Similar to sweet, bitter, and fatty foods, over consumption of umami tasting foods, may increase the risk of diseases such as obesity (Chamoun et al., 2018). Further research is required to better understand the impact of genetic variation on the preference and intake of umami foods that may reduce the risk for metabolic complications.

Salt and Sour Tastes

Several studies have shown that sour and salty tastants are modulated by Na+ and H+ which are ion channels through specialized membrane on the surface of the cell. Salty taste is elicited by NaCl which is also known as salt and is an ionic compound. Type I cells play a role in the salty taste sensation (Chandrashekar et al., 2006). Usually, salt is used to improve the palatability of foods. There are channels associated with salty taste: the specific channels (ENaC) and the non-specific channel (TRPV1). In rodents, ENaCs located on TRC membranes in fungiform papillae are made up of two alpha, one beta, one delta, and one gamma subunit, and play an essential role in the perception of Na+. Salty taste responses to NaCl are significantly inhibited by amiloride (ENaC blocker). However, this blocker does not impact other taste modalities. Amiloride represses salt taste intensity in the mouth (Feldman et al., 2003).

Consumption of dietary sodium (salt) may cause increases in hypertension and developing cardiovascular diseases; however, it is an essential micronutrient and is required for electrolyte balance (Havas, Dickinson, & Wilson, 2007; Johnson, Johnson, Peyton, & Durante, 2005). Salty taste and sodium intake might be influenced by genetic variations and eating behaviors (Chandrashekar et al., 2006). The sour taste comes from the acids in some foods such as fruits and foods containing vinegar. Polycystic kidney disease (PKD) 2L1 protein is a member of the transient receptor potential ion channel family, and PKD2L1 and PKD1L3 interact through their transmembrane domain (Ishii et al., 2012). The PKD2L1 and PKD1L3 genes have SNPs coding that may impact sour taste perception and food intake, and is an essential area for future research (Chamoun et al., 2018).

The Relationship between Taste Receptors and Body Composition

There are several factors that can influence appetite and food intake independent of genetics, such as socioeconomic factors, environment, eating behaviors, depression, and medical treatments. Humans are born with an inherent revulsion for bitter compounds and a liking for sweet foods, and the ability to taste bitterness and sweetness are varied between humans. Genetic variation can play an essential role in tasting bitter and sweet compounds (Dinehart, Hayes, Bartoshuk, Lanier, & Duffy, 2006). Some studies showed that children who have a TAS2R83 haplotype should be non-tasters, and BMI was not different between tasters and non-tasters; however, there were differences in food selection of young preschool children (Golding et al., 2009; Keller et al., 2014).

There is no direct relationship between TAS2R38, and body composition such as BF% and BMI. However, there is a direct pathway between TAS2R38 and PROP taster

status (Bouthoorn et al., 2014). Individuals known to be supertasters are less likely to consume coffee and green leafy vegetables. Most studies used PTC and PROP to determine who is a supertaster, taster, or non-taster. Many factors such as gender, age, and oral diseases may influence the sense of taste; more women are supertasters than men and women are more responsive to PROP due to having more fungiform, papillae, and taste buds. However, other studies indicated that supertasters have more papillae as well as anatomical differences (Bartoshuk, Duffy, & Miller, 1994; Grimm & Steinle, 2011). DXA was used the first time by Bouthoorn et al. (2014) to find a relation between PROP status and fat mass in girls. They found that non-taster females had higher body weights and fat mass than tasters (Bouthoorn et al., 2014). Another study observed that adiposity among supertasters (37.2%) and medium (44.3%) tasters were significantly higher than in non-tasters (18.3%) with multi-ethnic participants aged 9-10 years and 17-18 years (Baranowski et al., 2010).

Two studies did not observe a relationship between TAS2R38 haplotype, BMI, and waist-to-hip ratio in females, but there was a weak association between PROP phenotype and BMI. The PROP phenotype was strongly correlated to adiposity in males more than TAS2R38 (Tepper et al., 2008; Timpson et al., 2005). According to Sharma, Kansal, and Chopra (2013), the TAS2R38 gene is significantly associated with premenstrual syndrome, and the prevalence of premenstrual syndrome was higher in PTC non-tasters. PTC tasters had a BMI slightly higher than non-tasters, but it was not statistically significant. The relationship of TAS2R38 gene with obesity-associated traits in some people may be due to some habits and behavioral traits concerning food intake (Sharma et al., 2013). Saraswathi, Najafi, Vineeth, Kavitha, and Malini (2011) reported

that a higher percentage of non-tasters were observed in overweight/obese children (13-17 years old) which were likely to consume more junk food, fatty food, and creamy dairy.

Bitter taste is mediated by the TAS2R family of receptors. Genetic factors influence food consumption and body composition and account for differences in food intake among individuals. Gene variation has been shown to be associated with differences in taste function and potentially dietary consumption and preference. SNPs in taste receptor genes such as TAS2R38 has been linked to variability in taste perception, food intake, nutritional habits, and health status.

Overall, there are many factors that influence food intake and body composition. Investigating the relationship of genetic factors related to food intake (genes in taste buds) with an impact on selection or avoidance of specific foods, may help to understand individuals' health status. Identifying super tasters, tasters, and non-tasters may help understand peoples' eating behaviors.

CHAPTER III

MATERIALS AND METHODS

Research Objectives

The present study assessed how SNP's of TAS2R38 gene impact food intake and body composition. The study objectives were three-fold (Figure 3.1).

1. Determine the prevalence of genotypes of taste receptor gene for bitter taste TAS2R38 (rs713598 and rs10246939) in a group of young adult participants.
2. Examine how genetic variation in bitter taste receptor TAS2R38 (rs713598 and rs10246939) influence food preference and consumption regarding carbohydrates (fruits and vegetables), proteins, and fats among a group of young adult participants (Figure 3.2).
3. Examine how genetic variation in bitter taste receptors TAS2R38 (rs713598 and rs10246939) influence BF% in a group of young adult participants (Figure 3.2).

The flow chart for this research is presented in Figure 3.3. The study was designed as a cross-sectional study consisting of 500 participants, 18-25 years old, males and females. Presumably healthy participants were recruited from the University's campus, via classroom announcements and emails. Students were invited for one visit to the Nutritional Performance Assessment Composition Testing lab which is located in

Ballew Hall at Mississippi State University. Participants were asked to avoid eating or drinking four hours prior to the visit, as well as not smoking for 12 hours prior to coming to the lab. In the lab, participants were given a consent form and if they agreed to participate, they were enrolled in the study and completed a diet history questionnaire (Web-DHQ II) and followed protocol for saliva collection. Confidentiality was maintained during the archive and analysis of data.

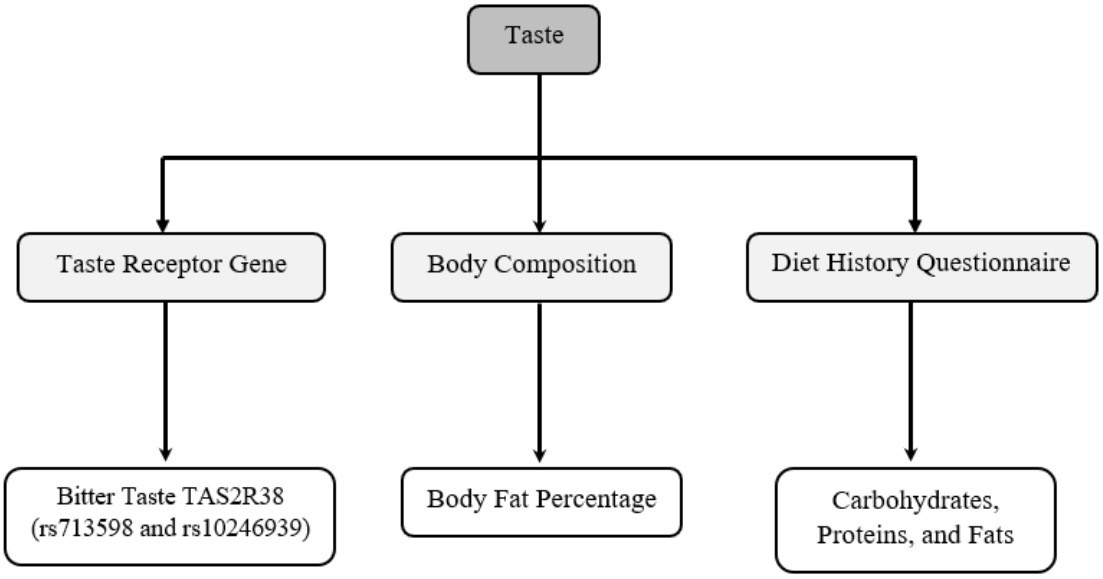


Figure 3.1 Outline of the objectives of the dissertation research

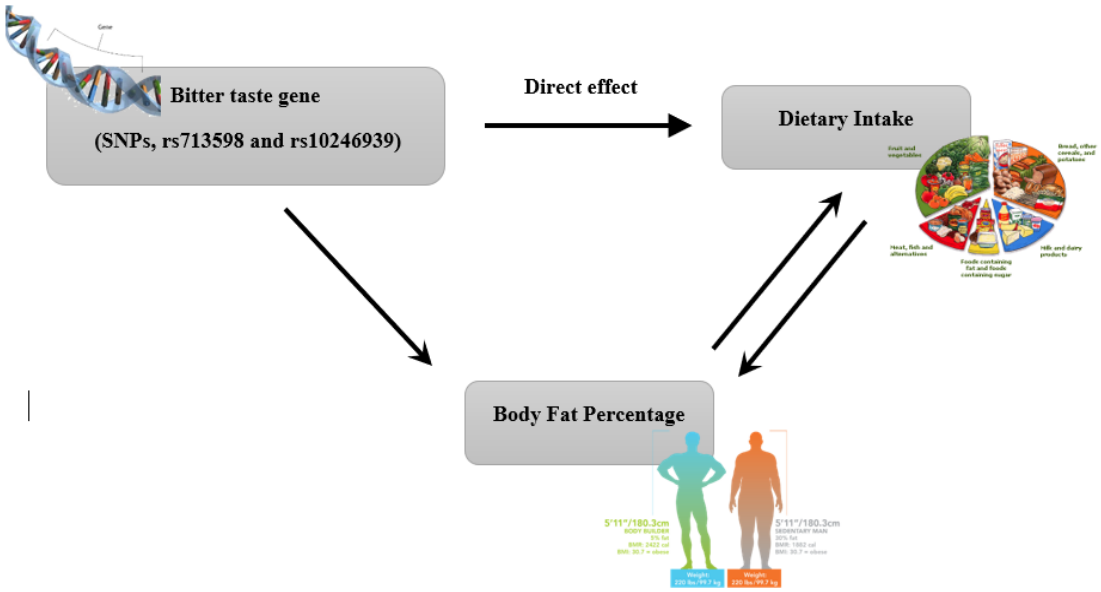


Figure 3.2 Effect of taste gene on food intake and body composition

Institutional Review Board Approval

Participants were males and females age 18-25 years old and presumably healthy. Approval for the study was obtained from the Mississippi State University Institutional Review Board (IRB# 17-664).

Inclusion Criteria:

- Young adults age 18-25 years
- Willingness to complete all parts of the study
- Ability to read and write English and understand the Informed Consent
- Ability to provide a saliva sample
- Presumably healthy

Exclusion Criteria:

- Younger than 18 years or older than 25 years
- Inability to read and write English or understand the Informed Consent
- Inability to provide a saliva sample
- Self-reported pregnancy for females

Diet History Questionnaire (Web-DHQ II)

All participants were asked to complete a 153-item electronic NIH DHQ II. The National Institutes of Health validated the DHQ which asks questions regarding types and portion sizes of foods and beverages over the course of the past 12 months. The participants estimated the average frequency and portion sizes of the foods consumed during the previous year. The information in the Web-DHQ II database was used to transform into daily food intakes. All items were analyzed to compute a total daily intake of macro- and micro-nutrients for each participant (Subar et al., 2001). Total fats, proteins, and carbohydrates were calculated by summing the mean grams and percentage of each nutrient. Carbohydrate included dietary fiber, alcohol, and sugar, in addition to vegetables and fruits. Participants had the option to complete the DHQ online before coming to the laboratory for body composition determination and saliva collection.

Body Composition

Body composition and weight were measured using a BIA system (TBF-300A, Tanita Corp, Tokyo, Japan). The Tanita® analyzer measures the lower-body resistance between the right and left legs as the individual stands on the electrode plates. It uses single-frequency (50-kHz) and provides a printout of measured fat mass, fat-free mass, BMI, impedance, and total body water. The procedure was conducted according to the manufacturer's instructions (Sociedad Española de Nutrición Parenteral y Enteral, 2012). Body fat percentage was calculated from body impedance values and the pre-entered personal data which included age, gender, and height. Body composition was estimated using the standard equation provided by the BIA device and added 2-3 pounds for clothing depending if the participant was clothed in heavy winter clothing or light summer clothing without shoes. A stadiometer was used for measuring each participant's height (QuickMedical Heightronic® Model 235, USA).

Physical Activity Assessment

Physical activity was assessed in a random sample of 100 of the students (20%). Participants were asked about their physical activity levels during a normal week as well as what kind of activity they performed. Physical activity level was classified into four groups according to World Health Organization (WHO) physical activity guidelines; group one, participants who engaged in ≤ 150 min/week of moderate or ≤ 75 min/week of vigorous activity which were classified as 'inactive' group or 'none'. Group two, participants who met the guidelines, which is at least 150 minutes of moderate or 75 minutes of vigorous intensity aerobic activity/week, were classified as low-intensity. Group three, participants who engaged in 150–299 min/week of moderate or 75–149 min/week of vigorous activity, which were classified as moderate-intensity. The last group, participants who engaged in ≥ 300 min/week of moderate or ≥ 150 min/week of vigorous activity were classified as high-intensity. A minute of vigorous activity is equal to two minutes of moderate activity (World Health Organization, 2010).

Saliva Collection and DNA Sampling

Saliva is an elaborate biospecimen and the gold standard for biological testing. Saliva samples can be collected in a convenient, minimally-invasive, and repeated manner. Participants were asked to rinse their mouths thoroughly using water before saliva collection. Two saliva samples were provided by each participant. The passive drool method was used for collecting salvia, and collection tools were acquired from the Salimetrics Company (SalivaBio, CA, US). Each participant used two 2ml cryovials and one collection aid adapter. The manufacturer's instructions were followed for collection with some modifications. Collected saliva was blotted on Fisherbrand^{TM}grade P5 filter paper using a method developed at the Craniofacial Genetics Laboratory (CFGL) at the University of the Pacific Dugoni School of Dentistry in San Francisco, CA. On each paper, six circles were drawn to show the location of all saliva samples. Filter papers were air-dried and then packaged in coded envelops to prevent cross-contamination of specimens. Saliva was analyzed for genotyping by using q-PCR for SNPs.

Genotyping Technique

SNP genotyping can be accomplished through many techniques with different principles, abilities, and cost. This study was carried out using the allelic discrimination assay using the Applied Biosystems (7300/7500/7500 Fast) Real-Time PCR System. This system uses fluorescent-based PCR chemistry and detects variants of a single nucleic acid sequence in the samples (Livak, Marmaro, & Todd, 1995). DNA extraction from saliva samples was performed using the TaqMan® Genotyping Master Mix kit (Applied Biosystems, Foster City, CA, USA) following the manufacturer's instructions with some

modifications. Sample-to-SNP™ Kit and GTXpress™ Master Mix were used to prepare, extract, and analyze DNA samples (genotyping of SNPs) from the saliva. The first step was to cut out the 5-mm punch from the filter paper containing the DNA without including the pencil marks, place in a micro-centrifuge tube 1.5 ml with 50µL lysis solution and briefly vortex. The samples were incubated at 95 °C, then cooled at room temperature for 30 seconds to stabilize the DNA. To each tube, 50µL of DNA Stabilizing Solution was added and then centrifuged briefly. Sample lysate was stored at -20 °C before use. GTXpress™ Master Mix was used for the next step. Any frozen genomic DNA or sample lysates were thawed by removing them from the freezer and placing on ice. After the samples were thawed, they were mixed by vortexing and then centrifuged. The polymorphism was amplified by a set of forward and reverse primers with the following sequences - forward primer: 5'–CCTTCGTTTCTTGGTG AATTTTTGGGATGTAGTGAAGAGGCGG–3'; reverse primer: 5' AGGTTGGCTTGGTTTGCAATCATC -3'. The amplification reaction for an individual PCR tube was performed in a total volume of 20 µl reaction mixture. PCR reaction mix volume was determined by using the TaqMan® GTXpress™ Master Mix (2×) 12.50µL, TaqMan genotyping assay mix (20×) 1.25µL, DNase-free water 6.25µL, and 5µL sample. The tube was vortexed and centrifuged briefly to spin down the contents and to eliminate air bubbles from the solution. The MicroAmp™ Optical 96-well plate on the Real-Time PCR System was used with a sealed plate with a MicroAmp™ clear adhesive film. PCR conditions were cycling (40 cycles) for denature (3 sec) and anneal/extend (30 sec). Finally, the SDS software recorded the results of the allelic discrimination analyzed on a scatter plot of Allele 1 (VIC) versus Allele 2 (FAM).

Hardy-Weinberg Equilibrium

Hardy-Weinberg equilibrium principle (HWE) is used to compare allele frequencies in a given population over a period of time (Hosking et al., 2004).

There are two equations necessary to solve a Hardy-Weinberg equilibrium question:

$$p + q = 1 \quad (2.1)$$

$$p^2 + 2pq + q^2 = 1 \quad (2.2)$$

p is the frequency of the dominant allele.

q is the frequency of the recessive allele.

$\mathbf{p^2}$ is the frequency of individuals with the homozygous dominant genotype.

2pq is the frequency of individuals with the heterozygous genotype.

$\mathbf{q^2}$ is the frequency of individuals with the homozygous recessive genotype.

Statistical Analysis

Participants were divided into two groups, males and females. Demographic information was collected and included gender, age, race/ethnicity, height, and weight. Additionally, BMI and BF% were determined. All analyses were adjusted for the effects of SNPs, body composition, and dietary intake. Descriptive statistics for mean values, standard deviations, and standard errors of mean were used for typical distribution. Differences between means were separated by one and two-way ANOVA. Categorical variables were reported as frequencies and percentages while continuous variables were reported as means ± SEM. The difference in continuous variables between two groups was analyzed using students t-test, and the difference between multiple groups was analyzed by analysis of variance (MANOVA), with post hoc multiple comparisons. The chi-square test was used to analyze differences in genders. The frequency of the recessive and dominant allele was determined by using the Hardy-Weinberg equilibrium as well as chi-square. All statistical analysis of data was conducted using the Statistical Package for Social Sciences (SPSS) version 23.0 (SPSS Inc. USA) software for Windows. $P \leq 0.05$ was considered as significant.

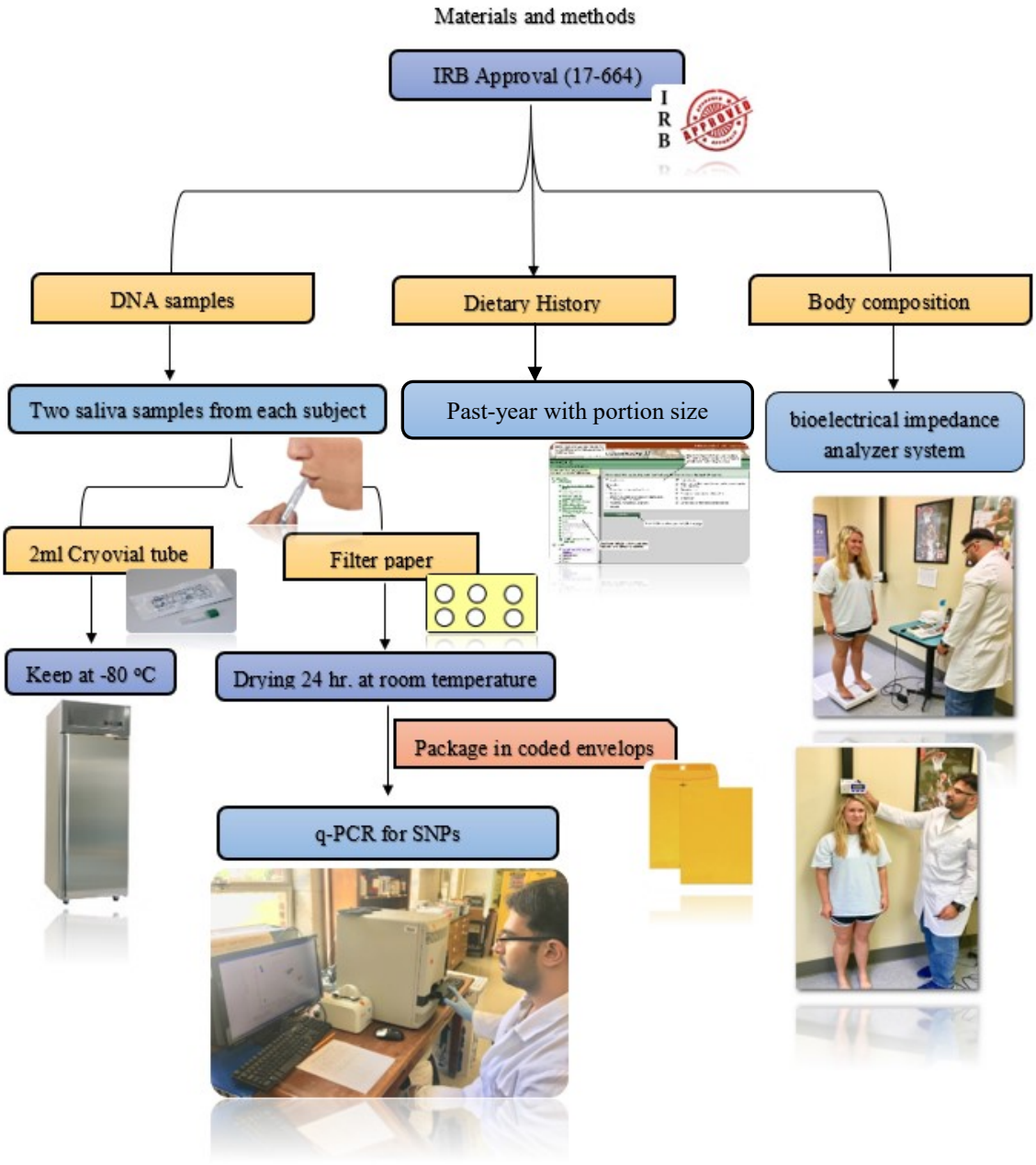


Figure 3.3 Outline of the materials and methods of the dissertation.

CHAPTER IV

RESULTS AND DISCUSSION

Determine the prevalence of genotypes of taste receptor gene for bitter taste TAS2R38 (rs713598 and rs10246939) in a group of young adult participants.

Results

Anthropometric and physical activity results of the 500 study participants are shown in Table 4.1 and Figure 4.1. All participants were presumably healthy undergraduate and graduate college students. Of these, 414 were women (83%) and 86 were men (17%). Mean age and standard error of the mean (Mean± SEM) of this study were 20.56± 0.230 years for men and 20.36± 0.146 years for women. Differences were observed in body composition parameters for height, weight, BMI, BF%, fat mass, fat free mass, and total body water between male and female participants ($p<0.05$). Males had higher values for height, weight, BMI, fat free mass, and total body water. Females had higher values for fat mass and BF%. The mean BF% for females was 28.12± 0.44 and 19.10± 0.86 for males ($p<0.001$).

Table 4.1 Age, anthropometric measurements, and body composition in males and females.

	Males (n= 86) Mean ± SEM	**Females (n= 414) Mean ± SEM**	**p-value**	**95% Cl**
Age (years)	20.56± 0.230	20.36± 0.146	.560	-0.47, 0.86
Height (feet, inches)	5.79± 0.026	5.46± 0.012	<.001*	0.28, 0.39
Weight (pounds)	176.86± 3.92	144.29± 0.53	<.001*	24.04, 41.10
BMI (kg/m^2)	25.99± 0.28	24.12± 0.28	.005*	0.57, 3.17
Body fat (%)	19.10± 0.86	28.12± 0.44	<.001*	-11.13, -7.03
Fat Mass (pounds)	36.20± 2.7	43.70± 1.32	.018*	-13.67, -1.30
Fat Free Mass (pounds)	140.54± 1.90	103.10± 2.50	<.001*	26.58, 48.47
Total Body Water (pounds)	103.0± 1.40	73.64± 0.40	<.001*	27.45, 31.67

* Significant at $p<0.05$, 95% Cl: 95% confidence interval.

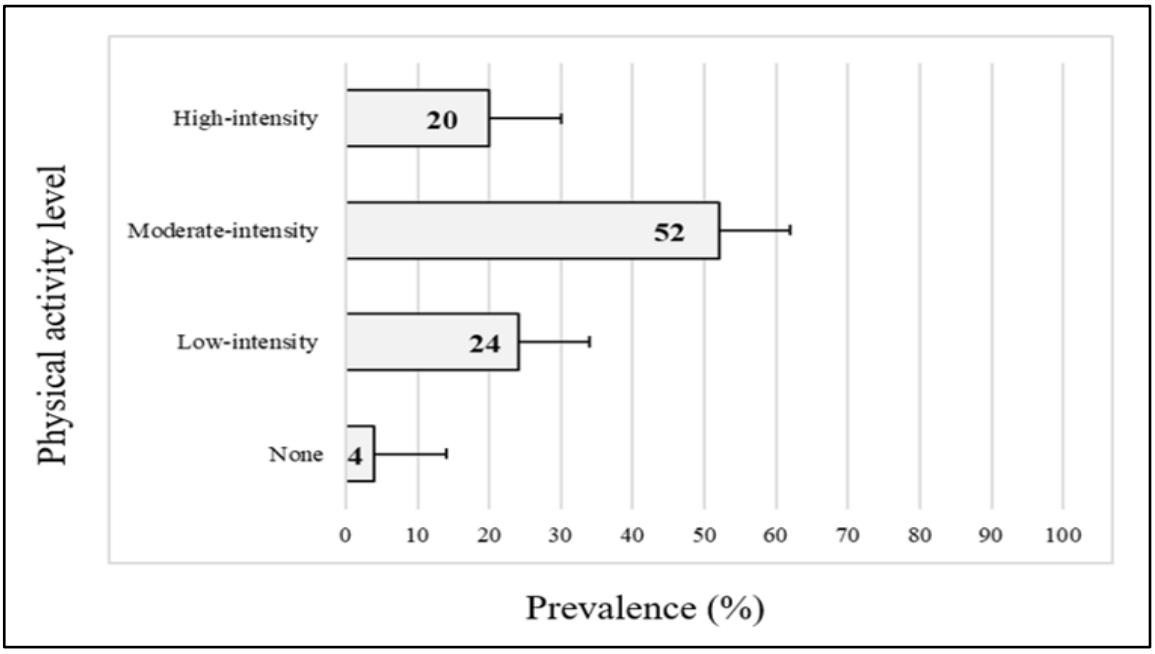


Figure 4.1 Prevalence of physical activity level in males and females.

As shown in Figure 4.2, the allele frequency (%) in TAS2R38 gene polymorphism rs713598 in our study was C, 0.58 and G, 0.42, which was similar to the American population (dbSNP) (C, 0.66 and G, 0.34). However, the results of another SNP (rs10246939) was different (C, 0.47 and T, 0.63) compared to American population (dbSNP) which was C, 0.69 and T, 0.31.

Two nonsynonymous cSNPs in TAS2R38, rs713598 and rs10246939 were genotyped in the genetic samples. The two polymorphic SNPs were in Hardy–Weinberg equilibrium. Variation in the frequency was observed in different ethnic groups. It did not differ between allele frequency in ethnic groups such as Caucasian, African American, Asian, and Latino compared with American population. Allele C was dominant in ethnic groups with rs713598. However, it was different in rs10246939 in the same group; Caucasian and African American compared with American population (C, 0.48 T, 0.52; C, 0.44 T, 0.56 and C, 0.69 T, 031, respectively). The minor allele T was dominant in this SNP in our study; however, C was dominant in the American population (Figure 4.3).

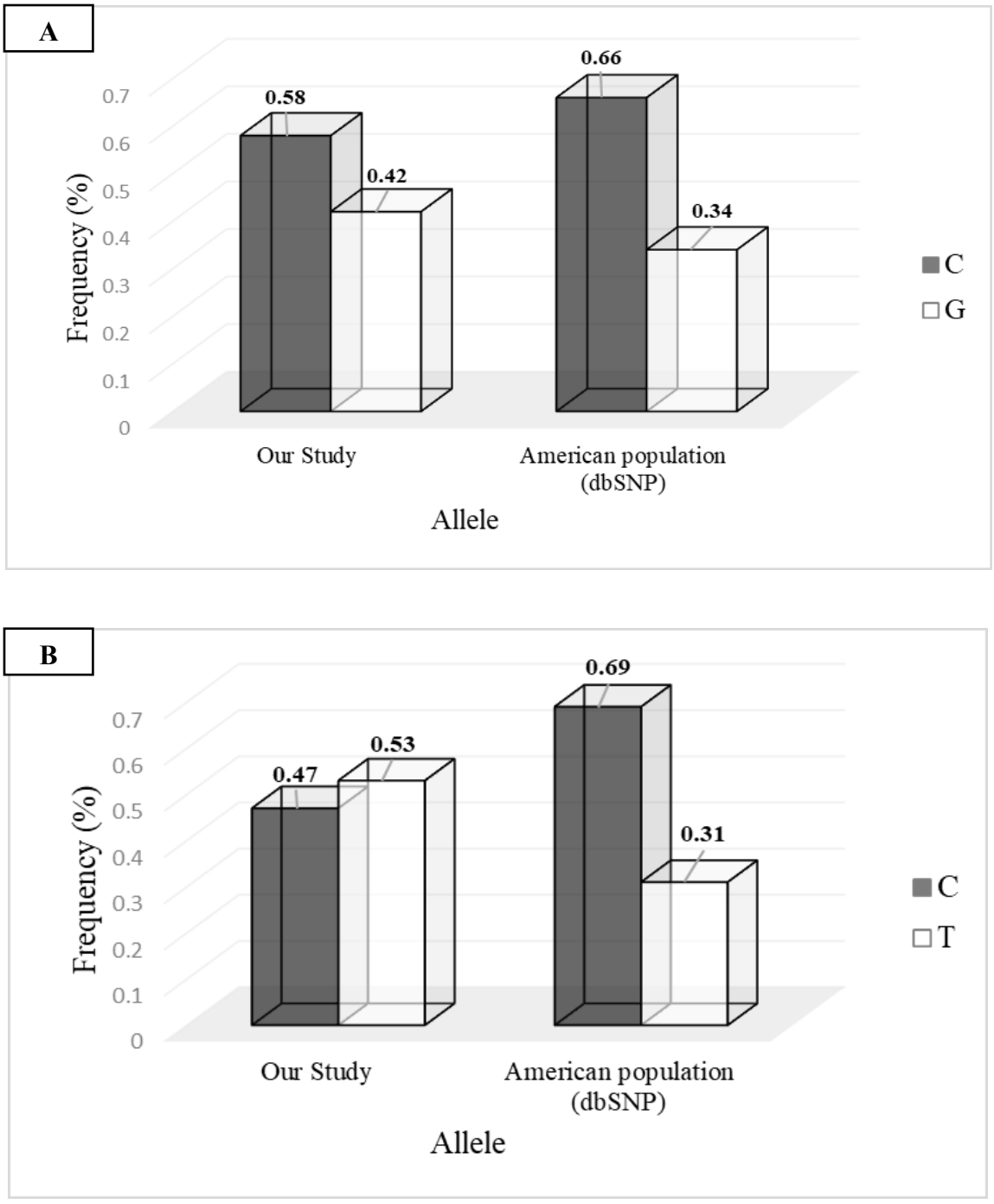


Figure 4.2 Comparison between our study and American population (dbSNP) in TAS2R38 gene polymorphism with allele frequencies (%): A) rs713598, B) rs10246939

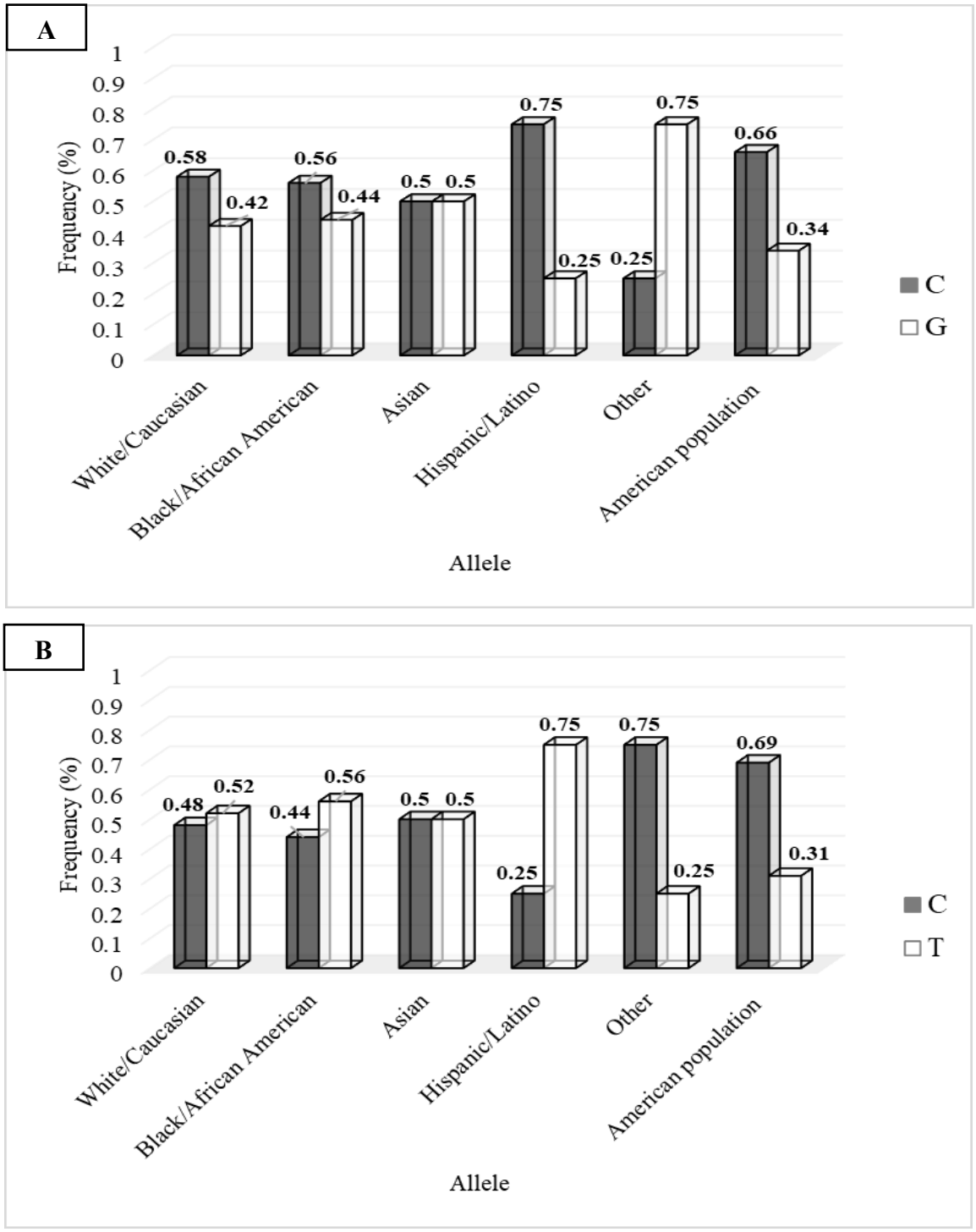


Figure 4.3 Comparison of race/ethnicity between our study and American population (dbSNP) in TAS2R38 gene polymorphism with allele frequencies (%) by race: A) rs713598, B) rs10246939

Both SNPs followed Hardy–Weinberg equilibrium. SNP rs713598, the allele frequency (%) for allele C was 0.54 and 0.46 in allele G in males (χ^2=0.365; p=0.83) whereas it was C, 0.58 and G, 0.42 in females (χ^2=0.067; p =0.96). For SNP rs10246939, the allele frequency (%) for allele C was 0.50 and 0.50 in allele T for males (χ^2= 0.418; p =0.811) whereas it was C, 0.46 and T, 0.54 in females (χ^2=0.475; p=0.788). There was not a significant difference in frequency of the genotypes between genders (Table 4.2).

In Table 4.3, we compare results of our study with American population (dbSNP) in TAS2R38 gene polymorphism with allele frequencies (%) in both SNPs, rs713598 and rs10246939. There was not a significantly different frequency of the genotype for rs713598 and rs10246939 in participants (N= 500) (C, 0.58; G. 0.42; χ^2= 0.368; p=0.87 and C, 0.47; T, 0.53; χ^2=0.139; p=0.93, respectively).

Hardy–Weinberg method was used to determine allele frequencies (%) in genotypes. CG and CT heterozygous were most commonly observed compared with CC, GG, and TT homozygous in rs713598 and rs10246939. CT genotype in males and females were 53% and 48%, respectively, in rs10246939, and was 46% and 48%, respectively, in CG genotype rs713598 (Figure 4.4).

Table 4.2 Distribution of TAS2R38 gene (rs10246939 and rs713598) between males and females.

Genotype rs713598	**Males n (86)**	**%**	**Allele* Frequency**	**χ^2**	**P-value**	**Females n (414)**	**%**	**Allele* Frequency**	**χ^2**	**P-value**
CC	26	30.23	**C,** 0.53	0.365	0.83	141	34.06	**C,** 0.58	0.067	0.966
CG	40	46.51				199	48.07			
GG	20	23.26	**G,** 0.47			74	17.87	**G,** 0.42		
Genotype rs10246939										
CC	20	23.26	**C,** 0.50	0.418	0.811	93	22.46	**C,** 0.47	0.475	0.788
CT	46	53.49				199	48.07			
TT	20	23.26	**T**, 0.50			122	29.47	**T,** 0.53		

*Allele frequency was calculated using Hardy-Weinberg Equation.

Table 4.3 Comparison between our study and American population (dbSNP) in TAS2R38 gene polymorphism with allele frequencies (%): A) rs713598, B) rs10246939

Genotype rs713598	**Participants N (500)**	**%**	**Allele* Frequency**	**χ^2**	**P-value**	**American population (dbSNP)**	**%**	**Allele* Frequency**	**χ^2**	**P-value**
CC	167	33.4	**C,** 0.58	0.268	0.87	-----	-----	**C,** 0.66	------	-----
CG	239	47.8				-----	-----			
GG	94	18.8	**G,** 0.42			------	------	**G,** 0.34		
Genotype rs10246939										
CC	113	22.6	**C,** 0.47	0.139	0.93	------	-----	**C,** 0.69	-----	-----
CT	245	49				------	-----			
TT	142	28.4	**T**, 0.53			------	-----	**T,** 0.31		

*Allele frequency was calculated using Hardy-Weinberg Equation.
----- Not available

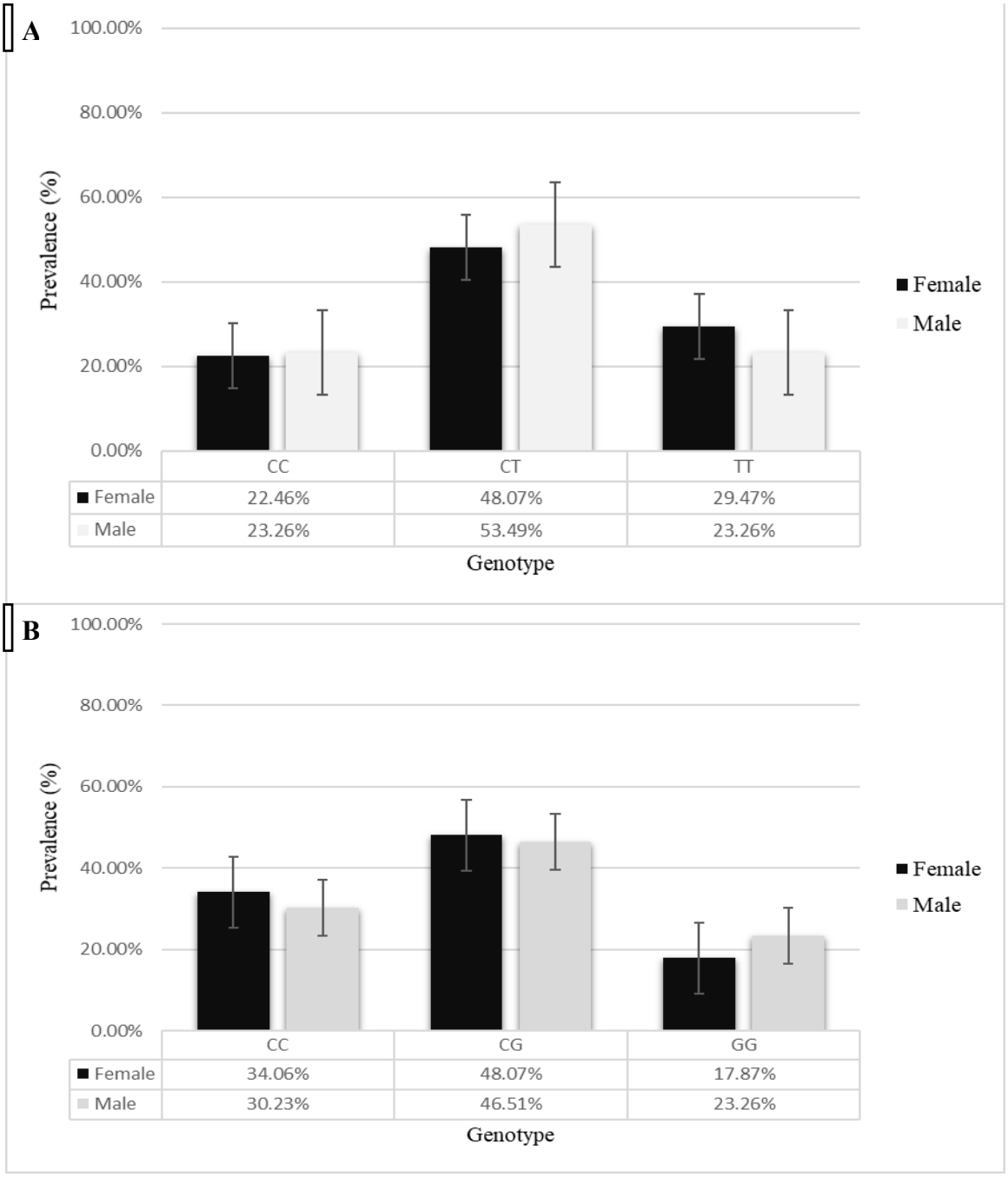


Figure 4.4 Distribution of TAS2R38 gene (rs10246939 and rs713598) in males and females: A) rs10246939, B) rs713598

Discussion

Body composition testing is an important key component of health in both individuals and populations. The technique is easily executed, non-invasive, and affordable. BIA is a practical method that is usually used for estimating BF% in males and females, which was used in this study with a single-frequency. BIA equipment can measure not only total body water and BF%, but also fat mass, fat-free mass, and fat distribution within the whole body. Body composition differs between males and females. Males have more muscle and lean mass, but females have more body fat (gluteal region) than males (abdominal region) of the same BMI. Usually, adipose tissue accumulates around the trunk and abdomen in males, whereas around the hips and thighs in females. All types of fat accumulate in the subcutaneous area as subcutaneous adipose tissue, and also accumulates as visceral adipose tissue (Bredella, 2017; Romero-Corral et al., 2008)

College-age young adults in this age group may be more likely to have BMI measurements in the normal range. The average BMI for men is 27 kg/m^2 and for women it is 26.5 kg/m^2 in the US (Carpenter et al., 2013). The average BMI in our study population was similar to the American population and Carpenter et al. (2013); their results showed that BMI for males was 24.4 kg/m^2 and 22.0 kg/m^2 for females. The average BMI for males in our study was 25.99 kg/m^2 and 24.12 kg/m^2 for females. Also, it was expected that females would have a higher BF% than males, which was 19.10± 0.86 in males and 28.12± 0.44 in females ($p<0.001$). Our results are in agreement with Ramírez-Vélez et al. (2017). They reported that women had significantly higher BF% than men ($p<0.05$), 27% and 15.7%, respectively (Ramírez-Vélez et al., 2017).

The nonsynonymous coding SNPs within TAS2R38 gene such as rs713598 (Ala49Pro) and rs10246939 (Ile296Val) with only two of haplotypes (Pro-Ala-Val (PAV) and Ala-Val-Ile (AVI)). These are commonly found in individuals, who have the PAV allele are significantly more sensitive to PROP, PTC, and bitterness of foods compared to who have homozygous for the AVI allele (non-taster) (Kim, Wooding, Ricci, Jorde, & Drayna, 2005).

Examine how genetic variation in bitter taste receptor TAS2R38 influences food preference and consumption regarding carbohydrates, proteins, and fats among a group of young adult participants

Results

The relationship of TAS2R38 gene and dietary intake is shown in Table 4.4. The multivariate ANOVA of the associations between genotypes and food consumption was used to determine realsionships. Total energy, dietary fiber (total fibers, soluble fibers, insoluble fibers), vegetables (total vegetables, dark-green vegetables, orange vegetables, white potatoes, starchy vegetables, tomatoes, and other vegetables), fruits (total fruit intake, citrus fruits, and other fruits), fat, protein, caffeine, dietary vitamin E intake and alcohol consumption were examined. There were no significant differences in the variables between the major allele and minor allele in males and females. Total energy (kcal), carboyhdrates, fat, and protein intakes (g/day) were higher in males who carried CG and CT genotype in rs713598 and rs10246939 (2758.54± 229.46, 340.01± 30.56, 105.83± 9.98, and 109.51± 10.65, respectively) in rs713598 (Table 4.4), and (2558.86± 218.29, 317.98± 28.92, 96.84± 9.49, and 100.42± 10.11, respectively) in rs10246939 (Table 4.7). No significant differences in those dietary variables were noted among TAS2R38 genotype.

Daily intakes of α-tocopherol (vitamin E, IU/day) were 21.79± 2.20 and 20.91± 2.06, respectively, for males who had CG and CT heterozygotes in rs713598 and rs10246939, respectively, (Tables 4.4 and 4.7). For females, it was 12.84± 0.76 and 12.83± 0.76, respectively, for CG and CT heterozygotes (Tables 4.5 and 4.6). There was not significance between intake of vitamin E and genotypes in TAS2R38 gene. This is the first known study investigating the association between dietary α-tocopherol intake

and genotype which may contribute to the development of a personalized recommendation for vitamin E intake.

Alcohol intake (g/day) was significantly different between genotypes (CC, CG, and GG; SNP, rs713598) in females who carried C allele (major allele), and there was a lower intake of alcoholic beverages compared with G allele (minor allele) in the groups; CC, 9.00± 1.95, CG, 6.89± 1.64, and GG 15.10± 2.70, respectively, p=0.035 (Table 4.5). However, it was not significant in the same group of females in the SNP, rs10246939 (p=0.127) (Table 4.6).

Alcohol and caffeine intakes were significantly different between genotypes (CC, CG, and GG; SNP, rs713598) in participants (N=500) who carried GG genotype. There was a trend in the CC genotype of participants in the SNP, rs10246939 with a higher alcohol intake (p=0.075) (Table 4.8). There was a higher intake of alcohol (g/day) in GG genotype compared with other genotypes (GG, 14.51± 2.24; CC, 9.13± 1.69; CG 6.94± 1.41; p=.018) (Table 4.9). Participants who carried CC genotype had a higher intake of caffeine (mg/day) compared with other genotypes (CC, 139.92± 12.69; CG, 136.76± 10.61; GG, 94.39± 16.83; p=0.05) (Table 4.9), but overall there were no significant differences between dietary variables.

Table 4.4 Dietary intake according to TAS2R38 (rs713598) in males.

Males (n= 86): TAS2R38; rs713598				
Dietary Intake	**CC n (%)** 26 (30)	**CG n (%)** 40 (47)	**GG n (%)** 20 (23)	**p-value**
	Mean ± SEM	**Mean ± SEM**	**Mean ± SEM**	
Energy (Kcal/day)	2037.04± 284.62	2758.54± 229.46	2317.52± 324.51	.137
Total Carbohydrates (g/day) Total carbohydrate (%)	255.9± 37.90 50.24± 3.8	340.01± 30.56 49.30± 4.5	287.56± 43.22 49.63± 3.4	.215 .160
• Total sugar (g/day)	134.71± 23.52	170.82± 18.96	152.65± 26.81	.489
• Grains (g/day)				
Total grains Whole grains Non-whole grains	4.30± .89 .65± .17 3.65± .80	6.72± .72 .95± .14 5.76 .64	4.89± 1.02 .41± .20 4.47± .91	.088 .091 .116
• Vegetables (cup/day)				
Total vegetables Dark-green vegetables Orange vegetables White potatoes Starchy vegetables Tomatoes Other vegetables	1.63± .25 .30± .10 .11± .03 .33± .07 .21± .06 .20± .05 .47± .09	1.84± .20 .44± .08 .11± .02 .32± .05 .14± .04 .31± .04 .50± .07	1.56± .28 .25± .11 .10± .03 .44± .08 .08± .06 .29± .05 .38± .10	.674 .364 .983 .483 .356 .218 .668
• Fruits (cup/day)				
Total fruit Citrus fruits, melons, and berries Other fruits	2.31± .55 1.10± .31 1.21± .27	1.57± .44 .62± .25 .95± .22	1.17± .62 .52± .35 .65± .31	.371 .386 .406
Total fats (g/day) Total fats (%)	74.97± 12.37 33.12± 4.2	105.83± 9.98 34.52± 3.2	81.94± 14.11 31.82± 2.8	.123 .820
Total proteins (g/day) Total proteins (%)	78.11± 13.21 16.64± 2.8	109.51± 10.65 16.18± 3.1	91.84± 15.06 17.85± 3.9	.179 .120
Dietary fiber • Total fibers (g/day) Soluble fibers Insoluble fibers	 18.93± 2.81 6.09± .83 12.78± 2.04	 21.27± 2.26 6.24± .67 14.95± 1.65	 15.01± 3.20 4.47± .94 10.38± 2.33	 .285 .288 .275
Caffeine (mg/day)	137.05± 29.96	118.11± 24.15	95.46± 34.16	.659
Alcohol (g/day)	9.80± 2.84	7.18± 2.29	12.73± 3.23	.370
Vitamin E (IU/day)	16.33± 2.73	21.79± 2.20	16.10± 3.11	.188

MANOVA was used to test for significant differences ($p<0.05$) across genotypes.

Table 4.5 Dietary intake according to TAS2R38 gene (rs713598) in females.

Females (n= 414): TAS2R38; rs713598				
Dietary Intake	**CC** **n (%)** 141 (34)	**CG** **n (%)** 199 (48)	**GG** **n (%)** 74 (18)	**p-value**
	Mean ± SEM	**Mean ± SEM**	**Mean ± SEM**	
Energy (Kcal/day)	1692.85± 113.84	1819.08± 95.83	1827.72± 157.15	.656
Total Carbohydrates (g/day)	213.82± 15.52	238.63± 13.06	229.78± 21.42	.473
Total carbohydrates (%)	50.52± 3.8	52.47± 2.9	50.28± 4.1	.320
• Total sugar (g/day)	109.42± 9.96	130.13± 8.38	121.20± 13.75	.283
• Grains (g/day)				
Total grains	4.19± .36	4.47± .31	4.25± .50	.835
Whole grains	.63± .05	.55± .04	.57± .07	.437
Non-whole grains	3.55± .34	3.91± .28	3.68± .47	.710
• Vegetables (cup/day)				
Total vegetables	1.39± .11	1.49± .09	1.67± .15	.339
Dark-green vegetables	.27± .05	.37± .04	.40± .07	.225
Orange vegetables	.11± .01	.10± .01	.10± .02	.811
White potatoes	.28± .03	.29± .02	.32± .04	.706
Starchy vegetables	.085± .00	.07± .00	.09± .01	.288
Tomatoes	.22± .02	.22± .02	.26± .03	.491
Other vegetables	.40± .03	.42± .03	.48± .04	.479
• Fruits (cup/day)				
Total fruit	1.54± .21	1.66± .18	1.71± .29	.874
Citrus fruits, melons, and berries	.67± .13	.69± .11	.60± .18	.927
Other fruits	.87± .10	.97± .08	1.11± .14	.409
Total fats (g/day)	63.33± 4.41	66.90± 3.71	64.60± 6.09	.810
Total fats (%)	33.6± 2.5	33.09± 3.1	31.81± 2.9	.283
Total proteins (g/day)	60.06± 4.64	62.81± 3.91	64.77± 6.41	.820
Total proteins (%)	15.88± 2.5	14.48± 1.4	17.88± 4.1	.340
Dietary fiber				
• Total fibers (g/day)	15.56± .99	15.86± .83	16.90± 1.37	.724
Soluble fibers	5.19± .30	5.12± .25	5.06± .42	.969
Insoluble fibers	10.31± .72	10.67± .61	11.79± 1.00	.478
Caffeine (mg/day)	140.44± 14.04	140.51± 11.82	95.36± 19.39	.110
Alcohol (g/day)	9.00± 1.95	6.89± 1.64	15.10± 2.70	.035*
Vitamin E (IU/day)	12.53± 0.90	12.84± 0.76	12.59± 1.24	.962

Table 4.6 Dietary intake according to TAS2R38 gene (rs10246939) in females.

Females (n= 414): TAS2R38; rs10246939				
Dietary Intake	**CC n (%)** 93 (22)	**CT n (%)** 199 (48)	**TT n (%)** 122 (30)	**p-value**
	Mean ± SEM	**Mean ± SEM**	**Mean ± SEM**	
Energy (Kcal)	1801.98± 140.10	1836.17± 95.78	1663.60± 122.32	.600
Total Carbohydrates (g/day)	226.72± 19.10	240.35± 13.05	210.87± 16.67	.378
Total Carbohydrates (%)	50.32± 3.2	52.35± 2.8	50.70± 2.4	.185
• Total sugar (g)	119.72± 12.25	131.06± 8.37	107.20± 10.70	.213
• Grains (g)				
Total grains	4.25± .45	4.48± .31	4.17± .39	.810
Whole grains	.57± .06	.56± .04	.63± .05	.576
Non-whole grains	3.67± .41	3.91± .28	3.53± .36	.706
• Vegetables (cup/day)				
Total vegetables	1.62± .13	1.50± .09	1.38± .12	.399
Dark-green vegetables	.41± .06	.35± .04	.27± .05	.225
Orange vegetables	.10± .01	.11± .01	.11± .01	.903
White potatoes	.30± .03	.30± .02	.28± .03	.887
Starchy vegetables	.09± .01	.07± .00	.08± .00	.427
Tomatoes	.25± .03	.22± .02	.22± .02	.626
Other vegetables	.46± .04	.42± .03	.40± .03	.616
• Fruits (cup/day)				
Total fruit	1.61± .26	1.66± .18	1.58± .23	.958
Citrus fruits, melons, and berries	.60± .16	.68± .11	.69± .14	.906
Other fruits	1.01± .13	.98± .08	.89± .11	.742
Total fats (g)	64.45± 5.43	67.655± 3.714	62.01± 4.74	.636
Total fats (%)	32.18± 3.7	33.16± 3.2	33.54± 2.9	.333
Total proteins (g)	64.65± 5.72	62.86± 3.91	59.32± 4.99	.761
Total proteins (%)	17.5± 2.1	15.01± 1.9	15.80± 2.7	.288
Dietary fiber				
• Total fibers (g/day)	16.55± 1.22	16.55± 1.22	15.34± 1.07	.749
Soluble fibers	5.02± .37	5.18± .25	5.13± .33	.940
Insoluble fibers	11.49± .89	10.78± .61	10.14± .77	.521
Caffeine (mg/day)	111.40± 17.34	142.72± 11.85	131.65± 15.14	.330
Alcohol (g/day)	13.33± 2.41	7.46± 1.65	8.47± 2.11	.127
Vitamin E (IU/day)	12.79± 1.11	12.83± .76	12.38± .97	.932

Table 4.7 Dietary intake according to TAS2R38 gene (rs10246939) in males.

Males (n= 86): TAS2R38; rs10246939				
Dietary Intake	**CC** **n (%)** 20 (23)	**CT** **n (%)** 46 (54)	**TT** **n (%)** 20 (23)	**p-value**
	Mean ± SEM	**Mean ± SEM**	**Mean ± SEM**	
Energy (Kcal)	2317.52± 331.05	2558.86± 218.29	2279.86± 331.05	.717
Total Carbohydrates (g/day) Total carbohydrate (%)	287.56± 43.86 50.0± 2.81	317.98± 28.92 51.02± 1.32	281.39± 43.86 50.1.3± 2.10	.729 .622
• Total sugar (g)	152.65± 27.03	159.80± 17.82	149.22± 27.03	.940
• Grains (g) Total grains Whole grains Non-whole grains	 4.89± 1.04 .41± .20 4.47± .93	 5.95± .69 .87± .13 5.07± .61	 5.34± 1.04 .73± .20 4.61± .93	 .680 .183 .838
• Vegetables (cup/day) Total vegetables Dark-green vegetables Orange vegetables White potatoes Starchy vegetables Tomatoes Other vegetables	 1.56± .28 .25± .11 .10± .03 .44± .08 .08± .06 .29± .05 .39± .10	 1.78± .19 .43± .07 .11± .02 .34± .05 .14± .04 .29± .03 .46±.06	 1.71± .28 .28± .11 .10± .03 .30± .08 .23± .06 .23±.05 .55± .10	 .826 .367 .961 .453 .257 .666 .541
• Fruits (cup/day) Total fruit Citrus fruits, melons, and berries Other fruits	 1.17± .62 .52± .35 .65± .31	 1.51± .41 .58± .23 .93± .20	 2.68± .62 1.35± .35 1.33± .31	 .189 .148 .302
Total fats (g) Total fats (%)	81.94± 14.40 31.31± 3.2	96.84± 9.49 34.12± 3.5	86.40± 14.40 33.32± 1.5	.647 .443
Total proteins (g) Total proteins (%)	91.84± 15.34 19.71± 2.9	100.42± 10.11 17.88± 1.9	89.59± 15.34 17.22± 3.1	.804 .711
Dietary fiber • Total fibers (g/day) Soluble fibers Insoluble fibers	 15.01± 3.21 4.47± .94 10.38± 2.34	 20.60± 2.11 6.06± .62 14.48± 1.54	 19.76± 3.21 6.47± .94 13.22± 2.34	 .343 .273 .349
Caffeine (mg/day)	95.47± 33.91	110.72± 22.36	159.73± 33.91	.362
Alcohol (g/day)	12.73± 3.24	7.94± 2.14	8.83± 3.24	.466
Vitamin E (IU/day)	16.10± 3.13	20.91± 2.06	16.70± 3.13	.332

Table 4.8 Dietary intake according to rs10246939 in TAS2R38 gene.

Participants (N= 500): TAS2R38; rs10246939				
Dietary Intake	**CC n (%)** 113 (22.6)	**CT n (%)** 245 (49)	**TT n (%)** 142 (28.4)	**p-value**
	Mean ± SEM	**Mean ± SEM**	**Mean ± SEM**	
Energy (Kcal/day)	1902.78± 130.29	1971.86± 88.88	1750.40± 116.74	.320
Total Carbohydrates (g/day)	238.11± 17.56	254.92± 11.98	220.80± 15.73	.222
Total carbohydrates (%)	51.91± 5.5	52.88± 3.2	50.12± 4.1	.162
• Total sugar (g/day)	125.47± 11.13	136.46± 7.59	113.12± 9.97	.175
• Grains (g/day)				
Total grains	4.39± .41	4.75± .28	4.33± .37	.609
Whole grains	.54± .06	.62± .04	.64± .05	.453
Non-whole grains	3.84± .38	4.13± .26	3.68± .34	.566
• Vegetables (cup/day)				
Total vegetables	1.63± .12	1.55± .08	1.42± .11	.445
Dark-green vegetables	.39± .05	.37± .03	.27±.05	.195
Orange vegetables	.10± .01	.11± .01	.11± .01	.939
White potatoes	.32± .03	.31± .02	.28± .03	.657
Starchy vegetables	.09± .01	.09± .01	.10± .01	.615
Tomatoes	.26± .02	.23± .01	.22± .02	.547
Other vegetables	.45± .04	.43± .02	.42± .03	.876
• Fruits (cup/day)				
Total fruit	1.53± .24	1.64± .16	1.74± .21	.820
Citrus fruits, melons, and berries	.58± .15	.66± .10	.78± .13	.596
Other fruits	.94± .11	.97± .08	.95± .10	.977
Total fats (g/day)	68.09± 5.18	73.13± 3.53	65.45± 4.64	.392
Total fats (%)	31.33± 3.2	31.66± 4.5	30.01± 6.2	.211
Total proteins (g/day)	70.12± 5.51	69.91± 3.76	63.58± 4.94	.549
Total proteins (%)	16.71± 2.9	15.36± 5.7	19.66± 3.8	.399
Dietary fiber				
• Total fibers (g/day)	16.41± 1.15	16.89± .78	15.96± 1.03	.772
Soluble fibers	4.94± .35	5.35± .24	5.32± .31	.610
Insoluble fibers	11.40± .84	11.48± .57	10.57± .75	.613
Caffeine (mg/day)	107.63± 15.40	136.71± 10.51	135.60± 13.80	.263
Alcohol (g/day)	13.17± 2.05	7.55± 1.40	8.52± 1.84	.075
Vitamin E (IU/day)	14.14± .63	14.96± .43	13.90± .56	.275

Table 4.9 Dietary intake according to rs713598 in TAS2R38 gene.

Participants (n= 500): TAS2R38; rs713598				
Dietary Intake	**CC n (%)** 167 (33.4)	**CG n (%)** 239 (47.8)	**GG n (%)** 94 (18.8)	**p-value**
	Mean ± SEM	**Mean ± SEM**	**Mean ± SEM**	
Energy (Kcal/day)	1746.44± 107.59	1976.31± 89.94	1943.00± 142.65	.243
Total Carbohydrates (g/day)	220.38± 14.50	255.60± 12.12	242.78± 19.23	.177
Total carbohydrates (%)	50.42± 3.5	52.12± 4.5	51.31± 2.1	.120
• Total sugar (g/day)	113.36± 9.19	136.94± 7.68	127.77± 12.18	.145
• Grains (g/day)				
Total grains	4.21± .34	4.84± .28	4.43± .45	.347
Whole grains	.64± .05	.62± .04	.54± .06	.505
Non-whole grains	3.56± .31	4.22± .26	3.88± .41	.276
• Vegetables (cup/day)				
Total vegetables	1.43± .10	1.55± .08	1.67± .13	.351
Dark-green vegetables	.27± .04	.38± .03	.38± .06	.181
Orange vegetables	.11± .01	.10± .01	.10± .01	.858
White potatoes	.28± .02	.30± .02	.35± .03	.414
Starchy vegetables	.10± .01	.08± .01	.09± .01	.527
Tomatoes	.22± .02	.23± .01	.27± .02	.389
Other vegetables	.41± .03	.43± .02	.46± .04	.693
• Fruits (cup/day)				
Total fruit	1.66± .20	1.64± .16	1.60± .26	.981
Citrus fruits, melons, and berries	.73± .12	.68± .10	.58± .16	.757
Other fruits	.92± .09	.96± .08	1.01± .13	.858
Total fats (g/day)	65.14± 4.27	73.41± 3.57	68.94± 5.67	.329
Total fats (%)	31.42± 3.2	34.33± 4.5	32.42± 2.2	.274
Total proteins (g/day)	62.87± 4.55	70.62± 3.80	71.30± 6.03	.361
Total proteins (%)	18.16± 5.2	14.16± 4.3	16.29± 5.2	.198
Dietary fiber				
• Total fibers (g/day)	16.08± .95	16.76± .79	16.65± 1.26	.856
Soluble fibers	5.33± .29	5.31± .24	4.96± .38	.706
Insoluble fibers	10.69± .69	11.39± .58	11.62± .92	.654
Caffeine (mg/day)	139.92± 12.69	136.76± 10.61	94.39± 16.83	.05*
Alcohol (g/day)	9.13± 1.69	6.94± 1.41	14.51± 2.24	.018*
Vitamin E (IU/day)	13.9± .52	14.97± .43	14.13± .69	0.289

Table 4.10 presents dietary intakes between males and females separately. Significant differences were observed between genders. Males had higher energy, carbohydrates, fats, proteins, total dietary fiber and α-tocopherol intakes than females (p<0.05). There was not a significant difference in caffeine intake although females had a mean intake of 132.42± 8.09 mg/day and males consumed 118.57± 17.76 mg/day (p=0.478).

Table 4.10 Dietary intake according to gender.

Dietary Intake	Males (n= 86) Mean ± SEM	95% CI	Females (n= 414) Mean ± SEM	95% CI	p-value
Energy (Kcal/day)	2437.85± 147.83	2147.39, 2728.31	1777.64± 67.38	1645.25, 1910.02	<.001
Total Carbohydrates (g/day) Total carbohydrates (%)	302.39± 20.05 49.15± 3.13	262.99, 341.80	228.60± 9.14 51.12± 1.21	210.641, 246.56	<.001
• Total sugar (g/day)	155.68± 12.78	130.56, 180.79	121.48± 5.82	110.03, 132.93	.015*
• Grains (g/day) Total grains Whole grains Non-whole grains	 5.56± .47 .73± .07 4.82± .43	 4.63, 6.50 .59, .87 3.96, 5.68	 4.33± .21 .58± .03 3.75± .19	 3.91, 4.76 .52, .65 3.35, 4.14	 .020* .058 .026*
• Vegetables (cup/day) Total vegetables Dark-green vegetables Orange vegetable White potatoes Starchy vegetables Tomatoes Other vegetables	 1.71± .14 .35± .06 .10± .01 .35± .04 .14± .01 .27± .03 .46± .04	 1.43, 1.99 .22, .48 .07, .14 .27, .43 .11, .18 .21, .33 .37, .55	 1.49± .06 .34± .03 .10± .00 .29± .01 .08± .00 .23± .01 .42± .02	 1.36, 1.62 .28, .40 .09, .12 .26, .33 .06, .09 .20, .25 .38, .47	 .158 .868 .989 .187 <.001 .155 .434
• Fruits (cup/day) Total fruit Citrus fruits, melons, and berries Other fruits	 1.70± .28 .74± .17 .96± .13	 1.15, 2.26 .40, 1.09 .69, 1.23	 1.63± .12 .66± .07 .96± .06	 1.38, 1.88 .51, .82 .84, 1.08	 .806 .681 .985
Total fats (g/day) Total fats (%)	90.94± 5.87 34.42± 1.2	79.40, 102.49	65.27± 2.67 33.11± 1.2	60.01, 70.53	<.001
Total proteins (g/day) Total proteins (%)	95.91± 6.19 18.33± 2.3	83.73, 108.08	62.22± 2.82 17.33± 2.3	56.67, 67.77	<.001
Dietary fiber • Total fibers (g/day) Soluble fibers Insoluble fibers	 19.11± 1.32 5.78± .40 13.23±.965	 16.51, 21.71 4.99, 6.58 11.34, 15.13	 15.94± .60 5.13± .18 10.75± .44	 14.76, 17.13 4.77, 5.49 9.89-,11.61	 .030* .144 .020*
Caffeine (mg/day)	118.57± 17.76	83.66,153.48	132.42± 8.09	116.51, 148.33	.478
Alcohol (g/day)	9.26± 2.38	4.58, 13.94	9.08± 1.08	6.94, 11.21	.944
Vitamin E (IU/day)	18.82± 1.22	16.41, 21.22	12.69± .55	11.59, 13.78	<.001

Discussion

Food intake is a complex behavior influenced by several factors such as environmental, physiological, sociocultural and economic. The tool used to measure dietary intake was the DHQ, which has previously been described (Subar et al., 2001). Rejection of bitter compounds is a natural response due to possible toxic compounds in the foods (alkaloid compounds), and this phenomenon was observed especially in children (Mennella, Bobowski, & Author, 2015). However, some bitter compounds in food can promote health such as phenols (tea and citrus fruits), organosulfur compounds (cruciferous vegetables such as broccoli) and phytonutrients in fruits and vegetables, such as grapefruit. There are some vegetables such as broccoli which are often disliked by children likely due to a bitter taste, and rejection of bitter taste is obvious in their rejection of some medications and certain foods such as dark green vegetables (Drewnowski, 2018). Bitter taste sensation may be associated with some diseases, for instance obesity, heart disease, and hypertension. Higher sensitivity to bitter taste could cause the avoidance of consuming some vegetables rich in anti-oxidant compounds. Consequently, it may lead to consuming a high intake of sweet and fatty foods which may increase the risk of obesity (Goldstein et al., 2005).

There are 25 different types of bitter taste perception (T2Rs); however, only T2R38 is related with a genetically predetermined bitter taste (U Kim et al., 2003). Also, there are three SNPs in this T2R38 gene, which results in three amino acid substitutions at residues A262V (rs1726866; MAF D 0.4255), P49A (rs713598; MAF D 0.4952), and V296I (rs10246939; MAF D 0.4794).

The bitterness in some vegetables and fruits are due to the taste of thiol compounds which was found to have an association with homozygous for the C allele at

the rs713598 and rs10246939 locus in the TAS2R38 taste receptor gene. Individuals who do not carry the C allele may not taste bitterness, and this may impact their perception of bitterness of foods (Chamoun et al., 2018).

Cowart, Yokomukai, and Beauchamp (1994) observed that young adults (n=52) were more sensitive to two bitter compounds (quinine sulfate and urea) than elderly adults (n=60) which indicated an effect of age on bitter taste. The ability to taste bitter declines gradually with age (Cowart et al., 1994). Also, Mennella, Yanina Pepino, and Reed (2005) found a strong association between TAS2R38 genotype and bitter taste sensitivity in children 5 to 10 years old (n=143) with no correlation among adults (Mennella et al., 2005).

Timpson et al. (2005) observed no significant associations between TAS2R38 (rs713598 and rs1726866) haplotypes and recent food intake (green vegetables, fat, and alcohol) in 3383 women from 23 British towns (aged 60–79 years). However, there was a marginally lower risk of diabetes in women who had the non-taster genotype (odds ratio:0.69; 95%CI: 0.48, 1.00). They suggested that these participants consumed a diet richer in bitter tasting foods such as vegetables earlier in life (Timpson et al., 2005).

An association between bitter taste and alcohol consumption has been investigated in some studies (Duffy, Davidson, et al., 2004). The results from these studies were contradictory showing relations between TAS2T38 and alcohol intake or no associations at all. Duffy et al. (2004) observed that no association was found between TAS2T38 and alcohol intake in a study involving more than 3000 females. The impact of SNPs (rs713598; rs1726866 and rs10246939) in TAS2R38 gene on alcohol and coffee consumption was not significant (p=0.903 and p=0.994, respectively) (Duffy, Peterson, &

Bartoshuk, 2004). However, in another study categorizing participants who carried at least one PAV haplotype (C allele- major) observed lower alcohol intake per day in the group of German Sorbs (males= 405 and females= 602) with mean age of 48± 16.2 years and BMI of 27.06 ±4.9 kg/m2 (Keller et al., 2013).

Our results showed a decrease in consumption of alcohol intake with participants who had C allele (major allele), which agrees with Duffy et al. (2004) who observed that TAS2R38 genotype was a significant predictor of alcohol intake. AVI/AVI homozygotes (minor allele) reported higher alcohol consumption compared with PAV/AVI heterozygotes or PAV/PAV homozygotes (major allele) in healthy adults (53 women and 31 men with mean age of 36 years old). Also, alcohol sensation is related to the number of fungiform papillae on the tongue tip (Duffy, Davidson, et al., 2004).

The perception of caffeine and PROP share a common genetic factor, TAS2R38 gene, and caffeine plays only a minor role in eliciting coffee bitterness. Perna et al. (2018) examined the relationship between TAS2R38 gene polymorphism (RS713598), G/G, C/G or C/C genotype, food preferences and body composition in a cross-sectional study in 118 adults (94 women and 24 men). The frequencies of genotype C/C, G/G and C/G were 20.3%, 29.7% and 50.0%, respectively. Participants who had G allele had a higher perception threshold compared with the C/C genotype for caffeine and beer ($p<.05$). However, there was not a difference in body composition between genotypes in the groups (Perna et al., 2018).

Examine how genetic variation in bitter taste receptors TAS2R38 (rs713598 and rs10246939) influence body fat percentage in a group of young adult participants

Results

We evaluated BF% in 500 participants (414 females and 86 males) with the allele frequency. The results of our study show there was no significant difference in allele frequency C/T between males SNPs, rs10246939 and BF% in the groups; underfat, healthy, overfat, and obese were C, 0.50; T, 50 ; p=0.60; C, 0.53; T, 47 ; p=0.18; C, 0.47; T, 53 ; p=0.10; and C, 0.45; T, 55 ; p=0.99, respectively (Table 4.11)

However, there was a significant difference in allele frequency C and G in SNPs, rs713598 with the overfat group of males (C, 0.57; G, 0.43; p=0.02). The percentage of CG genotype was high in healthy BF% males (n=27, 31%) compared to males who had CC and GG genotype (n=13, 15% and n=11, 13%), respectively (Table 4.12).

Polymorphisms in TAS2R38 were not associated with BF% in females. There were no significant differences in allele frequency C/G between females SNPs, rs713598 and BF% (Table 4.13). However, BF% was higher in females who carried C allele (major allele) in all groups were higher compared to G allele (minor allele); underfat, healthy, overfat, and obese (C, 0.65; G, 0.35; p=0.44) (C, 0.56; G, 0.44; p=0.94), (C, 0.54; G, 0.46; p=0.98), and (C, 0.63; G, 0.37; p=0.99), respectively (Table 4.13), whereas females who carried T allele (minor allele) in all groups were higher compared to C allele (major allele) (Table 4.14).

As shown in Figure 4.5, 500 participants (86 males and 414 females) were classified according to their BF% and genotypes. After genotyping assays, the CC genotype was found in 167 participants, the CG genotype in 239 participants, and the GG genotype in 94 participants in rs713598 whereas the CC genotype was found in 113

participants, the CT genotype in 245 participants, and the TT genotype in 122 participants in rs10246939. CG and CT genotypes were higher in the healthy BF% group (n= 139 and n=138, respectively). The percentage of SNPs and BF% were lower in the obese group who had GG and TT genotypes (13% and 5%, respectively) compared to the other groups (Figure 4.6).

Table 4.11 Association of TAS2R38 gene (rs10246939) with body fat percentage (BF%) in males.

Genotype: rs10246939					**Allele Frequency**		**P-Value**
Classification of BF%	**CC n (%)**	**CT n (%)**	**TT n (%)**	**Total n (%)**	**C**	**T**	
Underfat	0 (0)	1 (1.2)	0 (0)	1 (1.5)	0.50	0.50	0.60
Healthy	11 (13)	32 (37)	8 (9)	51 (59)	0.53	0.47	0.18
Overfat	5 (6)	3 (4)	6 (7)	14 (16)	0.47	0.53	0.10
Obese	4 (5)	10 (12)	6 (7)	20 (23.5)	0.45	0.55	0.99
Total	20 (23)	46 (54)	20 (23)	86 (100)	0.50	0.50	0.81

Table 4.12 Association of TAS2R38 gene (rs713598) with body fat percentage in males.

Genotype: rs713598					**Allele Frequency**		**P-Value**
Classification of BF%	**CC N (%)**	**CG N (%)**	**GG N (%)**	**Total**	**C**	**G**	
Underfat	0 (0)	1 (1.2)	0 (0)	1 (1.5)	0.50	0.50	0.60
Healthy	13 (15)	27 (31)	11 (13)	51 (59)	0.51	0.49	0.91
Overfat	7 (8)	2 (2)	5 (6)	14 (16)	0.57	0.43	0.02*
Obese	6 (7)	10 (12)	4 (5)	20 (23.5)	0.55	0.45	0.99
Total	26 (30)	40 (47)	20 (100)	86 (100)	0.53	0.47	0.83

*Significant at $p<0.05$

Table 4.13 Association of TAS2R38 gene (rs713598) with body fat percentage (BF%) in females.

Genotype: rs713598					**Allele Frequency**		**P-Value**
Classification of BF%	**CC n (%)**	**CG n (%)**	**GG n (%)**	**Total n (%)**	**C**	**G**	
Underfat	32 (8)	27 (7)	11 (3)	70 (17.5)	0.65	0.35	0.44
Healthy	68 (16)	112 (27)	42 (10)	222 (53)	0.56	0.44	0.94
Overfat	18 (4)	32 (8)	13 (3)	63 (15)	0.54	0.46	0.98
Obese	23 (6)	28 (7)	8 (2)	59 (14.5)	0.63	0.37	0.99
Total	93 (22)	199 (48)	122 (29)	414 (100)	0.47	0.53	0.78

Table 4.14 Association of TAS2R38 gene (rs10246939) with body fat percentage (BF%) in females.

Genotype: rs10246939					**Allele Frequency**		**P-Value**
Classification of BF%	**CC n (%)**	**CT n (%)**	**TT n (%)**	**Total n (%)**	**C**	**T**	
Underfat	14 (3)	28 (7)	28 (7)	70 (17)	0.40	0.60	0.37
Healthy	59 (14)	106 (26)	57 (14)	222 (54)	0.51	0.49	0.08
Overfat	13 (3)	34 (8)	16 (4)	63 (15)	0.48	0.52	0.81
Obese	7 (2)	31 (7)	21 (5)	59 (14)	0.38	0.62	0.68
Total	93 (22)	199 (48)	122 (29)	414 (100)	0.47	0.53	0.79

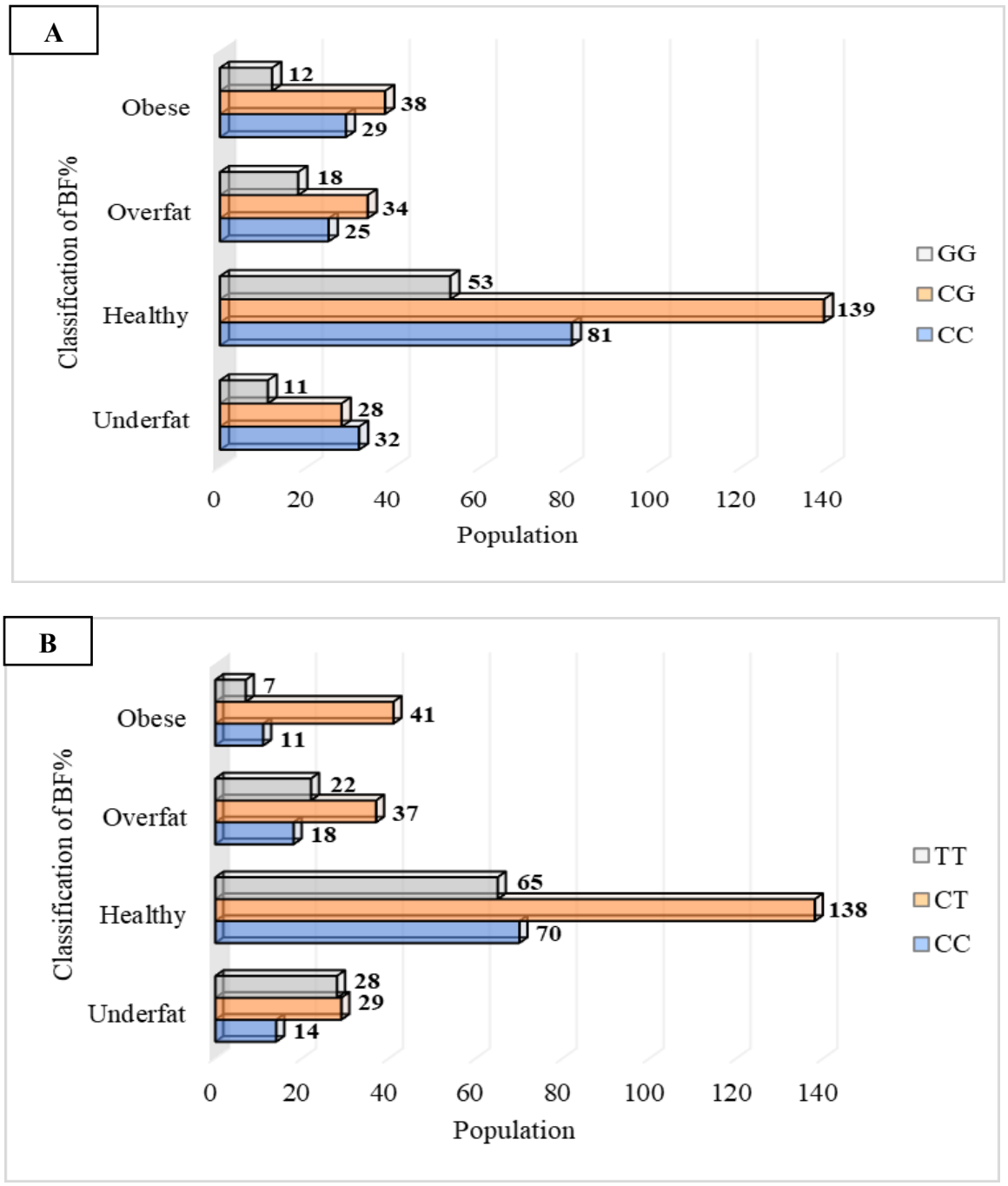
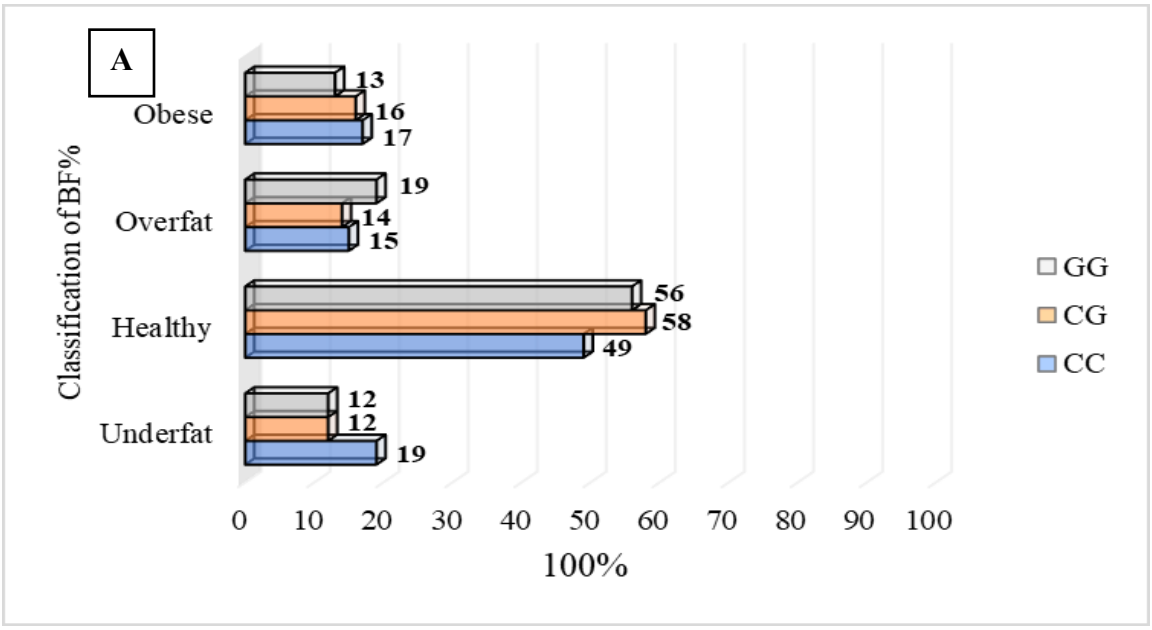


Figure 4.5 Relationship between SNPs genotyping and BF% in population (N=500): A) rs713598; B) rs10246939



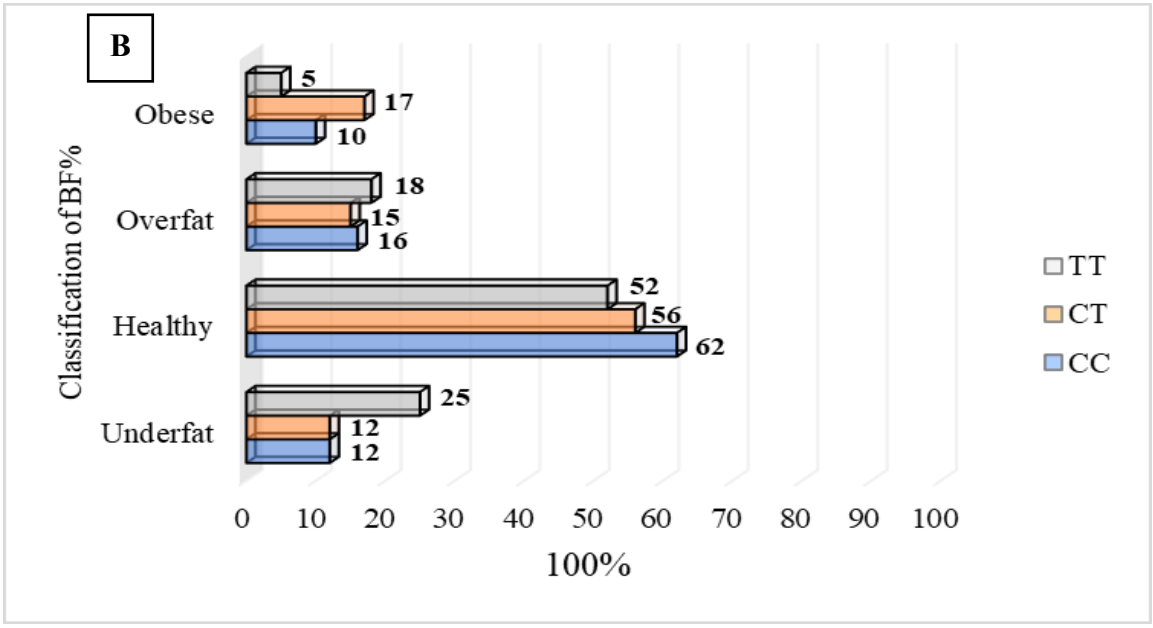


Figure 4.6 Relationship between percentage of SNPs genotyping and BF% in population (N=500): A) rs713598; B) rs10246939

Discussion

The TAS2R38 genotype (rs713598) was used to evaluate the relation between PROP taster status and body composition. Bouthoorn et al. (2014) found that non-taster girls had higher BMI and higher body fat mass compared with taster girls (BMI SDS: –0.09, p=0.023 and BF%: −0.49, p=0.028). However, there were no differences observed between tasters and non-tasters in boys. BMI and BF% were measured by DXA (Bouthoorn et al., 2014). Keller et al. (2013) observed a trend between the three TAS2R38 genetic variants (rs713598; rs1726866 and rs10246939) and BF% in non-diabetic women (p<0.056), but it was not significant between BMI and other anthropometrics (p<0.379) in the German Sorbs. The total of 1007 participants were 405 males and 602 females with a mean age of 48± 16.2 years and BMI of 27.06± 4.9 kg/m^2. Minor allele carriers showed lower BF% compared to homozygous major allele carriers (Keller et al., 2013).

More studies point in the opposite direction, reporting no influence of taste ability on BF%, BMI, and waist circumference when testing for an association between SNPs and body composition. Our results are in accordance with these negative studies rejecting the hypothesis that the TAS2R38 gene (SNPs, rs713598 and rs10246939) will have an influence on BF% or BMI. Two hundred and fifteen participants from Malaysia (100 males and 115 females) were examined to determine the influence of taste gene of the P49A SNP of TAS2R38 on food selection and body composition. However, there were no significant differences in BMI and total body fat between the genotypes (p<0.05). Therefore, the P49A SNP of the bitter taste receptor gene TAS2R38 could not serve as a predictor of anthropometric measurements such as waist and hip circumferences, total

body fat, and BMI (Ooi, Lee, Law, & Say, 2010). Hoppu et al. (2018) reported that the TAS2R38 genotype was not associated with body composition in a cohort study in Southwest Finland (757 women and 714 men) (Hoppu, Lagström, & Sandell, 2018). Sharma and Chopra (2013) observed that the bitter taste receptor gene (TAS2R38) could not serve as a significant predictor of anthropometric measurements for body fat, but this gene was significantly associated with premenstrual syndrome in adult Kullu females in Himachal Pradesh, India (Sharma & Chopra, 2013).

Limitations

The first limitation of our study is that participants were mainly females (83% of the total participants). Secondly, we used a SF-BIA device which may overestimate BF% in athletes and underestimate it in obese participants. However, the multi-frequency systems can correct this error by using low and high frequencies that calculate intracellular and extracellular fluids. Also, body water distribution may be different in severely obese individuals. Vegetable intake questions (DHQ II) did not specifically target vegetables that are high in bitter compounds such as glucosinolates, phenols, and isothiocyanates. Moreover, cooking methods were not evaluated such as boiling, steaming, blanching, or roasting. Variation in cooking temperatures can profoundly impact bitter compounds such as phenols in vegetables and fruits.

Applications in Public Health

The interaction between nutrition and genetics involves both nutrigenetics (how genetic variations modify an individual's response to food intake) and nutrigenomics (how nutrients effect gene expression). Nutrigenetics is defined as the science of the impact of genes such as SNPs on our potential health, which is strongly influenced by dietary intake, nutrition status, stress, and toxins whereas nutrigenomics is focused on the effect of diet and lifestyle factors on gene expression. It is important to note the difference between the terms nutrigenetics and nutrigenomics because although these terms are related, they are not interchangeable.

Obesity is an epidemic disease with the potential for improved prevention using nutrigenetics and nutrigenomics knowledge to develop a personalized calorie-controlled

diet such as dietary advice to individuals with a specific genotype to optimize nutrient intake during weight management. One example of a SNP affecting nutrient requirements is the TAS2R38 gene polymorphism which may impact vegetable and fruit consumption. Individuals who are supertasters are very sensitive to bitter taste and may have a diet lacking in vegetables rich in antioxidant and phenolic compounds such as broccoli and brussels sprouts. Therefore, carriers of this polymorphism may need to be aware of this and consume adequate amounts of vegetables and fruits in their diet.

Applications of genetic knowledge in public health interventions are a critical issue. For instance, SNPs can modify requirements for nutrients in an individual, but it may not translate to all populations. Registered dietitians and nutritionists can use genetic knowledge in public health interventions to incorporate a client's genetic profile into nutrition assessment, which allows for more precise dietary advice, control, and reduction of disease prevalence as well as contribute to the prevention of chronic diseases. It is desired that nutrigenetics and nutrigenomics will assist in creating new nutritional policies programs for diverse populations with patients and healthy people.

Future Research

It is important that future research focus on taste-driven food preferences in children and elderly, which may be genetic in origin in children but uncoupled in adults due to cultural impacts or lifestyle. Additional studies on taste preferences in children are desirable, which may help establish healthy eating habits unique for every child. Ethnicities are also important to be considered in future studies. Ethnicity is a complex construct which can affect BMI and body fatness; for example, African American individuals have less fat when compared with Caucasian individuals. Overall, new research in taste receptor genes may assist in tailoring food intake and reducing the risk for obesity and other chronic diseases. Additionally, taste research may improve our understanding of the association between taste perception and eating behavior, and body composition.

CHAPTER V

CONCLUSION

Our results do not support previous research on the influence of SNPs in taste receptor gene (TAS2R38) on food intake and BF%, which indicate that non-genetic factors may be of greater importance than genetics in determining dietary intake (specifically vegetables and fruits) and body composition in this group of participants. The correlation between TAS2R38 gene and food intake (bitter compounds) may not be straightforward. TAS2R38 gene is only one of the several genetic determinants likely to be involved in the pathways determining taste perception. Tasting ability is affected by several other sensory factors. Other factors may contribute to food intake and preference and modify the ability to sense bitter taste.

Males usually give lower priority to health compared to other considerations such as taste in selecting their food choices as well as eating more frequently, higher fat foods, and drink more alcohol than females. Females may be more aware of diet and health in general.

Our findings are consistent with studies that observed differential food intake according to the presence of bitter taste perception (Ooi, Lee, Law, & Say, 2010). Other studies suggest that bitter taste perception is not related to food intake due to other factors such as attitudes, culture, and food environment (Smith et al., 2016). More research is

required to have a better understanding of how genetic taste variations and other factors impact vegetable and fruit consumption, and how this information could help to teach people about a healthy diet. A previous study found that females showed higher sensitivity towards bitter-tasting compounds compared to males, due to the high number of fungiform taste buds and density of the fungiform papillae (Duffy, Davidson, et al., 2004). Some individuals who are born sensitive to bitter taste compounds may become less sensitive with age because of cultural, lifestyle, physiological changes, or disease. Furthermore, previous studies compared taster status of young adults with elderly and how age can affect food preferences (Mojet, Heidema, & Christ-Hazelhof, 2003).

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