SUCROSE THRESHOLDS AND GENETIC POLYMORPHISMS OF SWEET AND BITTER TASTE RECEPTOR GENES IN CHILDREN

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DEDICATION

I dedicate my dissertation work to my family and my loving partner.

"The best kind of people, are the ones that come into your life, and make you see the sun where you once saw clouds. The people that believe in you so much, you start to believe in you yourself. The people that love you simply for being you. The once in a lifetime kind of people."-~Unknown

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ABSTRACT

SUCROSE THRESHOLDS AND GENETIC POLYMORPHISMS OF SWEET AND BITTER TASTE RECEPTOR GENES IN CHILDREN

Paule Valery Joseph

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Background: Many illnesses of modern society are due to poor food choices. Excess consumption of sugars has been associated with obesity and diabetes. Children, due to their basic biology, are more vulnerable than adults to overeat foods rich in sugars. Little research has focused on whether there are individual differences among children in their sensitivity to sweet taste and if so the biological correlates of such differences.

Aims: The goal of this study was to determine whether variations in children's sucrose detection thresholds relate to their age and sex, taste genotype, added sugar or caloric intake, temperament or food neophobia and adiposity.

Methods: Sucrose detection thresholds in children age 7-14 years were tested individually using a validated two-alternative, forced-choice, paired-comparison tracking method. Genetic variants of taste receptor genes were assayed: *TAS1R2, TAS1R3* and *GNAT3* (sweet taste receptor genes; one variant each) and the bitter receptor gene *TAS2R38* (three variants). Children (n=216) were measured for body weight and height. A subset of 96 children was measured for percent body fat, waist to height ratio and added sugar and kcal intake.

Results: Mean sucrose threshold was 12.0 (*SD* 12.9), 0.23 to 153.8 mM. Girls were more sensitive than boys [t (214) = 2.0, p=0.047] and older children more sensitive than younger children [r (214) = -0.16, p = 0.016]. Variants in the bitter but not the sweet taste receptor genes were related to sucrose threshold and sugar intake; children with two bitter-sensitive alleles could detect sucrose at lower concentrations [F (2,165) = 4.55, p = 0.012; rs1726866]. Children with these variants also reported eating more added sugar (% kcals; [F (2, 62) = 3.64, p = 0.032]) than did children with less sensitive alleles. Sucrose detection thresholds predicted central adiposity [F (2, 59) = 6.1, p = 0.016), but not percent body fat [F (2, 58) = 1.4, p = 0.238]) when adjusted for added sugar intake, temperament, age, sex and negative reaction to foods.

Conclusions: Differences in sweet taste sensitivity may affect childhood dietary sugar intake with long-term health consequences, including obesity. There may be a more complex interplay between the bitter and sweet taste systems during development than previously appreciated. Understanding taste related parameters as well as other dimensions that may affect food consumption might help in developing weight management to minimize childhood obesity risk.

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LIST OF ABBREVIATIONS

A or Ala	Alanine
ANOVA	Analysis of variance
BIA	Bioelectrical impedance analysis
BMI	Body mass index
C	Cytosine
CDC	Centers for Disease Control and Prevention
dbSNP	Database of Short Genetic Variation
DNA	Deoxyribonucleic acid
DXA	Dual-energy X-ray absorptiometry
GNAT3	Guanine nucleotide-binding protein G (t) subunit alpha-3
GPCR	G protein coupled receptor
HWE	Hardy-Weinberg equilibrium
I	Isoleucine
NHANES	National Health and Nutrition Examination Survey
P	Proline
PROP	6-*n*-Propylthiouracil
PTC	Phenylthiocarbamide
PCR	Polymerase chain reaction
SD	Standard deviation
SNP	Single nucleotide polymorphism
T	Thymine

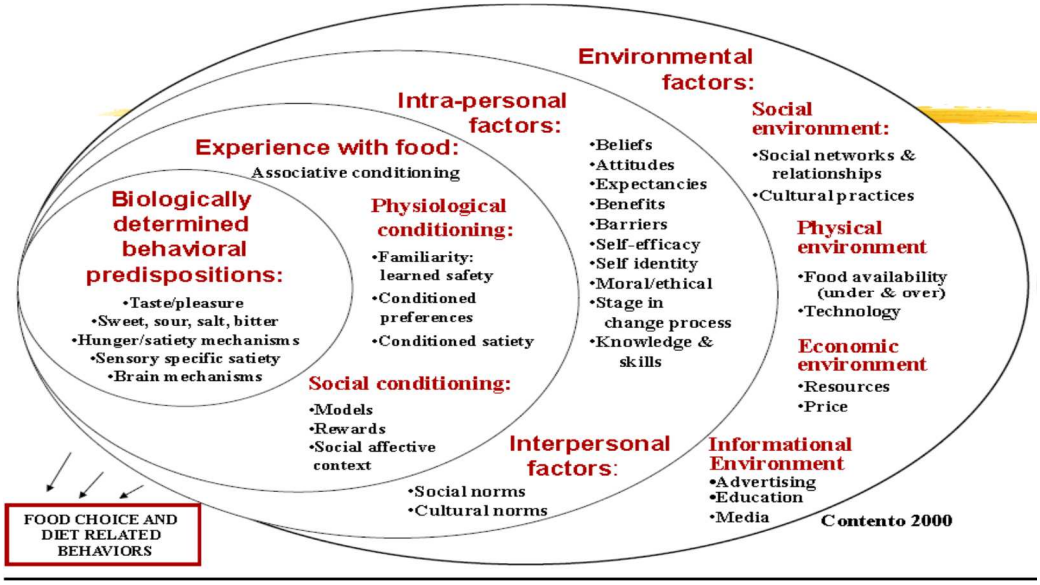
TAS1R2	Taste receptor type 1 member 2 gene
TAS1R3	Taste receptor type 1 member 3 gene
TAS2R38	Taste receptor type 2 member 38 gene
V	Valine
WHO	World Health Organization

CHAPTER 1

Introduction

The sense of taste plays a major role in food consumption. There are biological, inter/intra personal, and social-environmental factors that influence food intake, as shown in the Contento's model in **Figure 1** (Contento, 2007, 2008). Taste is a biologically determined behavioral predisposition that is linked to brain reward and sensory systems. The sense of taste is crucial in assessing a food's nutritional value and is important in the development of food preferences and appetite. Taste information is sent to the feeding and reward system of the brain (Katz & Sadacca, 2011). The food-reward system plays a significant part in regulating eating behavior. Understanding why an individual eats what he/she eats and the driving factors behind food choices is important to addressing the epidemic of obesity since food consumption has a noteworthy role in the development of this condition (Grimm & Steinle, 2011).

Figure 1. *Contento's Model of Influences on Food Choices*



People of all ages eat sugars in excessive amounts, often because these foods have a potent hedonic appeal, particularly for children (Mennella, Finkbeiner, Lipchock, Hwang, & Reed, 2014). In the childhood obesity literature, there is evidence that this over consumption of added sugars may contribute to obesity (Fiorito, Ventura, Mitchell, Smiciklas-Wright, & Birch, 2006; Lim et al., 2009; Linardakis, Sarri, Pateraki, Sbokos, & Kafatos, 2008). The increased BMI associated with the consumption of added sugars is long lasting, and that, when present in an early childhood, increased BMI will persist into adolescence (Fiorito, Marini, Francis, Smiciklas-Wright, & Birch, 2009). A prospective study conducted with African American children showed that increased of drinks with added sugar at baseline predicted increased weight gain when followed up 2 years later.

Through basic psychophysical research, we have learned that children live in different sensory worlds than adults when it comes to sweet taste (Mennella, Finkbeiner,

& Reed, 2012). Children's basic biology makes them more vulnerable than adults to overeating foods that are rich in sugars (Mennella, 2008, 2014; Mennella et al., 2012). Not only are children born into this world being able to detect and prefer sweet tastes, the predominant taste quality of mother's milk (Desor, Maller, & Turner, 1977), but also this heightened preference for sweet tastes persists throughout childhood and adolescence (Desor, Greene, & Maller, 1975; Mennella, Lukasewycz, Griffith, & Beauchamp, 2011; Pepino & Mennella, 2006).

This preference may have evolved to solve a basic nutritional problem of attracting children to mothers' milk and then fruits, sources of high energy and nutrient content, during periods of maximal growth (Coldwell, Oswald, & Reed, 2009; Drewnowski, 2000; Mennella, Finkbeiner, et al., 2014). However, we now live in an environment where sugars are abundant. Despite recommendations from organizations worldwide suggesting that we limit the intake of free sugars to less than 10% of total energy (U.S. Department of Agriculture, 2010; Welsh, Davis, & Shaw, 1993), recent estimates suggest that the levels of consumption far exceed recommended levels: US children and adolescents are consuming around 16% of their total caloric intake from added sugars (Ervin, Kit, Carroll, & Ogden, 2012). This overconsumption of sugars may lead to pediatric obesity.

Many illnesses of modern society are due in part to poor food choices (Mennella, Finkbeiner, et al., 2014). Excess consumption of sugars and simple carbohydrates has been implicated in metabolic diseases like obesity and diabetes (Ambrosini et al., 2013;

Battelino & Shalitin, 2014; Gross, Li, Ford, & Liu, 2004; Wang, 2014). Metabolic syndrome is more prevalent in those with central adiposity, and this fat pattern may be exacerbated by a diet high in added sugar (Koh, 2010; Parikh & Mohan, 2012; Wang, 2014). The obesity epidemic is plaguing the youngest members of our societies, affecting more than 42 million children globally (World Health Organization, 2015a). Overweight and obesity in children are important public health problems in the United States. One third of US children between the ages of 2-19 years are overweight and 16.9% are obese (Ogden, Carroll, Kit, & Flegal, 2014). Obese children develop many of the obesity-related complications such as diabetes and metabolic syndrome that adults do. Even if they don't develop these complications as children, they are at greater risk as adults (Centers for Disease Control and Prevention [CDC], 2014; Ogden et al., 2014).

As this daunting truth emerges, recognizing that obesity and other weight-related conditions are largely preventable is important. The identification of risk factors is one key to prevention (Dietz, 2004; Dietz & Gortmaker, 2001). Eating behavior is among those risks. It has been well established in the literature that the amount of calories consumed by an individual impacts their weight (Birch & Fisher, 1998; Crowell et al., 2015; Lee et al., 2011). Whether food habits of children are determined by genetic or environmental factors, there is no doubt that children have a higher affinity for sweet foods (Pepino & Mennella, 2006). During the past few decades, age-appropriate psychophysical methods have been developed to determine the level of sweet taste most preferred by individuals of varying ages (Mennella et al., 2011).

The sensation of sweet taste starts on the tongue and engages several signaling

proteins that are coded by specific genes in the human genome. Sucrose stimulates a receptor on taste cells; the resulting signal is conducted via G proteins and eventually produces a signal interpreted centrally as sweet taste (i.e., taste transduction). The sweet taste receptor has two parts; the gene *TAS1R2* encoding the first part was discovered in 1999, and the second gene, *TAS1R3*, was discovered in 2001 (for a review, see Reed & McDaniel, 2006). The respective proteins from these genes are T1R2 and T1R3. Among the G proteins, the one associated with sweet signaling is gustducin (G_{α} protein subunit), encoded by *GNAT3* (McLaughlin, McKinnon, & Margolskee, 1992). The bitter taste receptor has also been linked with sweet taste. Previous work has shown that variation in the bitter taste receptor gene has been associated with individual differences in sweet taste preference (Mennella et al., 2012; Mennella, Pepino, & Reed, 2005) and children's selection of sweet tasting foods (Keller et al., 2014).

When compared to adults, children prefer a more concentrated sweet tasting solution than adults (Liem & Mennella, 2002; Mennella, Finkbeiner, et al., 2014; Mennella et al., 2012; Mennella et al., 2011), with the switch-over to adult like patterns of preferences occurring during mid adolescence (Desor & Beauchamp, 1987; Mennella et al., 2011). The level of sucrose most preferred was related to measures of growth; children who were taller for their age preferred sweeter solutions than did those that were shorter (Mennella, Finkbeiner, et al., 2014). Most research has focused on sucrose preference, and to date there is a paucity of research on children's taste sensitivity, which is the ability to perceive sweetness at low sugar concentrations.

In adult populations, variation in the *TAS1R2*, *TAS1R3* and *GNAT3* genes relates

to differences in the ability to perceive sweet tasting stimuli. For *TAS1R2*, adults with one or two copies of the V alleles had a lower habitual sugar intake (Eny, Wolever, Corey, & El-Sohemy, 2010). *TAS1R2* showed no significant effect with sucrose taste sensitivity (Fushan, Simons, Slack, Manichaikul, & Drayna, 2009).

For *GNAT3*, adults with two C alleles (CC) were better able to sort low concentrations of sucrose into the correct order of concentrations than those with two T alleles (TT; rs7792845) (Fushan, Simons, Slack, & Drayna, 2010).

For *TAS1R3*, adults with one or two copies of the T nucleotide (TT) were less sensitive to the taste of sucrose than were those with two copies of the alternative C allele (CC; rs35744183) (Fushan et al., 2009). The *TAS1R3* genotype is also related to with differences in sweet preference. Adults with the TT genotype of the *TAS1R3* gene also preferred higher levels of sweetness than those with the CC genotype (Mennella, Finkbeiner, et al., 2014; Mennella et al., 2012; Mennella, Reed, Mathew, Roberts, & Mansfield, 2014), possibly because they need more sucrose to obtain the same hedonic effect.

To our knowledge, whether genotype-related differences in sweet taste sensitivity exist among children has not been investigated. Although some studies have examined these genes and their variants in children, these were studies of preference and not thresholds (Mennella et al., 2012; Mennella, Reed, Mathew, et al., 2014) and none found a relationship between genetic variation and sweet taste preferences among children. We do know, however, that variation in the *TAS1R3* gene does not relate to differences in levels of sucrose preference in children, as it does in adults (Mennella, Finkbeiner, et al.,

2014; Mennella et al., 2012; Mennella, Reed, Mathew, et al., 2014).

Variation in the bitter receptor gene *TAS2R38* may also explain individual differences in sweet preferences among children. *TAS2R38* contains three variant locations, best known for their association with the bitter perception of thioureas, such as propylthiouracil (Bufe et al., 2005; Kim et al., 2003). Children with the bitter-sensitive genotypes (AP, PP; rs713598, A49P) prefer significantly higher levels of sucrose than those with the bitter-insensitive genotype (AA) both in laboratory-based measures and in reported preferences of real-world foods like cereal and beverages (Mennella et al., 2012; Mennella et al., 2005). Other investigators also report that children who are bitter sensitive consume diets higher in sugar than do bitter-insensitive children (Keller & Tepper, 2004).

Purpose and Significance

Significant progress has been made in understanding the interactive role of genes and environment in the development of obesity across the lifespan. For reviews see the following references (Huang & Hu, 2015; Qi & Cho, 2008; Speakman, 2004; Thomas, 2010). In behavioral genetics research, taste science has focused on how variation in taste receptor genes accounts for individual differences in a variety of psychophysical measures in adults, such as taste detection thresholds, taste preference, and diet related food behaviors. Most of what is known to date regarding sweet taste in children and adolescents relates to preference. Measuring detection thresholds, the lowest concentration of a substance (i.e. sugar) that can be reliably detected (Bartoshuk, 1991; Bartoshuk, 1978), add a new dimension to our knowledge of children. If an individual's

detection threshold is high (a higher concentration is needed to detect a substance), it means that they have less sensitivity to the given stimulus. Equally, if the detection threshold is low, they are more sensitive; it means that they require a lesser concentration to detect the stimulus. With the increased prevalence of childhood obesity, a basic understanding of determinants of taste threshold differences could give us insights to potential preventative measures. But little is known about the role of genetics and sucrose detection thresholds in children. While several investigators have examined taste thresholds in children, this study was among the first to examine sucrose detection thresholds in the context of the unique approach of assessing genetic variants known to show sweet sensitivity in adults (Eny et al., 2010; Fushan et al., 2010; Fushan et al., 2009). An understanding of the factors associated with individual variability in sweet thresholds may provide insight into why some children over consume sweet foods or are overweight/or obese.

In addition, there are many factors that may contribute to difference in food consumption (called diet-related food behaviors in this study). Of these factors, the role of temperament has been associated with eating behaviors (Haycraft, Farrow, Meyer, Powell, & Blissett, 2011) as well as obesity in infancy (Carey, 1985; Darlington & Wright, 2006; Faith & Hittner, 2010), childhood (Agras, Hammer, McNicholas, & Kraemer, 2004; Carey, Hegvik, & McDevitt, 1988), and in adulthood (Fassino et al., 2002; Pulkki-Raback, Elovainio, Kivimaki, Raitakari, & Keltikangas-Jarvinen, 2005). In addition, temperament has been associated with sweet taste preference in children (Liem & Mennella, 2002) but not sucrose detection thresholds. Some dimensions of child

temperament particularly relate to eating behaviors (Forestell & Mennella, 2012; Haycraft et al., 2011; Pliner & Loewen, 1997). Furthermore, prior work revealed a significant effect of taste genotype and race/ethnicity on mothers' perceptions of the child's temperament, activity in particular (Mennella et al., 2005).

With these points in mind, we examined the degree of variation in children's sucrose detection thresholds and whether sweet and bitter taste receptor-related genotypes might partially account for variation in taste thresholds. Genotypes that were related to sucrose threshold and sweet food consumption were examined for the propensity of children to consume part of their calories as added sugars. In addition, estimates of dietary intake of added sugars (g) and daily caloric intake (kcal/day) were available for a subset of the children. We also hypothesized that if sweet taste sensitivity, diet and obesity share a common etiology, then sweet sensitivity could potentially provide insights into obesity risk. To that end, we examined how sensitivity to sweet taste varies with adiposity measures as assessed by BMIz (a ratio of weight to height compared with national norms by age and sex); percent body fat (an index of overall adiposity), and central obesity [waist-to-height ratio (WHtR)]. Considering that there are other factors that affect both obesity and taste, we considered child's personal characteristics as measured by the temperament and food neophobia scales. Those dimensions of the scale that were associated in the literature with eating behaviors were assessed (negative reaction to foods and food neophobia). Then all dimensions of temperament and food neophobia were considered when looking at multiple factors that may contribute to obesity. This work addressed a gap in the literature of chemosensory science. The study

described herein also served as groundwork for future studies that will further elucidate these relationships.

Specific Aims

The consumption of sweet foods by children likely contributes to obesity, but little attention has been paid to how children differ in their sense of sweet taste and how these differences might affect their health and behavior. A paired-comparison, forced-choice psychophysical method described herein was used to phenotype children for sweet taste sensitivity (Mennella et al., 2011). Relationships between sucrose detection thresholds and the sweet and bitter taste receptor genes have had limited examination in children.

This study was framed with the following three aims:

Aim 1a: We determined whether sucrose detection thresholds, personal characteristics (temperament, food neophobia), diet-related food behaviors (caloric or added sugar intake) or adiposity measures varied among children by demographic variables (age, sex).

H1a: We hypothesized that sucrose detection thresholds, personal characteristics (temperament, food neophobia); diet-related behaviors (caloric or sugar intake) and adiposity measures would vary in children by age and sex.

Aim 1b: We determined whether sucrose detection thresholds in children correlate with personal characteristics (negative reaction to foods and food neophobia), diet-related food behaviors as measured by intake (caloric or added sugar intake) and adiposity measures.

H1b. We hypothesized that sucrose detection thresholds would be associated with personal characteristics (negative reaction to foods and food neophobia), diet-related behaviors (caloric or added sugar intake) and adiposity measures.

Aim 2: We determined whether sweet and /or bitter taste receptor genes (*TAS1R2*, *TAS1R3*, *GNAT3*, and *TAS2R38)* predict differences in sucrose detection thresholds and diet-related behaviors (caloric or added sugar intake) in children.

H2: We hypothesized that allelic variation in *TAS1R2*, *TAS1R3*, *GNAT3*, and *TAS2R38* genes would partially account for differences in sucrose detection thresholds and diet-related food behaviors among children while adjusting for covariates of age, sex and adiposity.

Aim 3: We determined whether sucrose detection thresholds, sweet and bitter taste receptor genotype, personal characteristics (temperament, food neophobia) or sweet food diet related behaviors (added sugars as g/kg of body weight, % kcal as added sugars) were related and predicted measures of adiposity (waist to height ratio and percent body fat) in children.

H3: We hypothesized that adiposity would be predicted by sucrose detection thresholds, sweet or bitter taste receptor genotype, personal characteristics (temperament and food neophobia) or sweet food diet-related behaviors (added sugars g/kg of body weight, % kcal as added sugars).

Theoretical Framework and Conceptual Model

Considering the multiple factors that influence taste and health (i.e. adiposity), two theoretical frameworks were used to frame this study. First, Contento's model of

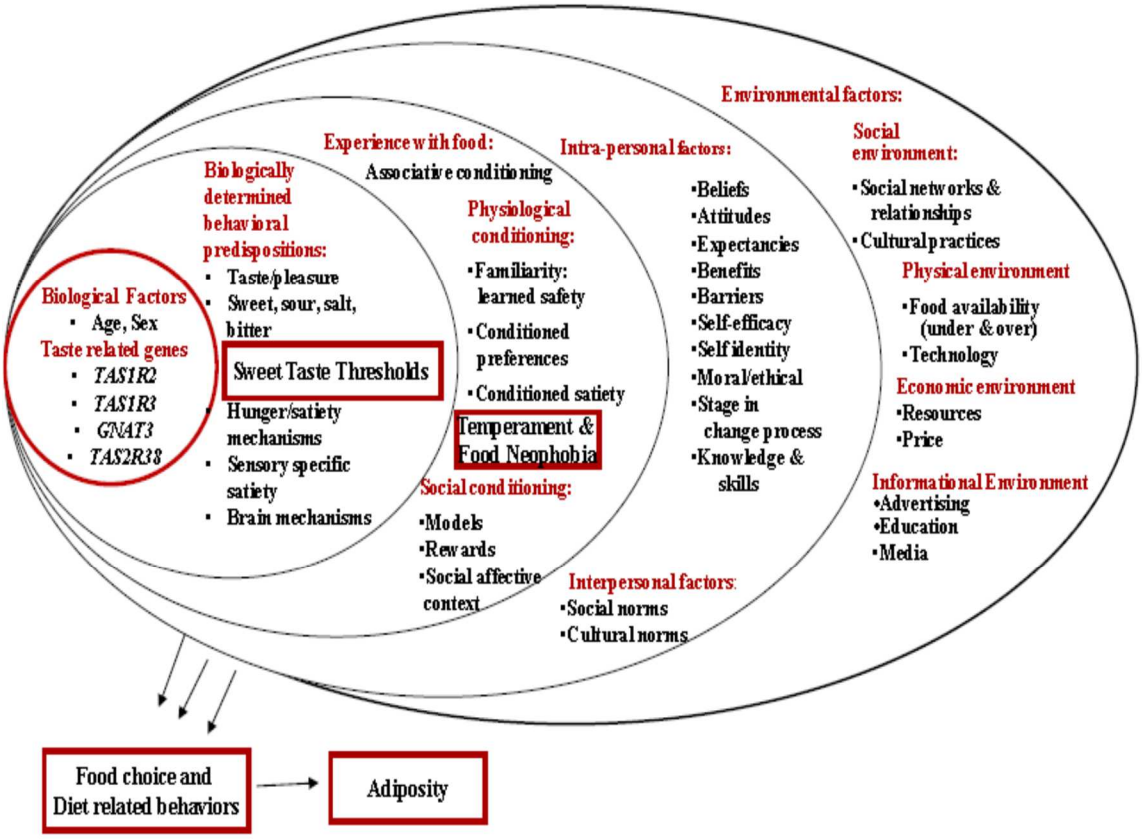
influences on food choices (**Figure 1**) was used as a lens to examine the intersectionality of biology, behavior, and environmental factors, as they play a role in taste and adiposity. Although Contento's model was first designed with a focus in explaining components or forces that may impact nutrition education programs, it fits nicely to study individual choices and behaviors about food. In Contento's model "biologically determined behavioral predispositions" refers to individuals' preference for sweet and dislike of bitter and sour tasting foods as well as a mechanism for the interplay of the food-reward system with sensory specific satiety. The second component "experience with food" focuses on the learned experience with food whether it is by physiological or social associations. The third part "personal factors" highlights both intra and interpersonal factors that influence food choice such as knowledge, attitudes, and beliefs, as well as families and social networks. Lastly, "environmental factors" states that food access and availability of foods also plays a role in food choices (Contento, 2007). Here the terms "food choice" and "diet-related behaviors" are referred to as outcomes. A key acknowledgement of this model is that all these influences interact dynamically with each other and they are not happening independent of each other.

Second, the behavioral genetics framework was used to examine genetic underpinnings of behavioral phenotypes (i.e. taste thresholds). The gene-environment interaction is defined as the diverse influence of a genotype on risk for disease for individuals with different environmental experiences (Ottman, 1996). This model served as a lens to understand the gene-environment interaction that can affect both taste and adiposity, since this framework proposes that the interaction between genes and the

environment affects the development of behavior (Fuller & Thompson, 1960; Maxson, 2002). In this model, the environment is defined as an exposure, which can be physical, chemical, biological, a behavioral pattern, or a life event among others. In this model, genetics is defined as genes and their genotypes. Phenotypes are the observable traits of an organism (Wojczynski & Tiwari, 2008) resulting from the interactions of genotypes and the environment. Phenotypes can be somatophenes or psychophenes, which are behavioral phenotypes (Fuller, 1979; Fuller & Willmer, 1973), the latter characterizing behavioral genetics. Somatophenes can further be classified as chemophenes (i.e., hematocrit, platelets) or morphenes (i.e. body size). However, one of the limitations of this model is that it doesn't take into account an individual's personal characteristics; in addition, the phenotype is limited to behavioral ones only.

To create a comprehensive model, the underpinnings of the two theories were merged (shown in red **Figure 2**), an additional sphere was added to Contento's model named biological factors highlighting taste related genes that were measured in this study, as well as age and sex, since these measures are related to "biologically determined behavioral predispositions". Under the section of biologically determined behavioral predispositions, sucrose detection threshold was added, since it might be associated with diet related food behaviors. Other behavioral phenotypes measured in this study-included: temperament, food neophobia, adiposity and dietary related behaviors. In this revised model, diet related behaviors were operationalized as dietary intake. To further complete the framework, the adiposity was added to the diagram as a body of research indicates that diet-related food behaviors are associated with adiposity.

Figure 2. *Integrated Theoretical Frameworks*

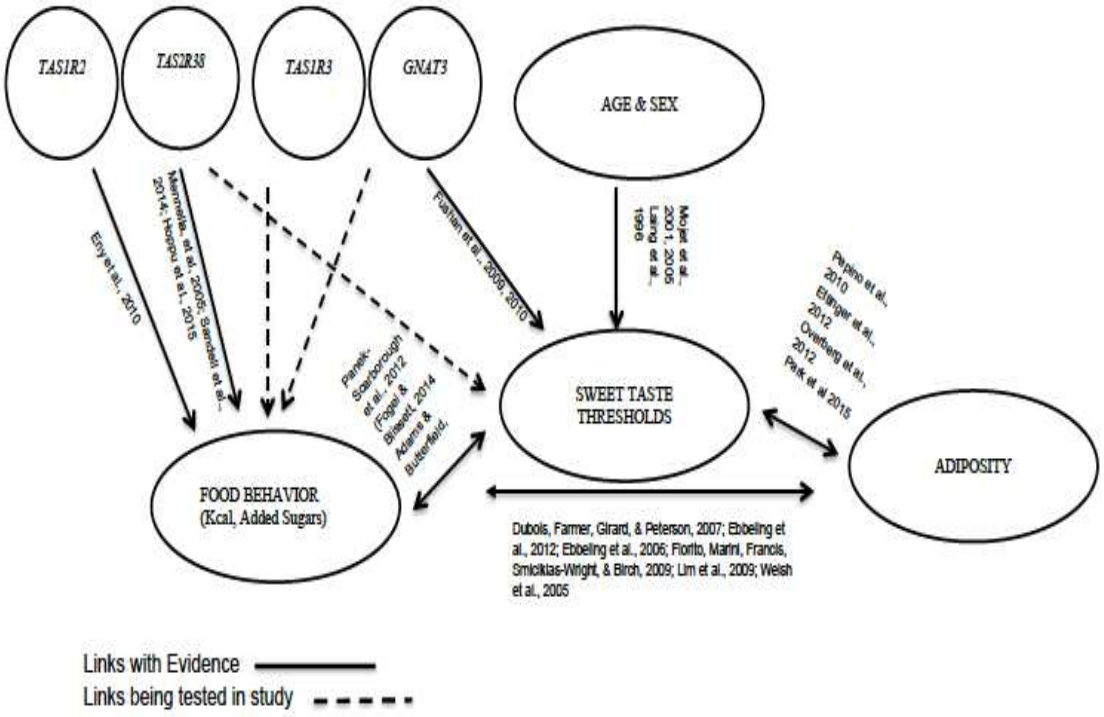


To further address the aims of this study, a conceptual map was developed with the variables used in this study to further hypothesize whether or not children's genetic differences affect sucrose detection thresholds and to assess the effects of age, sex, diet, temperament and adiposity (**see Figure 3**). This was used as lens to understand how genes and environments work together to influence behaviors, and what specific genes might be responsible for the behavior studied herein. We understand that genetics alone is not the sole contributor to taste perception and adiposity; therefore other important

determinants such as age, gender and personality characteristics are included in this framework (Eysenck, 1990; Freedman, Khan, Serdula, Ogden, & Dietz, 2006; Freedman et al., 2007; Keskitalo, Tuorila, et al., 2007). The new model assumes that adiposity and taste thresholds arise from a complex interplay between genetics, temperament, food neophobia and diet related food behaviors. The first intention of this conceptual model was to develop testable hypotheses that informed this study. Secondly, this conceptual model illustrates the hypothesized links (shown in dotted arrows) and guided the analysis of the variables chosen for this study (see Specific Aims section).

Some of the hypothesized links are based on what have been published in the literature to date; both in adults and children and some are being tested in this study.

Figure 3. *Conceptual Model: with Hypothesized Links*



Concepts and Definitions

The purpose of the following section is to briefly define a few concepts that are used throughout this document. It is divided between taste related definitions, genetics concepts, obesity and diet related terms (**Table 1**).

Table 1. *Concepts and Definitions*

Taste-Related Terms	
Basic Tastes	Refers to the five taste dimensions: sweet (i.e. sugars), sour (i.e. acids), salty (i.e. sodium chloride), bitter (i.e. alkaloids) and umami (i.e. monosodium glutamate)(Guyton, Hall, & Hall, 2006).
Flavor	Refers to the combination of taste and smell or the combination of taste, smell, and chemical irritation. Flavor describes the sensation of the food or substance being ingested (A. K. Bartoshuk, 1991; L. Bartoshuk, 1991; Dominguez, 2011; Weiffenbach & Bartoshuk, 1992). Flavor is a complex phenomenon brought forth by a multimodal sensory response that includes taste, olfaction, and perception (e.g., the burn of capsaicin or the tickle of carbonation) (Beauchamp & Mennella, 2011; Small, 2012).
Hedonic Response to Foods	Refers to the degree of pleasure an individual experience from a food. Foods or substances can be qualified as pleasant, appetizing (e.g., positive hedonic qualities: sweeter, creamier) or unpleasant and aversive (Lowe & Butryn, 2007).
Palatability	Refers to how an individual perceives a particular substance to be likeable. Palatability takes into account factors such as taste, an individual's physiological state, and learning history (Yeomans, Blundell, & Leshem, 2004).
Psychophysics	It is a quantitative science that studies the associations among physical stimuli and perceptions (Anderson, 1990).
Taste	Defined as the sense that individuals are able to recognize when a substance contacts the taste buds and subsequently triggers nerve responses to the taste centers in the brain. Taste is essentially the chemical reaction that allows us to detect whether the food being consumed contains bitter, salty, umami, sour or sweet compounds (Barlow & Klein, 2015; Bartoshuk, 1991).
Taste Buds	They are well-defined structures composed of taste receptor cells and supporting cells. It is considered the smallest functional element of the gustatory system (Barlow & Klein, 2015; Fabian, Beck, Fejerdy, Hermann, & Fabian, 2015; Jung, Akita, & Kim, 2004).
Taste Intensity	Refers to the strength of the perceived taste of a substance. It can be measured as no flavor or extremely strong flavor (Stevens, 1969).
Taste Thresholds	Taste thresholds refer to the minimum amount of a stimulant that elicits a response to our sense of taste; therefore, concentrations that are below the detection thresholds of an individual are not perceived. It is the lowest concentration of a substance (e.g., sugar) that can be reliability detected. People differ on how sensitive they are to certain compounds. For example: we can have an individual taste two substances (water and sucrose at a certain concentration) and determine at what concentration they are able to detect the substance (Bartoshuk, 1991; Bartoshuk, 1978).
Taste Preference	A test used to compare two substances and subjectively evaluate if there is a difference in the liking of the compound being tested. For example: we can provide two cups of sucrose with different concentrations and ask which one they prefer (Drewnowski, 1997).
Taste Receptor Cells	A sensory receptor cells that transmits information from a substance into a nerve signal and carries gustatory information to the brain (Li et al., 2002).
Genetics-Related Terms	
Alleles	They are polymorphisms of the same gene (Hart & Jones, 2005).
Diplotype	A pair of haplotypes from homologous chromosomes (Zuo, Wang, & Luo, 2014).
Gene	A segment of DNA, known to be the molecular unit of heredity. It controls the

	production of specific proteins that conduct a function in the body (Pearson, 2006).
Haplotype	A combination of alleles at multiple loci occurring on the same chromosome. They are inherited together (Malats & Calafell, 2003; Zuo et al., 2014).
RefSNP (rs) Numbers or rs#	The symbols mean that the SNP has been officially registered and given a reference SNP identifier by dbSNP (National Center for Biotechnology Information, 2005).
Genotype	Refers to a collection of genes responsible for an individual's observable traits. It is also the two alleles inherited for a specific gene (Malats & Calafell, 2003).
Phenotype	The actual characterization of physical traits observed in an individual's, such as taste (Malats & Calafell, 2003).
Homozygous	An individual that has two identical alleles for the gene in question (i.e.: CC, cc)(Strachan & Read, 2010).
Heterozygous	An individual who has two different alleles for a gene (i.e.: Cc, Bb) (Strachan & Read, 2010).
Locus (Loci)	A gene location on a chromosome (Malats & Calafell, 2003).
G- Protein Coupled Receptors	A large family of protein-coupled receptors that sense molecules outside the cell and activate inside signal transduction pathways and, ultimately, cellular responses (Venkatakrishnan et al., 2013).
Hardy-Weinberg Equilibrium (HWE)	The theory that states that allele and genotype frequencies will continue to be constant from generation to generation in a given population (Crow, 1999; Malats & Calafell, 2003).
Single Nucleotide Polymorphisms (SNPs)	SNPs are variations of a single base pair in a distinct DNA structure, called a nucleotide (i.e.: a SNP replaces the nucleotide thymine (T) with a nucleotide cytosine (C) within a DNA segment (Strachan & Read, 2010).
Obesity and Diet-Related Terms	
Added Sugars	Refers to the additional sugars, syrups, and other caloric sweeteners added when foods are processed or prepared. Examples of added sugars include brown sugar, cane sugar, corn sugar, corn sweetener, corn syrup, dextrose, fructose (when not naturally occurring), fruit juice concentrates, glucose, high-fructose corn syrup, honey, invert sugar, lactose (when not in milk or dairy products), maltose (U.S. Department of Agriculture, 2010).
Adiposity	In this document, adiposity refers to any body weight-related measure by which body fatness is assessed, such as waist-to height ratio; percent body fat, and BMI.
Body Mass Index (BMI)	BMI is a measure of body weight relative to height. BMI; calculated as weight in kilograms divided by the square of height in meters (Janssen, Katzmarzyk, & Ross, 2002).
BMI z Score	Body mass index z-scores, also called BMI standard deviation (SD) scores, are measures of relative weight adjusted for child age and sex (Kuczmarski et al., 2002).
BMI Percentile	For children, BMI is reported as sex- and age- specific percentiles and as z-scores. BMI charts compare their height and weight to other children of their same sex and age. For children ages 2 to 19 years, those who are at or above the 85th percentile are considered overweight. Those who are at or above the 95th percentile are considered obese (Bartok, Marini, & Birch, 2011; Kahn, Imperatore, & Cheng, 2005).
Metabolic Syndrome	A group of risk factors that increases an individual risk for heart disease, diabetes, and stroke among others. Metabolic risk factors include a large waist size (abdominal obesity), high blood pressure, high blood sugar levels, high levels of

	triglycerides, and low levels of high-density lipoprotein (HDL) (Despres & Lemieux, 2006).

CHAPTER 2

The sense of taste is produced when a chemical stimulus or substance, comes into contact with taste cells in the oral cavity and triggers nerve impulses to special taste centers in the brain (Purves et al., 1997). Taste is essentially the chemical reaction that allows us to detect whether the food being consumed contains salty, sweet, sour, bitter, or umami compounds. It is the sense that alerts an individual to recognize and ingest nutrients while avoiding toxins (Chaudhari & Roper, 2010).

Taste plays a role in eating behaviors. Taste detection and differentiation of taste stimuli regulate how people distinguish food and develop dietary habits (Cruickshanks et al., 2009; De Jong, Mulder, De Graaf, & Van Staveren, 1999). This is possible by the development of taste perception, which allows people to distinguish between different flavors and help in determining their taste preferences and dislikes. These factors affect not only food selection but also the amount of food a person ingests (El-Sohemy et al., 2007; Garcia-Bailo, Toguri, Eny, & El-Sohemy, 2009). Individual variations in taste perception may therefore affect dietary status and diet- related diseases such as obesity (Cicerale, Riddell, & Keast, 2012). However, eating behaviors can also be affected by taste preferences; both are influenced by social, physiological, genetic, and psychological factors. These can vary among cultures, age groups, and sexes.

Early studies pinpointed taste as one of the prominent elements in food consumption and selection (Drewnowski, Kurth, Holden-Wiltse, & Saari, 1992; Glanz, Basil, Maibach, Goldberg, & Snyder, 1998; Nasser, 2001). Although studies of taste have been around for hundreds of years, new discoveries in this area of research hold much

promise for explaining a person's food choices and eating behaviors that may lead to the development of overweight and obesity.

In the United States, the prevalence of childhood obesity has tripled in the past 30 years. Specially, one- third of preschool-aged children are at risk for obesity ((CDC), 2012; Ogden et al., 2014). The long term and short-term effects of obesity on health and well being of children are concerning. Children are at risk of developing conditions such as the likelihood to develop diabetes, hypertension, high blood pressure, and the greater risk for bone and joint problems and sleep apnea ((CDC), 2014). In addition to the physical effects, they are also at high risk of experiencing social stigmatization and discrimination (Li, Ford, Zhao, & Mokdad, 2009). Understanding the factors driving their food choices is important in this health context.

American children age 2 and above ingest over 15% of total energy from added sugars (Welsh & Cunningham, 2011; Welsh, Sharma, Grellinger, & Vos, 2011). There is a growing concern that this overconsumption of added sugars has contributed to the obesity epidemic (Drewnowski & Rehm, 2014; Ervin et al., 2012; Ervin & Ogden, 2013).

The alarming consumption of added sugars has become an important issue. Efforts have been made by several organizations to set recommendations to limit added sugars in diet. The Institute of Medicine (2002) recommends that added sugars be less than 25% of total calories in the diet. Other organizations such as the American Heart Association have recommended that children 2- 8 years should not consume more than 3-4 teaspoons of added sugars a day (Johnson et al., 2009). The latest report by the World Health Organization recommended that added sugars be less than 10% of total energy

intake, and suggested that an additional decrease to below 5% or approximately 25 grams (6 teaspoons) per day might produce further health benefits (World Health Organization, 2015).

Children are born with a liking for sweet tastes. Sweet taste is a sign that a food has calories and nutrition. Foods that are sweet tend to be eaten in order to provide energy needed to promote growth and development. Preference for sweet tasting foods is related to environmental effects, evolutionary needs and genetics (Keskitalo, Knaapila, et al., 2007; Liem & Mennella, 2002; Reed & McDaniel, 2006). As much as children are drawn to sweet taste, they vary in their sensitivity to sweet taste. This variation may explain individual differences in their consumption of sweet tasting foods.

Genetic factors that regulate the gustation system and its function may possibly account for individual differences in sweet taste perception in children. Genetic diversity of sweet taste receptor genes (*TAS1R2, TAS1R3, GNAT3*) has been shown to play a role in sweet taste sensitivity in adults (Eny et al., 2010; Fushan et al., 2010; Fushan et al., 2009), but no study to date has assessed this influence on sweet taste sensitivity in children. However, studies have shown that sweet taste preference in adults is associated with *TAS1R3* genotype (Mennella, Reed, Mathew, et al., 2014). In addition, studies have found that genetic sensitivity to bitter taste may also influence preferences for sweets. Mennella et al. (2005) reported that children with the bitter-sensitive *TAS2R38* genotype had higher preferences for sweet tasting foods and beverages. Children with one or two bitter sensitive alleles (AP or PP) preferred higher concentrations of sucrose solutions

than those children who had two copies of the bitter insensitive allele (AA). The association between sweet taste sensitivity and food preferences and consumption is not clear. However, bitter taste sensitivity may explain the negative reaction of children and adults to bitter foods such as vegetables (Garcia-Bailo et al., 2009). In the sweet taste literature, some studies hypothesize that sweet taste sensitivity may explain sweet food consumption (Eny et al., 2010; Wasalathanthri, Hettiarachchi, & Prathapan, 2014).

Development of Taste

The development of the senses of taste happens parallel to the development of the nervous system in the embryonic stage (weeks 1-8 of gestation), at the beginning of the fetal stage, and matures at variable rates (Lawless, 1985; Northcutt, 2004). By the culmination of gestation, the taste system is activated by the compounds carried by the amniotic fluid (Nickalaus, Boggio, & Issanchou, 2005). Studies specifically examining the diversity of tastes and scents experienced by individuals have found sweet taste compounds to be transmitted through the amniotic fluid to the fetus (Mennella, Jagnow, & Beauchamp, 2001). The moment that the fetus starts to swallow, taste receptors are activated, around twelfth week of gestation (Mennella & Beauchamp, 1996). Early studies conducted in the area of taste development revealed that taste buds are found after 8 weeks of gestation, and by 13 weeks they are similar to those of adults (Forestell & Mennella, 2012; Witt & Reutter, 1996). There is evidence that sweet taste is the first sense to develop (Mela, 2001; Mennella & Beauchamp, 1996).

Foods that taste sweet are often highly preferred over those that taste bitter by children and adults. Human preference for sweetness and how this manifests during development has been of interest to many investigators (Berridge & Robinson, 2003; Drewnowski, Mennella, Johnson, & Bellisle, 2012; Mennella, Forestell, Morgan, & Beauchamp, 2009; Ventura & Mennella, 2011). These studies have reinforced the hypothesis that the sense of sweet is present before birth—sweet taste is distinguished by unborn infants. Newborns clearly sense and show pleasure in sweet tastes (Mennella et al., 2005). Despite these known innate preferences for sweet taste, the degree of preference for sweet foods varies across individuals. These differences in sweet preferences are influenced by age, gender, previous exposures, and hormonal fluctuations, as well as genetics (Faas, Melgert, & De Vos, 2010; Overberg, Hummel, Krude, & Wiegand, 2012; Reed & McDaniel, 2006).

Taste and Age

Fetus

A baby's first experiences with taste occur long before birth. Studies have examined taste programming *in utero*, focusing on maternal diet during pregnancy and whether it has an impact on infant food preferences. Foods consumed by the mother affect the content of the amniotic fluid—the unborn infant swallows this fluid and is thus exposed to different flavors (Mennella, Johnson, & Beauchamp, 1995). In fact, neonates may identify and prefer flavors they were exposed to before they were born (Beauchamp & Mennella, 2011; Forestell & Mennella, 2012; Mennella, 1995; Schaal, Marlier, &

Soussignan, 2000). Mennella (1996) showed that neonates born of mothers who consumed carrot juice during pregnancy preferred a carrot-flavored cereal during infancy compared to the control group whose mothers had not consumed carrot juice (Mennella et al., 2001).

Newborn

From the earliest studies on the investigation of taste in humans, researchers have observed that newborns can detect and have an affinity towards sweet taste (Beauchamp & Moran, 1982; Maller & Desor, 1973; Tatzer, Schubert, Timischl, & Simbruner, 1985). Facial gestures showed that the newborn could distinguish several taste qualities (bitter, salty, sour, sweet and umami). Responses to sweet and umami by neonates are usually thought to express satisfaction (Mennella et al., 2009). Desor, Maller, and (1973) have shown that from birth human babies prefer sugar solutions to water, which suggested that sweet taste preference might be manifested before any cultural and environmental factors are active. Steiner (1979) also showed in earlier studies that newborns preferred sugary solutions to water. More recent studies have concluded that newborn infants display preferences for high sugar concentrations and select solutions that are sweeter (Drewnowski et al., 2012; Pepino & Mennella, 2006). Newborns prefer sweet tastes from birth and will choose to drink from bottles of sweetened water but will reject substances that taste bitter or sour (Liem & Mennella, 2002).

Sweet taste has been found to also have analgesic effects in children. The combination of sweet taste and a pacifier has been found to have a calming effect on

newborns, and it has been used as an analgesic for infants (Mennella, Pepino, Lehmann-Castor, & Yourshaw, 2010; Pepino & Mennella, 2005). The calming effect of sweet tasting substances has also been observed in both preterm and full term newborns (Smith & Blass, 1996). Overall, the desirability for sweet and the aversion towards bitter and sour tastes come to be more marked during childhood but tend to decline in adult life. The early attraction to sweetness is reinforced by exposure to sweet stimulation (Nicklaus et al., 2005).

Infant

Age differences in sweet taste preference have been widely studied (Green, Jacobson, Haase, & Murphy, 2013; Mojet, Christ-Hazelhof, & Heidema, 2005; Mojet, Heidema, & Christ-Hazelhof, 2003; Overberg et al., 2012). Taste preferences continue to develop during a child's first year of life. Early studies indicated that this innate receptiveness to sweets has evolutionary origins and is inherent (Berridge & Robinson, 2003; Drewnowski et al., 2012; Mennella & Beauchamp, 1998). Early exposure to sweets leads to an increased preference for these foods and a preference for higher concentrations of sugar (Harris, 2008; Pepino & Mennella, 2008).

Although preference for sweet is present at birth, this preference it is known to decrease with age. Schwartz, Issanchou, and Nicklaus (2009) evaluated acceptance of the five tastes (salt, sour, sweet, bitter, and umami) in the same infants at 3, 6, and 12 months of age, based on facial expressions during consumption, and found that taste preferences did indeed change with age. They found that at each age sweet and salty tastes were the

most preferred. Beauchamp and Moran (1982) studied taste preferences for sucrose solutions and water in human infants at birth and at 6 months of age. They found that the dietary exposure of sweetened water maintained their preference for sucrose solutions at age 6 months, and the acceptance for sweet taste was slightly decreased at 6-12 months of age. Several hypotheses have been proposed to explain this developmental change in sweet preference. One explanation is that dietary experiences modify the degree of the preference for sweet taste as early as 6 months after birth. The introduction of solid foods, which may be less sweet than milk, might be driving the noted decrease in preference, which may be a continuous process through adulthood. A second explanation is that maternal diet may be a contributory factor, especially for babies who are breast-feed. Human breast milk often contains flavors from compounds that a breast-feeding woman takes in through her diet. Flavors from foods consumed by the mother such as vanilla are detectable in breast milk 1-2 hours after consumption (Mennella & Beauchamp, 1996). Therefore, the taste of breast milk may also have an effect on the later preferences of newborns (e.g., sweet). Babies whose mother's breast-feed may perhaps be more tolerant of a range of flavors once they begin consuming solid foods (Beauchamp & Mennella, 2011; Maier, Chabanet, Schaal, Leathwood, & Issanchou, 2007).

Childhood and Adolescence

Segovia et al. (2002) compared male children 8-10 years of age to adult males. The children had a higher density of anterior papillae than did the adults, a factor that

might make them more sensitive to sucrose or sweet flavors. A later study involving 8,900 Danish school children showed a perceptible change in taste perception as the children developed into teenagers. In this study, teenagers showed an increased capacity to distinguish flavors, as well as a decreased preference for sweet flavors, compared with younger children.

A study by Mennella et al. (2012) analyzed individual's differences in sucrose and fat preference related to age, genotype, and lifestyle. In this study, children and their mothers chose the concentration of sucrose mainly preferred in water using identical, two-alternative forced-choice procedures and ranked samples based on intensity of sweetness. In general, while children were found to prefer higher concentrations of sucrose in water and pudding than did their mothers, children preferred a lesser concentration of fat in pudding. Further studies revealed that the perception of sweet taste remains finely tuned throughout childhood and adolescence (Desor, Greene, & Maller, 1975; Mennella & Beauchamp, 2005; Pepino & Mennella, 2006) and declines with age (Desor & Beauchamp, 1987). The rationale for the changes in preference with age continues to be explored both in human and in animal models (Mennella, 2008).

Foods that taste sweet are often highly preferred over those that taste bitter. The sense of taste is essential to one's capacity to obtain needed nutrients. Despite this established preference for sweet taste (Berridge & Robinson, 2003; Keskitalo, Knaapila, et al., 2007; Mennella et al., 2009), the degree of preference for sweet foods varies across individuals. These differences in sweet preferences are influenced by age, gender,

previous exposures, and hormonal fluctuations, as well as genetics (Faas et al., 2010; Overberg et al., 2012; Reed, Tanaka, & McDaniel, 2006).

When it comes to taste detection thresholds, many investigators have reported a decrease in sucrose sensitivity with age (Bartoshuk, Rifkin, Marks, & Bars, 1986; Cooper, Bilash, & Zubek, 1959; Fikentscher, Roseburg, Spinar, & Bruchmuller, 1977; Hermel, Schonwetter, & Samueloff, 1970; Moore, Nielsen, & Mistretta, 1982; Richter & Campbell, 1940) and some included children in their taste sensitivity studies (Cooper et al., 1959; Fikentscher et al., 1977; Hermel et al., 1970; Richter & Campbell, 1940). Sensitivity for the basic taste qualities decreased more in men than in women with aging (Fikentscher et al., 1977). Investigators report that children have higher taste thresholds than adults for sweet. A study compared taste thresholds in children (8- to 9-year-olds) and adults. They found 8 to 9-year-old boys' mean threshold for taste detection was significantly higher than both adult men and women, suggesting that the detection thresholds of boys of this particular age may not be fully developed. But girls of the same age had similar thresholds to adults, and there were no gender differences between the adult participants (James, Laing, & Oram, 1997). Other studies have reported that taste detection can change not only with age, but also with hormonal status (Alberti-Fidanza, Fruttini, & Servili, 1998; Allesen-Holm, Frøst, & Bredie, 2009) and temperature (Talavera, Ninomiya, Winkel, Voets, & Nilius, 2007).

Taste and Race

Racial differences in sweet taste preference have also been noted in several studies (Beauchamp & Moran, 1984; Pepino & Mennella, 2005; Salbe, Delparigi, Pratley, Drewnowski, & Tataranni, 2004; Schiffman, Graham, Sattely-Miller, & Peterson-Dancy, 2000). African American children (Beauchamp & Moran, 1984) and adolescents (Desor et al., 1975) have been reported to have a higher preference for sucrose solutions, as well as increased preference for sugary fat solutions (Bacon, Miles, & Schiffman, 1994). Studies have suggested that sustained preference for palatable sweet tastes may possibly contribute to eating patterns that lead to obesity. In a study of the degree of habituation to sweet-tasting foods, African American children had a prominent and constant desire for sweet taste, a probable risk factor for the development of obesity (Schiffman et al., 2000). African-American children age 9-15 years favored more sweetness in sugar and sucrose in water solutions than Caucasian children (Desor et al., 1973). African American children preferred higher concentrations of sugar in liquids and solid foods and added more sugar to foods and drinks (Mennella, Pepino, and Reed 2005). A different study observed that African-American children within the same age group preferred higher concentrations of sucrose in liquid dairy products (Bacon et al., 1994).

Few studies have evaluated the effects of race and cultural on taste sensitivity. Salbe et al. (2004) reported that Pima Indians rated sucrose solutions tasting sweeter than whites. A study looking at taste perception in Taiwanese and European reported that Taiwanese individuals rated sucrose solutions as pleasant but sweetened cookies less appealing compared to those of European descent (Bertino, Beauchamp, & Jen, 1983).

Taste and Gender

Investigators have found that there are no major differences in sweet taste sensitivity between adult men and women (Dinehart, Hayes, Bartoshuk, Lanier, & Duffy, 2006). One study showed no significant difference in the ability for men and women to detect and differentiate sucrose from other taste qualities and water (Chang, Chung, Kim, Chung, & Kho, 2006). Girls were better at perceiving different tastes than boys, and better at distinguishing all concentrations of sour and sweet tastes (Allesen-Holm et al., 2009). Boys needed an approximately 20 percent more sweetness to recognize the taste. Changes in sucrose detection thresholds for women may be due to hormonal effects, with women having increase sweet taste sensitivity with during pre-ovulation (Than, Delay, & Maier, 1994).

Taste and Disease

Aging and diseases such as cancer, infections, trauma, medication, malnutrition and surgical procedures can influence taste. Chemosensory dysfunctions increase with age (Boyce & Shone, 2006; Murphy, 1985). An often-cited etiology for taste disorders is primarily a defect of olfaction resulting in alteration of taste quality and intensity. For example, upper respiratory infections, idiopathic causes, head injury or other conditions can affect the sense of taste (Hummel, Landis, & Hüttenbrink, 2011). Lesions to the taste buds or mucosa, demyelination of the nerves, or cranial nerve damage may impair gustation. For example, the chorda tympani nerve is known to function in eliciting taste response; therefore, damage to this nerve may reduce taste sensitivity, which

consequently may affect food choices (Bartoshuk, 1993).

Taste disorders can lead to altered perceptions and taste threshold. Taste perceptions become somewhat impaired with normal aging, with older individuals having higher thresholds than younger ones (Moore et al., 1982). Problems with taste can have a big impact not only in an individual's food selection but also their quality of life. Healthy People 2020 Goals have highlighted chemosensory health as an area for attention. A goal is to decrease the percentage of adults with chemosensory disorders who experience adverse impact on health status, work or quality of life (U.S. Department of Health and Human Services, 2013). In addition, the 2012 National Health and Nutrition Examination Survey added a chemosensory component to assess normal variation and prevalence of dysfunction of taste (CDC, 2012).

Taste disorders may affect the amount and type of food eaten, leading to under and over consumption of foods. This can be a problem for people with illnesses such as diabetes, high blood pressure or obesity (Mattes et al., 1990). Taste disorders are classified as ageusia which refers to the absence of taste; hypogeusia or decreased perception of taste; dysgeusia when the taste capacity is distorted; parageusia, which is the incomplete sense of taste in the presence of stimulus; and lastly phantogeusia which is the distortion of taste perception, without the manifestation of a stimulus (Schiffman, 1983a, 1983b).

Nutritional deficiencies are also found to affect taste perception. Individuals with anorexia, malabsorption disorders and kidney function alterations as well as those with low zinc, and copper serum levels have reported decreased taste perception (Lynch,

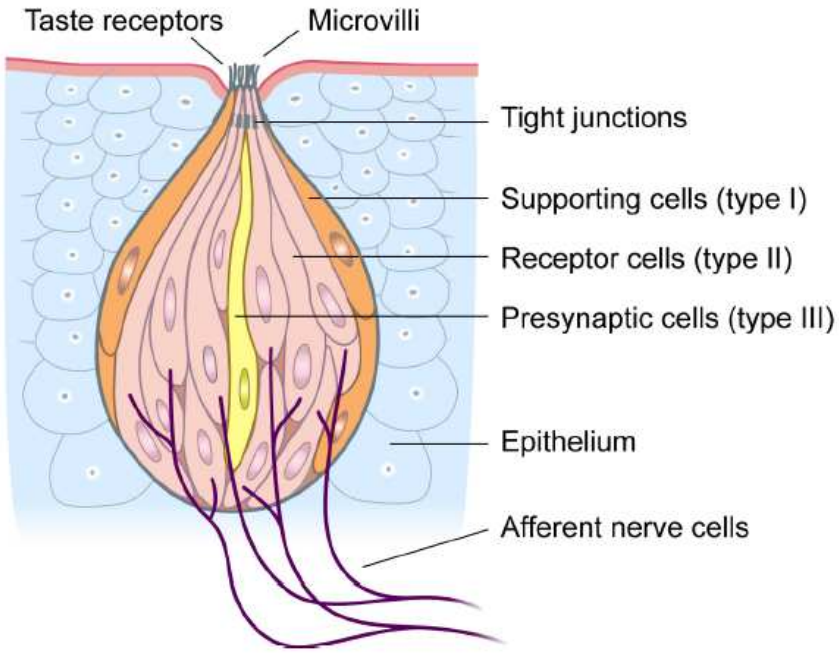
Lynch, Curhan, & Brunelli, 2013; Stewart-Knox et al., 2005). In children, a loss of sense of taste might cause a decrease in food intake, possibly resulting in eating disorders, which can affect physical growth and overall development (Moura, Cunha, Caldas, & Da Silva, 2015). A recent study looking at differences in taste perception in obese and non-obese children reported that obese had more difficulties identifying taste qualities than children and adolescents of normal weight (Overberg et al., 2012).

Biology of Taste

Anatomy of the Gustatory System

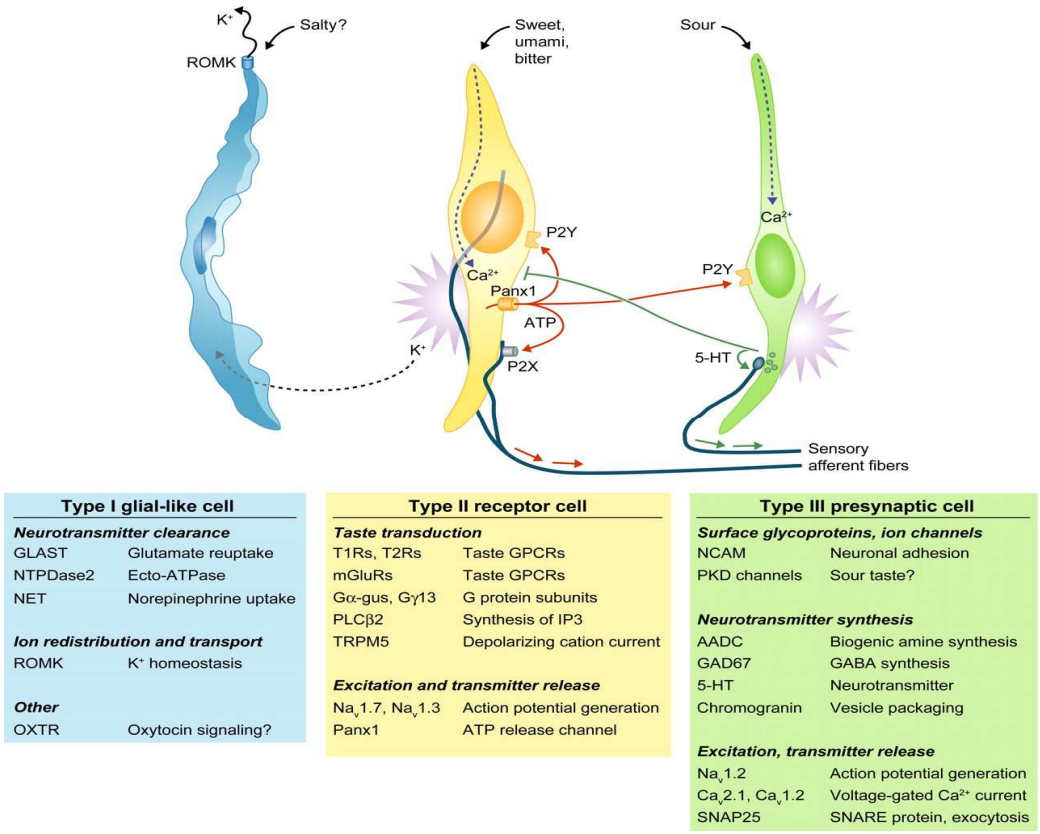
Our sensory system for taste is remarkably sensitive. Not only we can detect substances at extremely low concentrations, we are also able to differentiate between molecular compounds that are closely related. This amazing sensitivity is made possible by the taste buds, which are at the forefront of the taste system (**Figure 4**). These are onion-shaped structures on the tongue, throat, and pharynx as well as different places in the mouth. Humans have an average of 5,000-10,000 taste buds (Miller, 1995; Purves et al., 1997), which are found in aggregates of 50-100 taste receptor cells, composed of epithelial cells with some neuronal properties (Defazio et al., 2006; Finger et al., 2005). The taste buds are innervated by the chorda tympani and glossopharyngeal nerves carrying the taste messages to the brain, generating the sensation of taste (Bradbury, 2004; Chaudhari & Roper, 2010; Miller, 1995). Each taste bud has three cell types: type I (supportive), type II (receptor), and type III (presynaptic) cells (Herness & Gilbertson, 1999; Northcutt, 2004).

Figure 4. *Taste Buds*



Type I cells have a supportive function and they are the most abundant (Chaudhari & Roper, 2010; Finger et al., 2005). Type II cells are needed for transduction, they are known for being associated with the receptors for bitter, sweet, and umami taste (Adler et al., 2000; Romanov et al., 2007; Zhao et al., 2003). Type III cells are called presynaptic or synaptic cells (Huang, Maruyama, Stimac, & Roper, 2008; Roper, 2006, 2007). In **Figure 5**, Chaudari and Roper (2010) show a depiction of the cells and some of the proteins that are expressed in each cell type. From the activation of these cells, humans can distinguish five major tastes groups: salt, sour, sweet, bitter, and umami, each activated by specific receptors (Chaudhari & Roper, 2010).

Figure 5. *Type of Taste Cells*

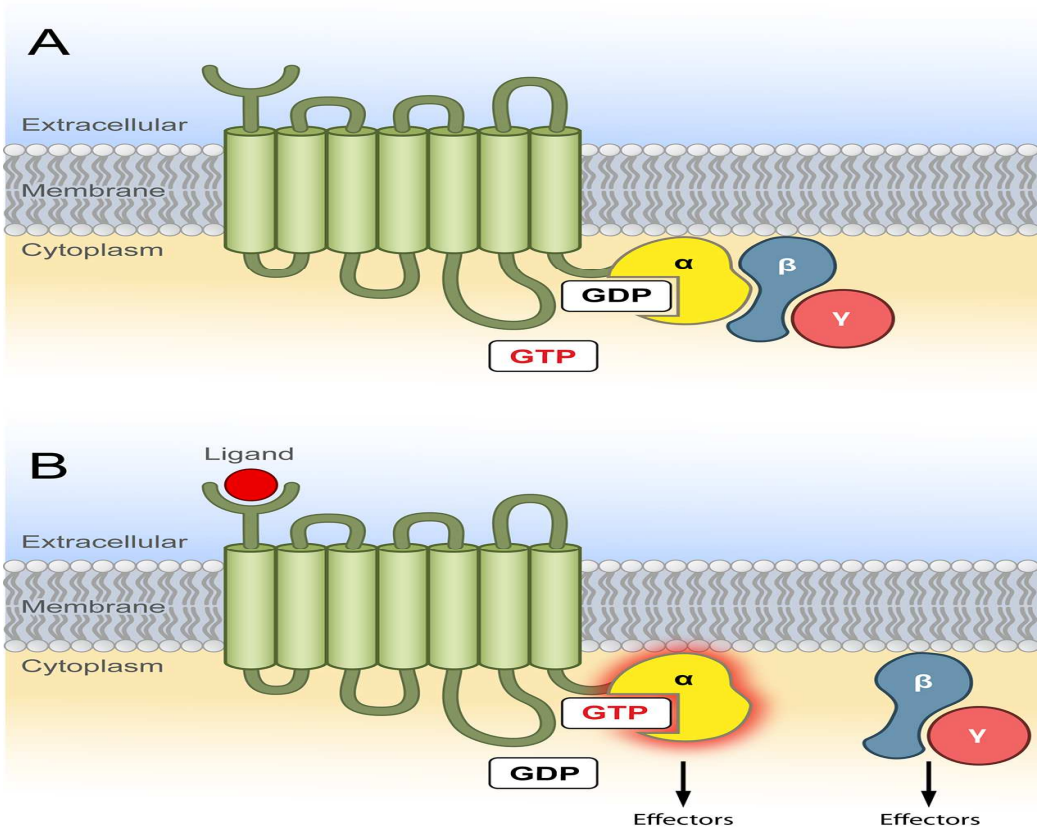


Genetics of Sweet and Bitter Taste

Taste receptor cells in the tongue are at the start of the sensory detection pathway of the gastrointestinal tract. These receptors provide vital information that affects both innate behaviors and aversive behaviors to foods (Kim, Breslin, Reed, & Drayna, 2004). Each taste modality is recognized by G-protein-coupled receptor (GPCR) dimers or through membrane channels (**Figure 6**). GPCRs (shown in green) have seven trans-membranes domains and are known to activate heterotrimeric G proteins, which are

composed of three subunits σ, β and γ, **Figure 6A** shows a GPCR not activated. Ligand binding (shown in red) activated the receptors and a conformational change occurs that facilitates the dissociation of the G proteins that interact downstream the effectors. The alpha subunit of the G protein attaches to either to GTP or GDP contingent on whether the protein is inactive (GDP) or active (GTP). Beginning with receptor activation by a ligand, the receptor exchanges the GDP for GTP bound to the alpha subunit while releasing the beta-gamma (βγ) subunit. These two subunits result in activation of effectors within the cell (Li et al., 2002) (**Figure 6B**), that in turn leads to several kinds of cellular and physiological responses. A detailed explanation of the transduction pathway for taste is explained in a later section.

Figure 6. *G-Protein Coupled Receptors (GPCRs)*



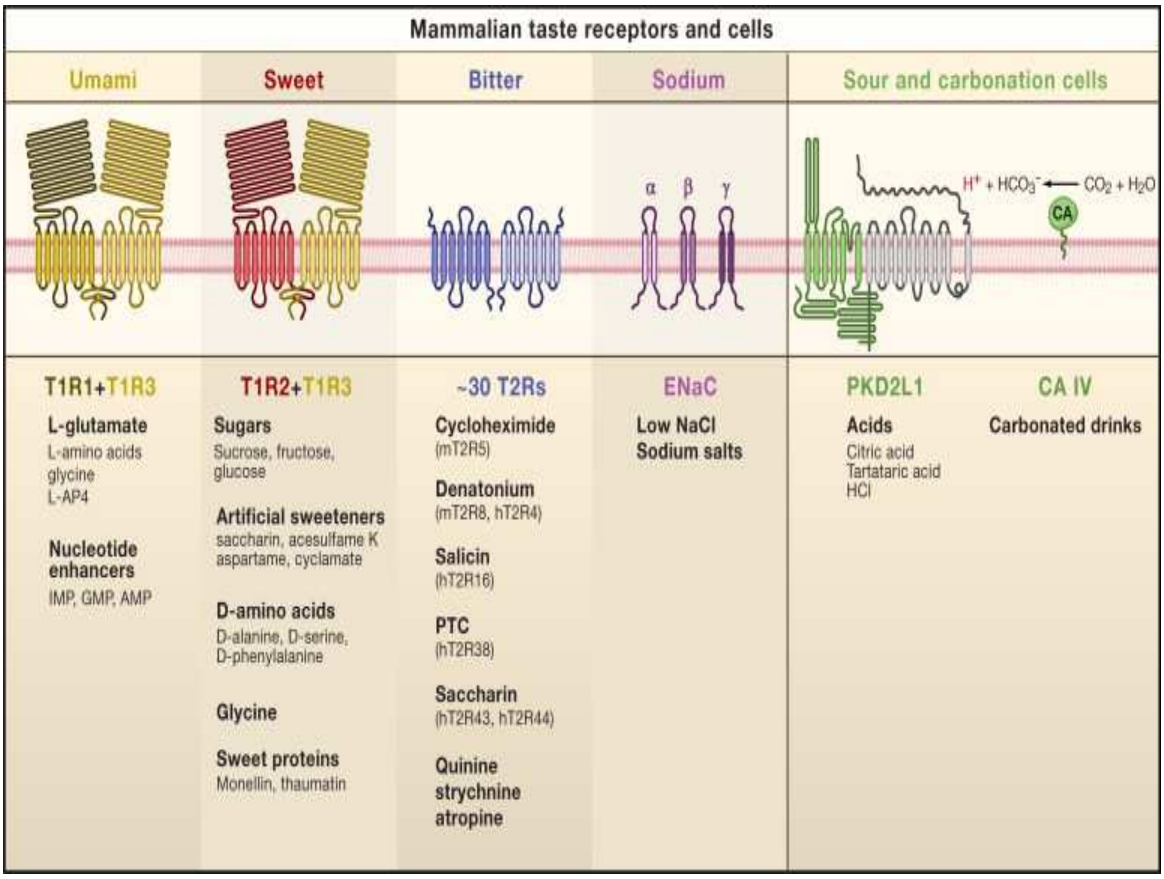
For taste, there are two GPCR groups. The first one consists of three members (T1R1, T1R2, and T1R3), which facilitate the perception of sweet and umami tastes. The second group is the TAS2R, which has around 30 different GPCRs, and the dimers create the receptors for different bitter-tasting compounds (Chandrashekar, Hoon, Ryba, & Zuker, 2006; Zhao et al., 2003).

Sweet taste is mediated by two types GPCRs that are part of the taste receptor family 1 (T1R2 and T1R3) (Chandrashekar et al., 2006; Zhao et al., 2003). These receptors are located within chromosome 1p36 (Liao & Schultz, 2003). The

heterodimeric combination of the two proteins (T1R2 and T1R3), are required for the sweet taste receptor to be functional, and begin the process to sense sweet taste (Nelson et al., 2002; Zhao et al., 2003). This heterodimer receptor helps recognize all types of sweet substances, including simple sugars, artificial sweeteners, amino acids and proteins (**Figure 8**) (Yarmolinsky, Zuker, & Ryba, 2009).

T2Rs are associated with bitter perception (Conneally, Dumont-Driscoll, Huntzinger, Nance, & Jackson, 1976; Reed et al., 1999), and are expressed in taste receptor cells that have gustducin, a G protein α subunit associated in bitter transduction (Ming, Ruiz-Avila, & Margolskee, 1998). T2Rs recognize a wide variety of compounds; some are very specific, while others are tuned to a particular class of compounds or respond broadly to many bitter tastants (**see Figure 7**).

Figure 7. *Mammalian Taste Receptors and Cells*



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Fuller (1974) identified the Sac locus linked with saccharin preference. The murine Sac locus is the main genetic factor that establishes the differences between sweet-preferring and sweet-indifferent strains of mice (Fuller, 1974; Lush, Hornigold, King, & Stoye, 1995). Several investigators reported that Sac determines both behavioral and electrophysiological responsiveness to saccharin, sucrose, and other sweeteners

(Bachmanov et al., 1997; Ninomiya, Tanimukai, Yoshida, & Funakoshi, 1991). Later studies identified the T1R G-protein-coupled receptor family in rats and humans (Hoon et al., 1999; Li et al., 2002). This receptor contains the two proteins responsible for creating a sweet taste receptor, T1R2 (taste receptor type 1, member 2) and T1R3 (taste receptor type 1, member 3) (Stone, Barrows, Finger, & Kinnamon, 2007; Zhao et al., 2003), which together form the T1R2/T1R3 heterodimer necessary in the perception of sweet taste (Nelson et al., 2001; Reed & McDaniel, 2006).

Genetic differences in the sweet receptor proteins partially determine two types of taste traits in adults, affecting sweet sensitivity (Fushan et al., 2010; Fushan et al., 2009) and preference (Mennella, Finkbeiner, et al., 2014). The *GNAT3* protein has been associated with sweet taste sensitivity in adult humans (Fushan et al., 2010). The *TAS1R2* gene, encoding the protein T1R2, is distinguished by high levels of genetic diversity, but as yet these variants have not been linked with individual differences in taste perception, although they have been linked to the intake of sweet foods (Eny et al., 2010). Humans differ in their sensitivity to certain tastes and these thresholds vary in humans, though the reason is not fully established. Miller and Reedy (1990) noted that some of the differences observed in human taste sensitivity may be affected by the numbers of taste buds a subject has, making them more or less sensitive to flavors; however, people differ not only in the number of taste receptors but also in the DNA sequences of particular receptors or their transduction molecules (described below) (Reed et al., 2006). Individuals who have heightened sensitivity to taste are called "supertasters" (Hayes, Bartoshuk, Kidd, & Duffy, 2008; Miller & Reedy, 1990), and those who have

minimal or complete lack of taste papillae due to a genetic disease or age-related decline (Guo & Reed, 2001) are categorized as "nontasters." Studies have shown that supertasters need smaller amounts of fat and sugar in their food to become satiated compared with nontasters (Bartoshuk, Duffy, Hayes, Moskowitz, & Snyder, 2006; Hayes et al., 2008). However, due to their keen sense of the taste of bitterness, supertasters have an increased intake of salt (Hayes et al., 2008), which masks bitterness; this may place them at a higher risk for hypertension but potentially a lesser risk for obesity. Though it can be hypothesized that this physiological difference may be responsible for some of the food choices a person makes and/or correlate with such conditions as obesity, hypertension, and diabetes, no study was found that examined this hypothesis.

The T1R3 protein (coded for by *TAS1R3* in humans and *Tas1r3* in rodents) is responsible for the perception of sucrose (Bachmanov et al., 2001; Max et al., 2001; Montmayeur, Liberles, Matsunami, & Buck, 2001; Nelson et al., 2001). Using an *in vitro* approach, T1R2 and T1R3 were found to be co-expressed in cells. When the receptor and sugar interact, it sets off a chain of events that result in action potentials conveyed to the brain (Li et al., 2002; Nelson et al., 2001). Later, Liao and Schultz (2003) confirmed that T1R1, T1R2, and T1R3 genes are expressed selectively in human taste receptor cells, consistent with their role in taste perception that was previously described by other investigators. T1R1 (taste receptor type 1 member 1) is a GPCR that in humans is encoded by the *TAS1R1* gene. T1R1 and T1R3 are found to create a heterodimer that detects umami taste (Nelson et al., 2001).

To further investigate the sweet taste receptor phenomenon, Bachmanov et al. (2001) used the quantitative trait locus technique in animals to generate high-sweetener-favoring B6 mice and low-sweetener-favoring 129 mice. Using a positional cloning approach, a small section of the F2-generation Sac locus was characterized as containing the G-protein coupled receptor T1R3 in mice. In order to confirm the function of the *Tas1r3* gene in sweet taste perception, congenic (differ in only one locus of the chromosome) mice were used in these studies. The mice expressing the *Tas1r3* gene showed saccharin and sucrose preferences similar to those of the control taster mice, while the same generation without the transgene showed no response to the sweetener or sucrose, thus demonstrating T1R3's role in sweet perception (Bachmanov et al., 2001). In studies of human taste genetics, taste receptor proteins such as T1R1 and T1R3 have been linked to individual variations of sweet taste recognition thresholds in humans (Fushan et al., 2010; Fushan et al., 2009) but not mice (Lu et al., 2005).

For bitter taste, single-nucleotide polymorphisms (SNPs) in the genes that code bitter receptors (*TAS2R*s) result in different chemical responses. For example *TAS2R38* gene, which codes T2R38, has been associated with individual differences in taste sensitivity for compounds containing a thiourea, phenylthiocarbamide (PTC) and 6-n-propylthiouracil (PROP) (Bufe et al., 2005; Kim et al., 2003; Lipchock, Reed, & Mennella, 2011). The *TAS2R38* gene has three SNPs with differences in amino acids. The receptor gene, which detects bitter, contains a proline at position 49, alanine at position 262, and valine at position 296 (PAV) (Kim et al., 2003). The polymorphisms

and respective genotype for the three variants are: rs713598 AA, AP, and PP; rs1726866 VV, AV, and AA; and rs10246939 II, IV, and VV, respectively.

Sweet and Bitter Taste Transduction Pathway

Signal transduction refers to the flow of chemical indicators that occurs downstream from chemoreception (e.g., taste receptor cells) that ultimately stimulates the nervous system to send a signal to the brain (Margolskee, 2002). The taste transduction pathway and gustatory mechanisms play an important role in food intake and metabolic regulation.

When discussing sweet taste, the transduction path is unique (**Figure 9**). In mammals, once a sweet compound or ligand (sugar or sweetener) binds to a sweet taste receptor with a G-protein subunit, such as alpha-gustducin, the subunit becomes activated and causes a downstream intracellular second-messenger cascade (Kitagawa, Kusakabe, Miura, Ninomiya, & Hino, 2001; Margolskee, 2002). Prior to the discovery of the sweet taste receptors, investigators focused on cyclic adenosine monophosphate (cAMP) and inositol 1,4,5-triphosphate (IP3) in signal transduction pathways of the taste receptor cells, suggesting one model of sweet taste transduction. However, with advances in technology, investigators further explored the transduction pathway of sweet taste (Taruno et al., 2013). The GPCR taste receptors stimulate a transduction pathway that involves the activation of phospholipase C β2 (PCLCB2), IP3-mediated Ca^{2+} release and Ca^{2+} dependent activation of the transient receptor potential cation channel subfamily M member 5 (TRPM5) channels that depolarize the plasma membrane and generate action

potentials, passing through the calcium homeostasis modulator 1 (CALHM1) and subsequent release of Adenosine triphosphate (ATP) (Taruno et al., 2013). ATP serves as a neurotransmitter in taste cells (Finger et al., 2005). The generated signal is sent to the brain through one of three cranial nerves: VII (facial), IX (glossopharyngeal), or X (vagus). Of these cranial nerves, the facial nerve (VII), more specifically, the chorda tympani branch is responsible for the sensory perception of the anterior two-thirds of the tongue. The glossopharyngeal nerve (IX) is responsible for the sensory perception of the posterior one third of the tongue. These nerves are known to be directly involved with the sense of taste. It is also important to highlight that the olfactory nerve (I) is responsible for the sense of smell, which indirectly affects the sense of taste. The vagus nerve (X) receives a special sense of taste from the epiglottis, specifically conducting the sense of taste from the mouth to the larynx. The nerves connect to areas of the brain associated with energy homeostasis (Chaudhari & Roper, 2010; Reed et al., 2006) and visceral perception and palatability (McCaughey, 2008).

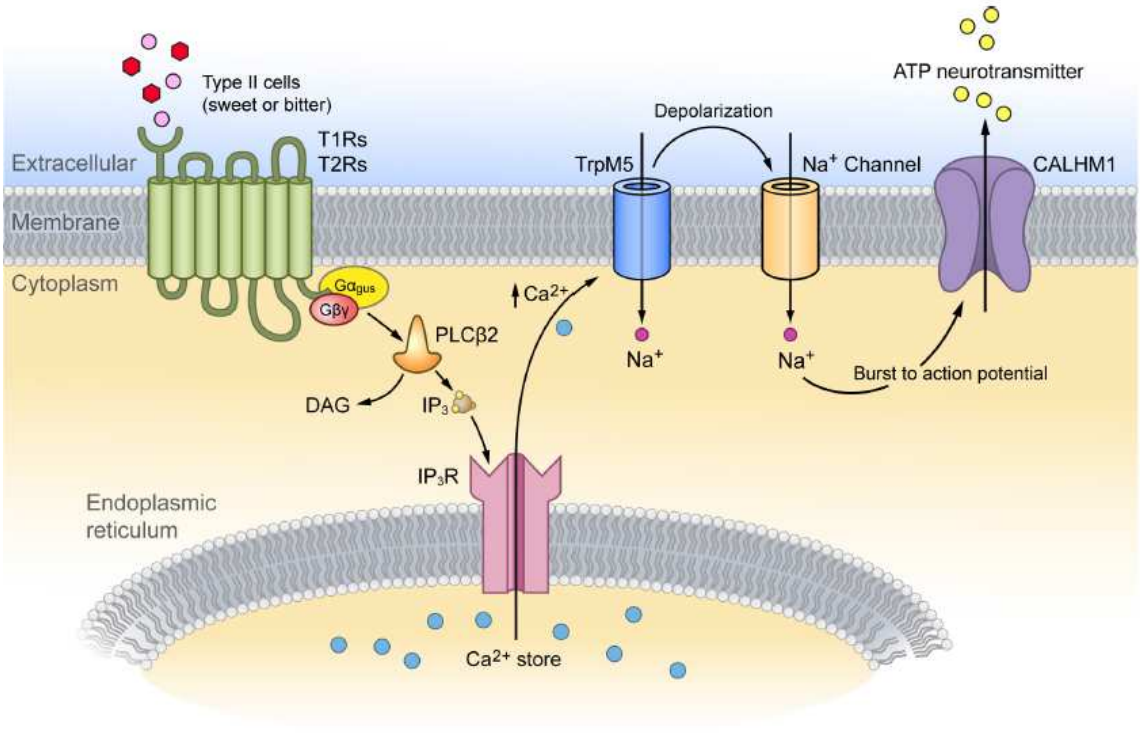
The same transduction pathway is activated when a bitter compound or ligand comes in touch with the bitter taste receptors (**Figure 10**) (Taruno et al., 2013). TAS2R proteins utilize the G-protein gustducin to elicit signal transduction. Both the α- and βγ-subunits are crucial to the transmission of the taste signal (Margolskee, 2002).

Alteration to the taste transduction proteins, such as T1R3, α-gustducin, or Trpm5 (transient receptor potential cation channel, subfamily M, member 5) may affect intake of and preference for caloric and noncaloric sweeteners (Sclafani & Ackroff, 2015; Zukerman, Glendinning, Margolskee, & Sclafani, 2013). Trpm5 is a protein that in

humans is encoded by the *TRPM5* gene. This protein is a key component of taste transduction, activated in the presence of high levels of calcium.

The sweet taste receptor is also expressed in other areas of the body, with additional biological responses (Egan & Margolskee, 2008; Kojima, Nakagawa, Ohtsu, Medina, & Nagasawa, 2014; Margolskee et al., 2007; Nakagawa et al., 2009). The sweet taste receptor cells located in human endocrine tissue contribute to incretin hormone secretion and glucose transport (Beauchamp & Mennella, 2011). Expression of these taste receptor genes is dysregulated in individuals with type 2 diabetes (for a review, see Depoortere, 2014). In the hypothalamus, the sweet taste receptor may provide signals for appetite regulation and food intake (Gerspach, Steinert, Schönenberger, Graber-Maier, & Beglinger, 2011). The sweet receptor is also expressed in adipocytes. When stimulated with appropriate ligands, such as high-potency sweeteners, this receptor plays an essential role in adipocyte proliferation (Laffitte, Neiers, & Briand, 2014). The pattern of sweet taste receptor expression among particular adipose depots in humans is not understood but may contribute to adipose tissue distribution (e.g., waist circumference). Individual differences in sweet taste thresholds may predict how well the sweet taste receptor functions in these other tissues, which may have a role in obesity, diabetes, and appetite. It is possible that sweet taste detection threshold may be proxy a measure for sweet receptor function in the brain, adipocytes, and endocrine cells.

Figure 8. *Sweet and Bitter Transduction Pathway*



Sweet Taste Detection in Children and Adults

A literature review was conducted to elicit what has been published in regards to sweet taste detection thresholds in children. Studies reported were from 1974 to 2015. The majority of these studies were conducted in adults (*N*=27) with some studies focused in both children and adults (*N*=4), while only a few studied children (*N*=8) (**Table 2**). When available the age range, numbers of subjects, methods used to measure sucrose detection thresholds and range of sucrose tested was provided. An attempt was made to report all thresholds in mM to ease comparison across the studies. In spite of the vast variety of populations studied and varied methodological approaches underpinning these studies, important insights and conclusions can be drawn.

Based on the studies reported in **Table 2**, clearly there is a lack of studies in taste thresholds in children; none of the ones reported for children take into consideration the taste receptors genes associated with sucrose detection thresholds. Most studies used traditional psychophysical methodology to assess taste perception, however, even the traditional methods used vary across studies.

The studies varied between whole mouth and spatial or regional test. A whole-mouth taste test is used to measure an individual's ability to detect, evaluate and identify the concentration of different sweet, sour, salty, and bitter taste solutions. With this methodology the liquid stimulus is presented in milliliters. The whole mouth procedure involves sipping a measured volume of a taste solution, keeping it in the mouth for an allotted time, and then spitting it out (Simon & Nicolelis, 2002). It is the technique most widely used in taste testing chemosensory procedures (Fushan et al., 2010; Meilgaard, Civille, & Carr, 2000; Simon & Nicolelis, 2002). By contrast, a spatial or regional test is used to evaluate diverse areas of the mouth. A cotton swab soaked in a taste solution is positioned in different areas of the tongue (Berling, Knutsson, Rosenblad, & Von Unge, 2011; Hummel, Erras, & Kobal, 1997; Meilgaard, Civille, & Carr, 2000; Snyder, Prescott, & Bartoshuk, 2006).

Some authors used a two alternative forced choice method (Pepino, Finkbeiner, Beauchamp, & Mennella, 2010), others used a three stimulus forced choice method (Kamath, Booth, Lad, Kohrs, & McGuire, 1983), a triangle method (Panek-Scarborough, Dewey, & Temple, 2012). Only one study used both electrogustometry data and chemical liquid stimuli (Park et al., 2015). The electrogustometry data is obtained using a device

that provides quantitative gustatory detection thresholds using an electrical current when exposed to sweet and bitter taste. The methods used by Pepino et al. (2010) were the only ones similar to the present study, which was validated to be used in children of diverse background (e.g., African-American, Caucasians) (Bobowski & Mennella, 2015; Mennella et al., 2011).

In adults sucrose detection thresholds have been studied in a variety of contexts, for example, in individuals with depression, smokers vs. nonsmokers, diabetics, obese vs. normal weight and in women with hormonal changes. Some studies only tested men, which did not allow assessing for differences between genders. In children, the vast majority of studies reported on populations of healthy children, but one study in children reported taste thresholds in obese and non-obese children. Many of the earlier studies had a focus on dental caries and sucrose detection thresholds, with a focus on dietary influences in later publications.

The sample size used in the studies varied greatly (i.e., 10-180 subjects). In addition, the range of sucrose used across studies was not standardized and the reporting of the data obtained varied (e.g., mM, M, g/mL, %), making it difficult to compare sweet taste thresholds reported among studies. In **Table 2** values that were not reported as milimolar concentrations in the literature were converted for easier comparison across studies. The range of sucrose detection thresholds reported is very wide; this could be because of the inconsistencies in methodologies.

Table 2. *Summary of Studies Reporting Sucrose Detection Thresholds in Children and Adults*

Subjects (children, adults, age range) N=number of subjects in study	Methods (Whole mouth; part of the tongue)	Sucrose detection thresholds as reported in manuscript	Sucrose detection thresholds reported (mM)	SNPs	Findings of the study	Citation
Studies in Adult Subjects						
Adults: 20.7 ± 0.3 years (N=70)	Whole mouth: Tested downward from a higher concentration to a lower concentration in decrements of 0.1%. Range of sucrose: not specified	Adults Male: 0.98 ± 0.09% Female students: 1.43 ± 0.21% in female students in the follicular phase Female: 1.27 ± 0.14% in the luteal phase.	Adults Male: 28.7 mM±26 mM Female Students: 41.8 mM±6.1 mM follicular phase Female students: 37.1 mM ± 4.1mM luteal phase	None	Sucrose detection was negatively correlated with depression symptoms and trait anxiety in women in the luteal phase. However findings were not significant for males and women in the follicular phase	Nagai, Matsumoto, Endo, Sakamoto, & Wada, 2015
Adults: 20-29 years old N=41	Electrogustometer Range: 3 uA (–8 dB) to 400 uA (34 dB) Whole mouth: chemical test Sucrose Range: 0.05g/ml-2g/ml	Obese group 0.70 g/ml Normal weight group: 0.33g/ml Smoker: 0.71 g/ml Nonsmoker: 0.39 g/ml Standard deviation not reported	Obese group: 2040 mM Normal weight group: 960 mM Smokers: 2070 mM Non-smokers: 1140 mM	None	With the chemical taste test, the obese group had higher thresholds for sweet. Smoking had an impact on taste threshold, with smokers having higher thresholds than non-smokers also for sweet.	Park et al., 2015
Adults: 20-60 years old N=40 prediabetic N=40 diabetics N=34 normal glycemic	Whole mouth: forced choice method Sucrose range: 1.25×10^3 mol/L to 6.4×10^1 mol/L	Diabetic: 0.025 mol/L ± 0.01 Pre-diabetic: 0.018 mol/L ± 0.01 Normoglycemic: 0.015 mol/L ± 0.01	Diabetic: 25 ± 10 mM Pre-diabetic: 18 mM±10 mM Normoglycemic: 15 mM ± 10 mM	None	The mean (SD) detection thresholds of diabetics were significant higher when compared to normoglycemic group and pre-diabetics.	Wasalathan thri et al., 2014

Subjects (children, adults, age range) N=number of subjects in study	Methods (Whole mouth; Part of the tongue)	Sucrose detection thresholds as reported in manuscript	Sucrose detection thresholds reported (mM)	SNPs	Findings of the study	Citation
Studies in Adult Subjects						
Adults: N=15	Regional taste recognition thresholds Sucrose Range: $\log_{10}$ molar concentration, from −1 to −3 (100 mM to 1 mM)	Adults: Bright light, (before = 13.4 mM (CI 10.9−16.4), after = 9.4 mM (CI 7.3−12.0) Dim light (before = 13.3 mM (11.3−15.7) and after = 11.3 mM (9.30−13.7)	Adults: Bright light, (before = 13.4 mM (CI 10.9−16.4), after = 9.4 mM (CI 7.3−12.0) Dim light (before = 13.3 mM (11.3−15.7) and after = 11.3 mM (9.30−13.7)	None	Sucrose thresholds were significantly lower after bright but not dim light exposure.	Srivastava, Donaldson, Rai, Melichar, & Potokar, 2013
Adults: 18-49 years old (Females only) N=72	Whole mouth: ascending forced choice trial method Sucrose Range:0. 2%, 0.3%, 0.5%, 0.8%, 1.0%, and 1.2% w/v	BMI group Normal: 0.32% Overweight: 0. 44% Body fat Normal: 0.41% Overweight: 0.44 %	BMI group Normal: 9.4 mM Overweight: 12.9 mM Body fat group: Normal: 12 mM Overweight: 12.9 mM	None	Women in both the overweight BMI and body fat groups had higher sucrose threshold than did women in the normal groups.	Ettinger, Duizer, & Caldwell, 2012
Adults: 18-50 years old N=50	Whole mouth: triangle test staircase procedure Sucrose Range: 0.065, 0.127, 0.25, 0.5, 1.0, 2.0, and 5.0 mg/L	Swallowed threshold: 1.04 mg/L Expectorated threshold: 1.41 mg/L No SD reported	Swallowed threshold: 3×10^{-3}mM Expectorated threshold: 41×10^{-3}mM		Sucrose detection thresholds predicted the reinforcing value of food. Those with poor detection thresholds had higher reinforcing value of food.	Panek-Scarborough et al., 2012

Subjects (Children, Adults, Age Range) N=number of subjects in study	Methods (Whole mouth; Part of the tongue)	Sucrose detection thresholds as reported in manuscript	Sucrose detection thresholds reported (mM)	SNPs	Findings of the study	Citation
Studies in Adult Subjects						
Adults: no age range provided N=160	Whole mouth. Threshold for sucrose were estimated using a storing test and signal detection analysis reported as *R*-index measures. Range of sucrose: 0, 0.5%, 1%, 2%, 2.4%, 2.8%, 3.2%, 3.6%, and 4% sucrose	R-index obtained for each pairwise comparison ranged from 71% to 94%. The lowest average R-index (71%) was obtained for the comparison of 0 and 0.5% sucrose whereas the largest R-index was obtained when comparing 1% and 2% sucrose solutions. Sucrose reported as AUC scores	CC 5.9 ± 1.4 AUC CT 6.8 ± 1.6 AUC TT 7.2 ± 1.0 AUC	*GNAT3*	*GNAT3* polymorphisms explained variation in sucrose thresholds. Individuals with one or two copies of the C allele have lower sucrose detection thresholds.	Fushan et al., 2010
Adults: no age range provided N=144	Whole mouth. Threshold for sucrose were estimated using a storing test and signal detection analysis signal detection analysis and reported as R-index measures. Range of sucrose: 0, 0.5%, 1%, 2%, 2.4%, 2.8%, 3.2%, 3.6%, and 4% sucrose	R-index obtained for each pairwise comparison ranged from 71% to 94%. The lowest average R-index (71%) was obtained for the comparison of 0 and 0.5% sucrose whereas the largest R-index was obtained when comparing 1% and 2% sucrose solutions. AUC scores	CC 6.90 ± 1.47 AUC CT 6.07 ± 1.08 AUC TT 4.36 ± 1.26AUC	*TAS1R3*	Individuals with T alleles have lower sucrose sensitivity thresholds.	Fushan et al., 2009

Subjects (Children, Adults, Age Range) N=number of subjects in study	Methods (Whole mouth; Part of the tongue)	Sucrose detection thresholds as reported in manuscript	Sucrose detection thresholds reported (mM)	SNPs	Findings of the study	Citation
Studies in Adult Subjects						
Adults: 41-75 years old N=33	Whole mouth, multiple forced-choice presentation with an ascending series Range: sucrose 1.25×10^{-3} to 6.4×10^{-1} mol/L	Pre-surgery: 5.1 mol/L± 1.8 mol/L Discharge: 1.7 mol/L± 1.2 mol/L	Pre-surgery: 5100 ± 1800 mM Discharge: 1700 ± 1200 mM	None	Sweet taste detection thresholds were lower at discharge compared to pre-surgery. Thresholds remained lower when checked 16 weeks post-op.	Keith, Mokbel, San Emeterio, Song, & Errett, 2010
Adults: 21-40 years old N=57	Whole mouth, forced choice staircase procedure Sucrose Range: 0.000056 to 1.0 M	Obese: Median ~0.00065M ± 0.0103M Lean: 0.0100 M ± 0.0074M	Obese: 0.65 ± 10.3mM Lean: 10mM ± 7.4 mM	None	Obese women had lower sucrose detection thresholds than normal weight women. Both obese and normal weight women preferred sucrose similarly. The level of sucrose preferred was not related to sucrose thresholds for either group or both groups combined	Pepino et al., 2010
Adults: 55-81 years old N=120	Side of the tongue: Forced choice, three-stimulus drop technique Sucrose Range: 34–342 mmol/L	Adults: 48mM± 38 mM	Adults: 48mM ± 38 mM	None	After an acute stroke, postmenopausal women had abnormal sucrose detection thresholds	Kim, Choi-Kwon, Kwon, & Kwon, 2009
Adults: 18-23 years old N=182	Whole mouth: forced choice method Sucrose Range: 2.5×10^{-4} to 0.5 M	Average: 10.83 mM ± 0.24 mM, Highest detection threshold: 19.88 mM ± 1.31 mM Lowest detection threshold: 5.85 mM ± 0.43mM	Average sucrose detection threshold: 10.83 mM ± 0.24 mM, Highest detection threshold: 19.88 mM ± 1.31 mM Lowest detection threshold: 5.85 mM ± 0.43 mM	None	The density of fungiform papillae and sucrose detection threshold were inversely related. The higher numbers of papillae was associated with lower detection thresholds (more sensitive).	Zhang et al., 2009

Subjects (Children, Adults, Age Range) N=number of subjects in study	Methods (Whole mouth; Part of the tongue)	Sucrose detection thresholds as reported in manuscript	Sucrose detection thresholds reported (mM)	SNPs	Findings of the study	Citation
Studies in Adult Subjects						
Adults: 21-30 years old N= 91 healthy, non-obese, non-diabetic	Whole mouth: Standard staircase methodology (modified from Pasquet et al., 2006) for various taste stimuli. Sucrose Range: 0.001–0.1 mol/l	Adults: 23.0 mM +2.5 mM in the morning 38.2 mM +4.0mMin the evening.	Adults: 23.0 mM +2.5 in the morning 38.2 mM +4.0 in the evening	None	Sweet taste detection thresholds varied by time of day with lowest thresholds in the morning and the highest thresholds at night.	Nakamura et al., 2008
Adults: 21-40 years old N=49	Whole mouth, two alternative staircase method Sucrose Range: 0.000056 to 1.0 M	Smokers Nicotine: 0.01M±0.006M Smokers non-nicotine: 0.010M±0.006M Nonsmokers nicotine: 0.008M ± 0.004M Nonsmokers non-nicotine: 0.012M±0.006M	Smokers non-nicotine: 10mM ± 6mM Smokers non-nicotine: 1mM ± 6mM Nonsmokers nicotine: 8mM ± 4mM Nonsmokers non-nicotine: 12mM ± 6mM	None	Smokers had higher sucrose thresholds (decreased sensitivity) than nonsmokers.	Pepino & Mennella, 2007
Adults: mean age 23.9 ± 1.2 N=69	Whole mouth: Standard two alternative forced choice Sucrose range: $3.2 \times 10^{-4} - 1.0$ M	Men: 0.22 x 10^{-2} M ± 0.27x10^{-2} M Women: 0.18 x10^{-2} ± 0.23 x10^{-2} M	Men 2.2mM ± 2.7mM Women: 1.8mM ± 2.3mM	None	Sweet taste sensitive increased with the ratio of PROP.	Chang et al., 2006
Adults: 28-78 years old N=21	Whole mouth: Test of limits 1.5 to 15.5 mM (in 1.0 mM increments) for sucrose	Sjögren's syndrome patients: Median 7.5 mM Control group: 5.5 mM	Sjögren's syndrome patients: Median 7.5 mM Control group: 5.5 mM No SD reported	None	Sucrose detection thresholds were higher in Sjögren's syndrome patients compared to controls. Detection thresholds for other taste compound were also higher	Gomez, Cassis-Nosthas, Morales-De-Leon, &

		No SD reported			in Sjögren's syndrome patients.	Bourges, 2004

Subjects (Children, Adults, Age Range) N=number of subjects in study	Methods (Whole mouth; Part of the tongue)	Sucrose detection thresholds as reported in manuscript	Sucrose detection thresholds reported (mM)	SNPs	Findings of the study	Citation
Studies in Adult Subjects						
Adults: 18-24 years old N=27	Whole mouth: constant stimuli with the order of presentation Counterbalanced using a Latin square. Sucrose range: 0.1, 0.08, 0.06, 0.04, 0.02, 0.01, and 0.008 M	#1Menstruation 0.025M± 0.002M #2Pre-ovulation: 0.015M ± 0.002M #3Post-ovulation: 0.027M± 0.002M Men measured during the same time frame as women. #1Men: 0.022M ± 0.004M #2 Men: 0.022M ± 0.003M #3 Men: 0.021M± 0.003 M	#1Menstruation: 25mM ± 2mM #2Pre-ovulation: 15mM ± 2mM #3Post-ovulation: 27mM ± 2mM Men measured during the same time frame as women. #1Men: 22mM ± 4mM #2 Men 22mM ± 3mM #3 Men 21mM ± 3mM	None	For women, there was an increase in sweet sensitivity during pre-ovulation phase and lower sweet sensitivity during post-ovulation. There was no variation in sucrose detection thresholds in men.	Than et al., 1994
Adults: 20-88 years old N=71	Whole mouth: forced choice procedure Sucrose range: 0.01-580mM	X of 6 reversals Younger group: 3.6mM Older group: 8.0mM 1st downward run Younger group: 5.5mM± Older group: 11.3 mM No SD reported	X of 6 reversals Younger group: 3.6mM Older group: 8.0mM 1st downward run Younger group: 5.5mM Older group: 11.3 mM No SD reported	None	Sweet taste sensitivity decreased with age (higher detection thresholds).	Moore et al., 1982
Adults: 23-88 years old N=81	Whole mouth: forced choice Sucrose range: 5.6 x 10^{-1} and 1.0x 10^{5}	5.92x10^{-3} mM± 5.92 mM	5.92x10^{-3} mM ± 5.92 mM	None	Sucrose thresholds were not significantly related to age.	Weiffenbach, Baum, & Burghauser, 1982

Subjects (Children, Adults, Age Range) N=number of subjects in study	Methods (Whole mouth; Part of the tongue)	Sucrose detection thresholds as reported in manuscript	Sucrose detection thresholds reported (mM)	SNPs	Findings of the study	Citation
Studies in Adults Subjects						
Adults: 65-87 years old Controls: 20-29 years old N=101	Method of Lagan and Yearisk (1976) Sucrose range: 0.01, 0.02, 0.05, 0.1, 0.2, 0.5 M	Mean thresholds not reported	Mean thresholds not reported	None	Elderly had higher sucrose thresholds than younger participants.	Lassila, Sointu, Raiha, & Lehtonen, 1988
Adults: 40-79 years old N=26	Whole mouth: 3 stimulus forced choice method Sucrose Range: 0.1, 1.3,6, 10, 30, 60, 100, 300, 600, 1000, and 3000 mmol/L	Unmatched controls: 22 ± 11.1 mM Range: 6-30 mM Patients: 58.8 ± 79.4 mM Matched controls: 54.5 ± 76.4 mM	Unmatched controls: 22 ± 11.1 mM Range: 6-30 mM Patients: 58.8 ± 79.4 mM Matched controls: 54.5 ± 76.4 mM	None	There were no significant differences between the groups for sucrose detection.	Kamath et al., 1983
Adults: 18-40 years old Females only N=22	Tip of tongue: Forced choice drop technique Sucrose range: 6, 12, 30, 60, 90, 150, 300, 500, 800, and 1000 mM	Mean thresholds not reported	Mean thresholds not reported	None	There was no difference among compared adult-onset obese, juvenile-onset obese, and never-obese females	Malcolm, O'Neil, Hirsch, Currey, & Moskowitz, 1980

Subjects (Children, Adults, age Range) N=number of subjects in study	Methods (Whole mouth; part of the tongue)	Sucrose detection thresholds as reported in manuscript	Sucrose detection thresholds reported (mM)	SNPs	Findings of the study	Citation
Studies in Adult Subjects						
Adults: 17-72 years old N=69	Whole mouth: three cup presentation Sucrose Range: 0.03-1.2 mg/100ml	Adult onset diabetes Diabetics: 0.5mg/100ml Control: 0.3 mg/100ml Non diabetic relatives: 0.3 mg/100ml Controls: 0.4 mg/100ml Juvenile onset diabetes: 0.45 mg/100ml Controls: 0.5 mg/100ml No SD reported	Adult onset diabetes Diabetics: 0.0146mM Control: 0.0087mM Non diabetic relatives: 0.0087mM Controls: 0.0116mM Juvenile onset diabetes: 0.0131mM Controls: 0.0146mM No SD reported	None	The adult onset diabetics demonstrated a higher sucrose threshold than controls.	Lawson, Zeidler, & Rubenstein, 1979
Adults: 21 young (19–33 years) and 21 elderly (60–75 years)	Whole mouth: Two-alternative forced choice, with concentrations presented in ascending order. Sucrose Range: 4.09×10^{-1}–1.63×10^{2}	Male elderly: 8.15709 g/l Female elderly: 4.56599 g/l Male young: 5.21838 g/l Female young: 3.90423 g/l No SD reported	Men elderly: 23.83mM Female elderly: 13.35mM Male young: 15.25mM Female young: 11.41mM No SD reported	None	The older men were less sensitive than the young men and women for sucrose. No sex differences observed.	Mojet, Christ-Hazelhof, & Heidema, 2001

Subjects (Children, Adults, age Range) N=number of subjects in study	Methods (Whole mouth; part of the tongue)	Sucrose detection thresholds as reported in manuscript	Sucrose detection thresholds reported (mM)	SNPs	Findings of the study	Citation
Studies in Adult Subjects						
Young adults: Mean age 21 years old N=12	Whole mouth: two alternative forced choice method Range of sucrose: not reported	All Anorexics: 0.49%± 0.50 Refeeding anorexics: 0.14%± 0.29 Low body weight anorexics: 0.85%± 0.40	All anorexics: 41.3±14.6 mM Refeeding anorexics: 4.1 ± 8.5 mM Low body weight anorexics: 24.9 ± 11.7 mM	None	Overall, anorexics did not have abnormal sucrose detection thresholds, but those anorexics in high caloric refeeding diets had lower sweet sensitivity compared to controls and those in low caloric diet.	Lacey, Stanley, Crutchfield, & Crisp, 1977
Adults: 17-19 years old (Males only) N=50	Tip of tongue: Force choice Sucrose range: 5mM-50mM	Caries free: 8.9 mM ± 0.5mM Control: 12.9 mM ± 0.9mM	Caries free: 8.9 mM ± 0.5 mM Control: 12.9 mM ± 0.9 mM	None	The caries-free group had a significantly lower mean sucrose detection threshold compared to that of the control group.	Catalanotto & Keene 1974
Adults: 17-25 years old (Males only) N=52	Tip of tongue: Forced choice 1-drop water and 1 drop tastant. Sucrose Range: 5 mM/L to 70 mM/L	Caries- free: 10.71mM ± 1.83 Caries-active: 16.79mM± 1.46	Caries- free: 10.71mM ± 1.83 mM Caries-active: 16.79mM ± 1.46 mM	None	Sucrose detection threshold for the caries-free subjects was significantly lower than that of the caries-active group.	Wrobel, Catalanotto, & Walter, 1978
Studies in Adults and Children						
With type I diabetes Adult: 16 years and older N=22 Children: 9-15 years old N=100 Without diabetes N=41-adults N=100- children	Tip of tongue: forced choice triangle method Sucrose range: 0.20, 0.40, 0.60, 0.80, 1.00, 1.20, 1.40, 1.60 %	Youth diabetic: 0.65% Non-diabetic: 0.520% Adults: Diabetic: 0. 860% Non-diabetic:0. 420% No SD reported	Youth Diabetic: 19 mM Non-diabetic: 15.2 mM Adults: Diabetic: 25.1 mM Non-diabetic: 12.3 mM No SD reported	None	Diabetes and age can decrease an individual's sucrose detection threshold	Hardy, Brennand, & Wyse, 1981

Subjects (Children, Adults, age Range) N=number of subjects in study	Methods (Whole mouth; part of the tongue)	Sucrose detection thresholds as reported in manuscript	Sucrose detection thresholds reported (mM)	SNPs	Findings of the study	Citation
Studies in Adults and Children						
Adults: 22.2 years, range not known (n=61) Children: 8-9 years old (N=68)	Whole mouth: A paired-comparison forced-choice procedure Range of sucrose: 1.171 × 10^{-4} − 7.5 × 10^{-2} M	Girls: 0.0072±0.0026 M Boys: 0.0170±0.0033M Adult women: 0.0062±0.0017M Adult men: 0.0068±0.0010M	Girls: 7.2mM +2.6mM Boys: 17mM +3.3mM Adult women: 6.2mM +1.7mM Adult men: 6.8mM +1mM	None	Boys had higher detection thresholds for sucrose. While female children had similar detection thresholds to adults.	James et al., 1997
Adults: 20-30 years N 10 Children 8 years N: 10	Different areas in the tongue: two forced alternative choice method with filter paper Sucrose rage: 0.2125 M	Data reported the number of correct responses based on the number of papillae and sensitivity to sucrose	Data reported the number of correct responses based on the number of papillae and sensitivity to sucrose	None	Children were significantly more sensitive to sucrose than adults. Counts of papillae were similar for both children and adults.	Stein, Laing, & Hutchinson, 1994
Adults: 16-25 years old Children: 6-15 years old N=103 adults N=37 children	Tip of tongue: three drop test Sucrose Range: 10, 15, 20, 25, 30,40, 50,100, 200 mmol/l	Children 6-15: 10-40 mM Adults: 10-100mM No SD reported	Children 6-15: 10-40 mM Adults: 10-100mM No SD reported	None	Sucrose thresholds were not related to the caries experience. Sucrose threshold decreased with age. Lower sucrose thresholds for sucrose were not related to sugar in beverages.	Adams & Butterfield, 1979
Studies in Children						
Children: 6-18 years old (N= 99 obese N=94 normal weight)	Whole mouth: Taste strips were used and ranked on a 5-point rating scale, with 1-No Taste, and 5 -Very Strong Taste. Range of sucrose: Four different concentrations for *sweet*, (sweet: 0.4, 0.2, 0.1, 0.05 g/ml sucrose).	Obese children: 12.6±3.0 Total Score Non-obese children: 14.1±3.0 Total Score	Not able to be calculated since the correct number of identified taste strips was reported.	None	Obese children had more difficulty identifying sweet as well as other taste qualities less compared to children and adolescents of normal weight	Overberg et al., 2012

Subjects (Children, Adults, age Range) N=number of subjects in study	Methods (Whole mouth; part of the tongue)	Sucrose detection thresholds as reported in manuscript	Sucrose detection thresholds reported (mM)	SNPs	Findings of the study	Citation
Studies in Children						
Children: 7-15 years old N=92	Tip of tongue: Up-down forced choice design Sucrose range: 5 to 70 mM	Caries susceptible: 13.7mM Caries free: 15.6mM No SD reported	Caries susceptible: 13.7mM Caries free: 15.6mM No SD reported	None	There was no significant difference in sucrose thresholds between the two caries groups. Results were different from the adult literature.	Catalanotto, Gaulin-Kremer, & Shaw, 1979
Children: 15-year-olds N=100 50 with a high and 50 with a low caries	Whole mouth Sucrose Range: 3.91mmol/L-88.39mml/L	Median 15.6 mM/L in both groups No SD reported	Median 15.6 mM/L in both groups No SD reported	None	There was not a statistically significant difference in thresholds between the groups. There was not an association between thresholds and preferences for sucrose.	Nilsson, Holm, & Sjostrom, 1982
Children: 5-12 years (N=40 years).	Whole mouth: the subjects tasted solutions at 2 concentrations and asked to identify the lowest concentration. Sucrose Range: 0.032M and 0.32 M.	Mean=1.56 M+ No SD reported	Mean=1560mM No SD reported	None	The sucrose solution was detected with the most diluted concentration. There were no gender differences for threshold.	Majorana et al., 2012
Children: 12 years old N=181	Whole-mouth technique Sucrose Range: 3.91 mmol/l to a maximum concentration of sucrose 88.39 mmol/l.	Median: 22.10 mM No SD reported	Median: 22.10 mM No SD reported	None	Girls were in the high sucrose perception taster group. There was no significant difference in sweet perception status between low and high caries groups.	Furquim, Poli-Frederico, Maciel, Gonini-Junior, & Walter, 2010
Children: 11-15 years old N=143	Whole mouth, up-down, two cup forced choice Range: 0.056M to 1.000M	Mean:0. 004 M ± No SD reported 4mM	Mean: 4mM No SD reported	None	Children with high and low sucrose preference patterns did not differ in sucrose perception.	Coldwell et al., 2009

Subjects (Children, Adults, age Range) N=number of subjects in study	Methods (Whole mouth; part of the tongue)	Sucrose detection thresholds as reported in manuscript	Sucrose detection thresholds reported (mM)	SNPs	Findings of the study	Citation
Studies in Children						
Children: 5-9 years old. N=99	Whole mouth: Forced choice triangle test Range of sucrose: 0%, 0.2%, 0.4%, 0.6%, 0.8%, 1.0%, 1.2%, 1.4% and 1.6%.	Median: 1.0% +0.37 Low sucrose threshold: (0.4 and 0.6%; n = 35) Moderate sucrose threshold:(0.8–1.2%; n = 36) High sucrose threshold: (1.4 and 1.6%; n = 28). No SD reported	Median: 29.2 mM± 10.8 mM Low sucrose threshold: 11.7 mM and 17.5mM Moderate sucrose threshold: 23.4 mM and 35.1 mM High sucrose threshold: 40.9 and 46.8 mM No SD reported	None	Children with moderate sucrose detection thresholds consumed the most non-bitter fruits. Children with high sucrose thresholds consumed more green leafy vegetables.	Fogel & Blissett, 2014
Children: 3-6 years old (N=45)	Whole mouth: Two alternative, forced-choice staircase Range of sucrose: 1.5, 3.0, 6, 12, 18, 24, 30, 45, 60, 120, 180, 240 and 300 mmol/l	Children: 31mM	Children: 31 mM	None	The goal of this study was to develop an instrument to measure thresholds in children younger than 6 years old. Found that accurate data can be obtained in children of this age group if method is short, easy to understand and fun.	Visser, Kroeze, Kamps, & Bijlevel d, 2000

CHAPTER 3

Research Design and Methods

Research Design

This dissertation study was a descriptive, secondary data analysis to which I contributed original genotype data analysis and interpretation for the candidate genes selected. It was based in part on data collected from two "taste studies" conducted by Dr. Julie Mennella and her research staff at the Monell Chemical Senses Center in Philadelphia, Pennsylvania, in 2004-2012. The goal of this study was to understand individual differences in taste sensitivity and genetics and their relationship to diet-related food behaviors and obesity. The study focused specifically on a sample of racially and ethnically diverse children 7-14 years of age from the Philadelphia area and the relationships among (a) psychophysical measures of sucrose detection thresholds (b) inherited forms of the sweet taste genes *TAS1R2*, *TAS1R3*, and *GNAT3* and bitter taste gene *TAS2R38*, (c) adiposity measures, (d) diet related food behaviors reported as dietary intake of total calories and added sugars, and (e) personal charactertistics measured as child temperament and food neophobia.

Recruitment, Setting and Consent

Recruitment, informed consent, and assent of subjects had already taken place in the parent studies. Mothers of children were recruited for a "taste study" from the metropolitan Philadelphia area using advertisements in local newspapers and magazines and via mass mailings. Screening procedures were implemented based on previous research published by the Reed and Mennella labs, all of which have been reviewed and

approved by the Office of Regulatory Affairs at the University of Pennsylvania. During the weeks prior to the study, a blank copy of the consent form was mailed to the home of each participant. Initial screening interviews were conducted with mothers over the telephone.

Before testing, written informed consent was obtained from the child's mother, and assent was obtained from each child. The mother of the child was provided with a copy of the consent form to read on the day of testing. During the consenting process with the parent, each section of the consent was reviewed. The experimenters explained the study goals and then verbally repeated the statement on the written consent form, emphasizing that the subject may, at any time and for any reason, withdraw from the participant group or experiment without penalty or prejudice. Before signing, each parent was encouraged to ask questions she might have regarding the experiment. To help the children achieve a developmentally appropriate awareness of the nature of the study, children were encouraged to ask questions and assent was obtained from each child. Questions were asked both in the presence of the mother and again in the private testing room a setting away from maternal influence.

A detailed description of the purpose of the research study as well as the risks and benefits of the study, compensation, and privacy policy were explained to parents. After signing the informed consent form, each parent was given a copy of the signed form and was invited to contact our laboratory at any time if any questions or concerns arose after study completion. Mother of participants were reimbursed for travel expenses and given a small incentive for their time and cooperation with study procedures.

Inclusion and Exclusion Criteria

Key inclusion criteria were age 7-14 years, ability to speak English, and reported good health at the time of study participation. Key exclusion criteria were medical conditions that interfere with eating or that alter taste perception (**Table 3**).

Table 3. *Inclusion and Exclusion Criteria*

Inclusion criteria	**Exclusion criteria**
Children age 7-14 years	Medical conditions that interfere with taste (i.e. cold or flu)
English-speaking	Taking medications that alter appetite
Good health	Unable to understand and complete psychophysical testing

Methods and Procedures

The following section focuses on the methods used to obtain the variables in this study. To further illustrate and clarify data obtained in the original "taste" studies and what was added in this study, see **Table 4**.

Table 4. *Variables Included in Analyses*

Measures collected in original studies	Demographic characteristics[a.b]
	Sucrose detection thresholds[a.b]
	Child's DNA[a, b]
	Height and weight[a, b]
	% body fat[b]
	Waist circumference (WC)[b]
	Dietary intake[b]
	Child's Temperament and Food Neophobia Scale[b]
Added in this study	SNP selection
	Genotyping of selected SNPs [a, b]
	Waist–Height Ratio (WHtR)[b]
Note: [a]Measures were collected in study #1; [b] Measures were collected in study #2	

Demographic Information

Demographic information was attained on each participant during the initial original parent "taste" studies. Age in months was calculated from the difference between reported date of birth of the child and the date of psychophysical testing. The child's mother reported the child's gender, race, and ethnicity. Mothers were also queried about their own race/ethnicity. Race ethnicity was assigned using standard US Census categories. Household income was also reported by the child's mother in categories (<$50,000, $50,000-$99,000, >$100,000, unknown).

Sucrose Detection Thresholds

Preparation of Sucrose Solutions

Prior to starting the sucrose detection thresholds testing, the tasting solution were prepared and stored in a cold room. The written protocol on how to prepare solutions was strictly followed to minimize potential systematic and random errors that can affect the results. Solutions were discarded after two weeks from the date prepared and replaced for future studies (see **Appendix A**)

Children's Testing and Training for Sucrose Detection Thresholds

Sucrose detection threshold using a two-alternative forced-choice staircase procedure was developed at the Monell Center for adults (Cowart & Beauchamp, 1990; Mennella et al., 2011; Pepino & Mennella, 2007; Pribitkin, Rosenthal, & Cowart, 2003) and later adapted for use among pediatric populations (Bobowski & Mennella, 2015; Mennella et al., 2011). The two-alternative forced choice is a psychophysical method developed to elicit responses about an individual's experience regarding a stimulus. It focuses on the evaluation of a single attribute (e.g., sweetness), and the stimulus is adjusted based on the individual responses (Bartoshuk, 1978; Jogan & Stocker, 2014). For this study, all testing took place in a private, comfortable room specially designed for sensory testing that was illuminated with red light to mask any visual differences among samples. Subjects consumed no food or drink other than water for at least one hour before the task and acclimated to the testing room and to the researcher for approximately 15 minutes before testing.

Prior to testing, all children were trained to become familiar with the method and to assess whether they understood the detection threshold task (**Appendix B**). Children were presented with a pair of 30 mL disposable medicine cups (Fisher Scientific, Inc.). One cup contained distilled water, and the other contained either a 0.056 mM or 18 mM sucrose solution (Bobowski & Mennella, 2015). Children were asked to taste both solutions in the order presented and to point to the solution that had a taste. The two pairs provided the children with the experience of tasting a pair of solutions for which they could not detect a difference (water vs. 0.056 mM sucrose) and for which the difference between solutions was easily discernible (water vs. 18 mM sucrose). This method eliminated the need for a verbal response and has been shown to be an effective method for assessing both taste and olfaction in children (Bobowski & Mennella, 2015; Mennella, Finkbeiner, et al., 2014). Training was repeated for those children who did not understand the task after one training session. If after training children did not comply with the procedures, became tired, or refused to continue, the testing was stopped and the data were not analyzed.

The solutions used for testing in the formal threshold detection protocol ranged in concentrations of 0.056-1000 mM and were equally diluted from the maximum concentration in quarter log steps. The order of presentation of solutions was randomized across subjects. The first pair of samples presented to the child was near the middle of the concentration series, starting at 3.2 mM (Pepino et al., 2010). During each trial, subjects were presented with pairs of solutions; within each pair, one solution was distilled water and the other the taste (sucrose) stimulus. Subjects were instructed to taste the first

solution presented within a pair, swish the solution in their mouth for 5 seconds, and expectorate. Subjects tasted the second solution within a pair using the same protocol, rinsing their mouth with distilled water once between solutions within a pair and twice between successive pairs. Time was monitored during interpair (5 s) and interseries (1 min) intervals using a digital clock, to prevent the introduction of traces of stimuli (i.e., sucrose) left from preceding trials (Bartoshuk, 1978). After tasting both solutions within a pair, subjects were asked to point to the solution that had a taste, as in the training task (**Appendix C**). The concentration of the tastant in the solution presented in the subsequent pair was increased after a single incorrect response and decreased after two consecutive correct responses. A reversal occurred when the concentration sequence changed direction (an incorrect response followed by a correct response or vice versa).

A tracking grid (**Figure 9)** was used to record subjects' responses. The testing procedure was terminated after four reversals occurred, provided the following criteria were met: (a) there were no more than two dilution steps between two consecutive reversals, and (b) the reversals did not form a consistent ascending or descending pattern such that positive and negative reversals were achieved at successively higher or lower concentrations (Pribitkin et al., 2003). For each subject, the calculated detection threshold for sucrose was calculated as the mean of the log values of the last four reversals (**Appendixes D & E**). The log transformation is used to make non-normally distributed data less skewed, to make patterns in the data more interpretable and to help meet the assumptions of inferential statistics (Bland & Altman, 1996; McDonald, 2014). Finally, the antilog value was determined to represent the mean sucrose detection threshold.

Although only a narrow section of the testing grid was required to test the subject illustrated in **Figure 9**, some children tasted stimuli over a wider range of concentrations before their threshold was determined.

Methodological sources of errors with this methodology have been considered. Careful attention to sources of both random and non-random error was taken into account in the development of this technique to reduce the potential for invalid and unreliable data. The two-alternative forced choice staircase approach maximizes precision by minimizing the number of trials (Bartoshuk, 1978; Jogan & Stocker, 2014). The methodology has been used in other studies as well where investigators obtained comparable data (Bobowski & Mennella, 2015; Pepino et al., 2010; Pepino & Mennella, 2012). The advantage of using this technique is that stimulates a large region of the anterior oral cavity, which has been used to make inferences about normal perception during eating and drinking. Steps have been taken to reduce limitations related to stimulus control and residual stimuli that may remain in the mouth by asking participants to rinse their mouth with water between test doses and measuring time between stimuli. During the preparation of the sucrose solution, ensuring adequate calibration of pipettes and scales has reduced some systematic errors.

Figure 9. *Forced Choice Sucrose Detection Threshold Tracking Grids*

Step	Sucrose Concentration (mM)	Pair											
		1	2	3	4	5	6	7	8	9	10	11	12
		Order of presentation											
		2	2	2	2	1	1	2	1	1	2	2	1
Step 0	1000												
Step 1	562												
Step 2	316												
Step 3	178												
Step 4	100												
Step 5	56												
Step 6	32												
Step 7	18												
Step 8	10				+	+							
Step 9	5.6		+	-			+	+		+	+		
Step 10*	3.2*	-							-			-	
Step 11	1.8												
Step 12	1.0												
Step 13	0.56												
Step 14	0.32												
Step 15	0.18												
Step 16	0.10												
Step 17	0.056												

The tracking grid used in the paired comparison threshold tracking method to determine sucrose detection thresholds. The order of presentation of pairs of solutions is determined at random (presentation order 1 signifies water is presented first within that particular pair, whereas 2 signifies the solution with a tastant (sucrose) is presented first within that particular pair). Testing begins at step 10, which corresponds to a concentration of 3.2 mM sucrose. Subjects are presented with a pair of solutions, asked to taste each solution according to the test protocol, and to point to the solution that has a taste. The tracking grid is used to record whether a subject correctly (+) or incorrectly (-) identified the solution with a taste. The concentration of the tastant in the solution presented in the subsequent pair is increased after a single incorrect response and decreased after two consecutive correct responses. Testing continues until the subject attains four reversals in performance (example is circled and marked with arrows to indicate the direction of reversal). A subject's threshold for a tastant was calculated as the mean of the log values of the last four reversals the final threshold for sucrose of this subject was 0.0042M. In this case, this subject's molar concentrations for each reserve were (0.010 M, 0.032M, 0.056M, and 0.0018M). The log of each were (-2.0) + (-2.49) + (-2.25) + (-2.74)/4 = -2.37 then we calculate the Antilog of (-2.37) = 0.0042.

Single Nucleotide Polymorphism (SNP) Selection

The taste receptor genes hypothesized to be related to sucrose detection thresholds were selected through a rigorous literature search limited to articles published in 2001-2014, written in English, using PubMed and Scopus databases.

Genes and their variants chosen for their relation to sweet taste or the consumption of sweet foods were *TAS1R2* (rs35874116), *TAS1R3* (rs35744813), and *GNAT3* (rs7792845). For *TAS1R2* gene I191V, the variants sites change amino acids in the proteins from Isoleucine (I) to Valine (V) at position 191.For the three variant sites within *TAS1R2*, genotyping results are shown as II, IV, and VV. For each of the regulatory regions of *TAS1R3* and *GNAT3*, genotyping results are shown as nucleotides (CC, CT, or TT) (**Table 5**). Of the three genotypes at this locus, those carrying the V allele have been associated with a lower habitual sugar intake (Eny et al., 2010). For the *TAS1R3* gene, the TT genotype is associated with a poorer ability to distinguish among low concentrations of sucrose compared with the CC genotype (Fushan et al., 2009). For the *GNAT3* gene, the CC genotype is associated with poorer ability to distinguish low concentration of sucrose compared with the CT or TT genotype (Fushan et al., 2010).

Table 4. *Taste Receptor and Signaling Protein Genes and Related Single Nucleotide Polymorphisms*

Sweet Taste Receptor Gene and Signaling Protein Single Nucleotide Polymorphisms								
Gene	**Marker**	**Official Name**	**Type**	**MAF**	**Type of Variant**	**Taste Association**	**Sample previously studied**	**Cite**
TAS1R2	rs35874116	Taste receptor, type 1, member 2	G protein-coupled receptor	C=0.266	Missense	Sugar consumption	Adults	Eny et al., 2010
TAS1R3	rs35744813	Taste receptor, type 1, member 3	G protein-coupled receptor	T=0.253	Upstream	Sweet taste sensitivity	Adults	Fushan et al., 2009
GNAT3	rs7792845	Guanine nucleotide binding protein, alpha transducing 3	G protein-signal transduction	T=0.320	Intron	Sweet taste sensitivity	Adults	Fushan et al., 2010
Bitter Taste Receptor Gene Single Nucleotide Polymorphisms								
TAS2R38	rs713598	Taste receptor, type 2, member 38	G protein-coupled receptor	C=0.495	Missense	Bitter taste sensitivity	Adults and Children	Mennella, Pepino, Duke, & Reed, 2010
TAS2R38	rs1726866	Taste receptor, type 2, member 38	G protein-coupled receptor	A=0.425	Missense	Bitter taste sensitivity	Adults and Children	Mennella, Pepino, Duke, et al., 2010
TAS2R38	rs10246939	Taste receptor, type 2, member 38	G protein-coupled receptor	C=0.479	Missense	Bitter taste sensitivity	Adults and Children	Mennella, Pepino, Duke, et al., 2010

Note: MAF = Minor allele frequency. A marker polymorphism is an alternative form of a gene, which is denoted with rs numbers that are unique identifiers of each variant.

Variations in the bitter taste receptor gene *TAS2R38* can explain differences in sweet taste preference in children. For the *TAS2R38* gene, the variant sites are expected to change amino acids in the proteins as follows: A49P, V262A, and I296V (Timpson et al., 2007). For *TAS2R38* gene A49P, the variants sites change amino acids in the protein from Alanine (A) to Proline (P) at position 49. For V262A, the change of amino acid occurs at position 262 with a Valine (V) to Alanine (A). Lastly, for I296V, an Isoleucine (I) changes to Valine (V) at position 296.The aforementioned *TAS2R38* variants sites form two common haplotypes with the amino acid combination of AVI and PAV. The diplotypes of these combinations predict bitter taste sensitivity. Commonly, individuals with the AVI/AVI diplotype are bitter nontasters; AVI/PAV are medium tasters; and PAV/PAV are tasters (Kim et al., 2003; Mennella, Pepino, Duke, et al., 2010). For A49P, the P allele is associated with higher sucrose preference in children compared with the AA allele (Mennella et al., 2005). Because of the tendency for closely linked alleles to be co-inherited, the V allele of rs1726866 and the I allele of rs10246939 would have a similar effect as the A allele of A49P (Kim et al., 2003).

The minor allele frequencies (MAF) for the chosen polymorphisms are also shown **in Table 5**, along with the type of variant. MAF refers to the frequency at which the least common allele occurs in a given locus and population. The MAF numbers reported here are based on dbSNP database (National Center for Biotechnology Information, 2005).

Genotyping

As part of the original parent studies, on the day of testing each child provided a saliva or buccal cell sample from which genomic DNA was extracted, purified, and quantified (BuccalAmp, Epicenter, Madison, WI, or Genotek, Kanata, Canada [**see appendix I & J**) and stored in an -80 °C freezer. Genotyping was conducted in the laboratory of Dr. Danielle Reed, director of genomics at the Monell Chemical Senses Center. The methods used and the genetic markers selected were validated in Dr. Reed's laboratory (Mennella, Finkbeiner, et al., 2014; Mennella, Pepino, Duke, et al., 2010). Genomic DNA samples were used as a template in TaqMan® assays (Applied Biosystems, Foster City, CA) in duplicate using previously established methods (Mennella et al., 2012). The extracted genomic DNA samples are diluted to 5 ng/µl, assayed for genotype and alleles identified (**see appendix K**).

For genotype quality assurance, the concentration of DNA in stored samples was checked using a Nanodrop® Spectrophometer ND100 prior to use. Subjects with known genotype from previous studies were added as controls. A random regenotyping of at least 5% of the sample was used to assess agreement of genotype between duplicate samples. A comparison of genotypes for those samples that were regenotyped resulted in 100% agreement. Samples that failed to amplify or cluster into genotype groups were genotyped once more. If no genotype could be obtained with three attempts, the value was treated as missing data.

We assessed the distribution of the expected and observed genotypes using the Hardy-Weinberg equilibrium (HWE). HWE, typically used to identify genotyping errors

(Teo, Fry, Clark, Tai, & Seielstad, 2007), assumes that the genetic variation in a population will remain constant from one generation to the next in the absence of other evolutionary factors, such as mutations, natural selection, nonrandom mating, gene flow, and genetic drift (Contento, 2008). For example, when there are two alleles for a particular gene (A and B) and their frequencies is p and q, then p + q must equal 1. The formula for calculating HWE is p2 + 2pq + q^2 = 1. Then, we can calculate the expected frequencies of the genotypes AA (p^2), AB (2pq), and BB (q^2) (Hosking et al., 2004; Ryckman & Williams, 2008).

Adiposity Measures

Height and weight were measured in children in the laboratory. BMI and BMI z-score measures were available in all children for whom we had height and weight. However, the measures of fat mass (percent body fat), waist circumference and waist-to height ratio (WHtR) were taken in a subset of 96 children from study 2. Details on validity and reliability of the instrumentation used in this study are provided in **Table 6**

Height and Weight

All but three children (n=232) were weighed (kg) and measured for height (cm) wearing light clothing and no shoes using a physician's scale (model 439, Detecto, Webb City, MO).

Body Mass Index (BMI) Categories and BMI z-Scores

BMI, the most widely used measure to screen for obesity (CDC, 2013), was computed from the measured weight and height as kilograms per meters squared (Cole, 2008). However, BMI is considered an imperfect tool to measure obesity in adults since

it does not discriminate between excess fat mass and excess lean mass (Romero-Corral et al., 2008). This concern is also true for children and adolescents when using BMI. BMI is a moderately sensitive measure of adiposity (Demerath et al., 2006; Freedman & Sherry, 2009) and thus measurement of adiposity by other means is indicated.

Participants were then classified into one of four BMI categories (i.e., underweight, healthy, overweight, and obese) following the Centers for Disease Control and Prevention charts for pediatric BMI by age and sex for children 2-18 years of age. The BMI-for-age reference in the United Stated is based on nationally representative sample from boys and girls ages 2–20 years (Kuczmarski et al., 2000).

BMI z- scores are the most common method to assess overweight and obesity in children and adolescents and are validated for use in this population (Inokuchi, Matsuo, Takayama, & Hasegawa, 2011; Kakinami, Henderson, Chiolero, Cole, & Paradis, 2014; Kuczmarski et al., 2002; Must & Anderson, 2006). BMI z-scores are measures of relative weight adjusted for child age and sex. The Z score characterizes the number of standard deviations from the mean and permits comparing the BMI of a given child to the BMI distribution for a population of children of the same age and sex (Must & Anderson, 2006). The BMI z score represents a child's BMI in a standard normal distribution with a mean of zero and a standard deviation of 1. For this study, BMI z-scores were calculated using EpiInfo 3.5 www.cdc.gov/epiinfo) (Kuczmarski et al., 2002).

Body Fat

Body fat was estimated by bioelectrical impedance analysis (BIA) using the Quantum X instrument (RJL Systems, Clinton Township, MI) and Body Composition 2.1 software using a new pediatric equation, validated for children 3.9 to 19.3 years of age (Chumlea et al., 2002; Kriemler et al., 2009; Wu et al., 1993). Body fat was estimated in kilograms and as a percentage of measured body weight. BIA is a commonly used method, based on the conduction of electrical current in the body and the differences in the resistance to conduct electricity between the fat and water components of the body. BIA has been validated with dual-energy X-ray absorptiometry (DXA) (Sun et al., 2005; Thomson, Brinkworth, Buckley, Noakes, & Clifton, 2007). The BIA method has been validated for use in the pediatric population (Houtkooper, Going, Lohman, Roche, & Van Loan, 1992; Okasora et al., 1999; Talma et al., 2013). Measures were obtained by following the manufacture's protocol (http://www.rjlsystems.com/documentation/how-electrodes-are-placed-on-the-hand-and-foot/). In this study, trained research assistants measured BIA in a subset of 96 children.

Waist Circumference (WC)

This measure has been used in previous studies and validated in children (Bergmann et al., 2010; McCarthy & Ashwell, 2006; Taylor, Jones, Williams, & Goulding, 2000). WC was measured to assess central adiposity measuring their abdominal girth recorded to the nearest 0.1 cm by the subject standing with his or her weight evenly distributed on both feet and with the feet about 25-30 cm apart.

In this study, trained research assistants measured waist circumference in the subset of 96 children using a plastic tape measure. The waist was defined as the point midway between the iliac crest (superior border of hip bone) and the costal margin (lower rib). Two measures were recorded; if the difference between the measures was greater than 1 cm, a third measure was performed; the mean of the two closest measures was calculated and reported.

Waist-to-Height Ratio (WHtR)

WHtR is defined as the ratio obtained from an individual's waist circumference (centimeters) divided by their height (centimeters) and is a measure of a central obesity; a high WHtR is commonly associated with poor metabolic health in adults and in children (Khoury, Manlhiot, & McCrindle, 2013; Park & Kim, 2012). It is considered by some to be a measure of body fat distribution that has greater validity than BMI (Khoury et al., 2013; Savva, Lamnisos, & Kafatos, 2013). WHtR was computed in the subset of 96 children who had waist circumference measured in this study. Children with a WHtR ratio >0.5 were considered as having central adiposity (Khoury et al., 2013; Mokha et al., 2010).

Diet Related Food Behaviors

Dietary Intake

In the subset of 96 children, a recall of the previous day's food intake was obtained using the Automated Self-Administered 24-Hour Recall System (ASA24), a web-based, validated, self-administered 24-hour dietary recall instrument developed by the National Cancer Institute (Bethesda, MD) (ASA24-Kids-2012; National Cancer Institute, n.d.; Kirkpatrick et al., 2014). Mothers and children sat side by side as the mother reported to a trained researcher the food intake from the previous day for her child. The child was asked about food eaten and to report any snacks or foods eaten outside the home (e.g., at school) (Kirkpatrick et al., 2014). After a subject reported a specific food or beverage, ASA24 provided a visual depiction of the item, which allowed subjects to estimate portion sizes. The ASA24 used a series of questions to establish a quick list of foods eaten, a query for long gaps between eating occasions, questions about preparation methods and serving sizes of food items, forgotten foods, and a final review. The information obtained was analyzed using the U.S. Department of Agriculture's Food and Nutrient Data System software, version 4.1.

From these data, we focused specifically on daily caloric intake (kcal/day), added sugar intake (g added sugar/day), and added sugars as a percentage the total caloric intake. The intake of calories and added sugar was expressed relative to body weight (kg) (National Cancer Institute, n.d.).

The Goldberg cutoff method was used to evaluate an individual's bias in reporting energy intake, the greatest risk of which is under-reporting energy intake

(Livingstone & Black, 2003). The diet information was eliminated for children identified as under-reporters prior to further analysis (Champagne, Baker, Delany, Harsha, & Bray, 1998). The child's reported energy intake (kcal) was calculated relative to the basal metabolic rate (BMR) based on their age and gender to obtain a ratio (**Appendix F**). Physical activity was not measured in this study. Children who reported energy intake/BMR <1.0 were considered under-reporters as children would not maintain a state of health with energy intake below basal energy needs. For this study, children who reported no intake of added sugar were also excluded.

Personal Characteristics

Child's Temperament Questionnaire and Food Neophobia Scale (FNS)

Temperament is defined as the set of biological characteristics that are present in an individual from birth to adulthood. A child's temperament has been associated with eating behavior (Haycraft et al., 2011) and taste preference (Liem & Mennella, 2002). In addition, temperament traits have been associated with overweight and obesity in children and adults (Agras et al., 2004; Haycraft et al., 2011). Children's temperament may explain why some children are at risk for overweight or obesity, and temperament may relate to sucrose detection thresholds in children. Therefore, we measured dimensions of child temperament particularly as they relate to eating behaviors (Pliner & Loewen, 1997).

A dimension of children's temperament that may translate into poor dietary intakes and unhealthy weight outcomes (underweight and obesity) in children is food neophobia. Food neophobia is considered the inborn personality attribute characterized

by the rejection of foods that are new or completely unknown to the child (Dovey, Staples, Gibson, & Halford, 2008; Pliner & Hobden, 1992). Food neophobia in children has been found to be associated with food consumption, particularly fruits and vegetables (Cooke, Wardle, & Gibson, 2003; Galloway, Lee, & Birch, 2003). Food neophobia has also been associated with bitter taste sensitivity (Carter et al., 2000).

For this study, mothers filled in their child's temperament and food neophobia questionnaires (**Appendix G & H**). The survey was comprised of 31 items, each rated on a five-point scale, asking parents whether they agreed or disagreed with the statements asked about their child. Higher scores indicated more of the characteristic. Twenty-five items in the scale measured the child's temperament, which is made up of five dimensions: emotionality, shyness, activity, sociability, and negative reactions to food. For example, activity in the temperament scale measures how physically active a child is and the level of activity a child engages in during a day, ranging from very low to very high. The original Food Neophobia Scale (FNS) has 10-items with good reliability and validity (Pliner & Hobden, 1992). In this study, Food Neophobia was measured with six items in the survey chosen because they seem to best capture responses to new foods. This short scale has been used in prior studies (Cooke, Carnell, & Wardle, 2006; Liem & Mennella, 2003).

Instrumentation: Validity and Reliability

Table 5. *Validity and Reliability of Instruments*

	Reliability and Validity
Two alternative forced –choice staircase procedure	The two alternative forced-choice procedures discourage response biases (Linschoten, Harvey, Eller, & Jafek, 2001). This method is brief and has evidence of reliability and external validity in children. When methodology was used for sucrose preference reliability was assessed by comparing the results of the first series (i.e., weaker stimulus presented) with the second series (i.e. stronger stimulus) Statistically significant correlations between the concentrations of sucrose chosen in the 2 series were observed for children (ICC = 0.42, *n* = 338), adolescents (ICC = 0.46, *n* = 168) (Mennella et al., 2011).
Height	Measures obtained in the laboratory by a trained technician were shown to me more reliable. In an adult study of self-reported height participants overestimated their height by 2.2±3.5 cm (mean ± standard deviation [SD]) (Griebeler, Levis, Beringer, Chacra, & Gómez-Marín, 2011). Likewise, children tend to overestimate their height (Beck et al., 2012). Systematic measured height has been reported to be more valid than self-report as discrepancy estimates between the two methods have been observed, with individuals overestimating their height (Powell-Young, 2012).
Weight	To ensure accuracy of measurements duplicate assessments are taken. With a third measure needed if the first two are very different. Measures were obtained in the laboratory since measured height by a trained technician was shown to me more reliable. In a self-reported study of adults where weight was obtained, weight was underestimated by 3.1±6.5 lb (1.5 ± 2.9 kg) (Griebeler et al., 2011). Likewise, children tend to underestimate their weight (Beck et al., 2012). Measured weight has been reported to be more valid than self-report, as underestimation of weight is often common (Powell-Young, 2012).
Body Mass Index (BMI)	BMI has been evaluated in the literature against the gold standard measure of adiposity dual x-ray absorptiometry (DXA) in children. The correlation of BMI with DXA total fat mass was 0.85 in children 3-8 years old (Eisenmann, Heelan, & Welk, 2004). Correlation between BMI and DXA total fat was 0.84 for boys and 0.90 girls. Bland-Altman plots of the methods indicated agreement (Boeke et al., 2013).
BMI z Score.	Association of BMI z score with DXA percent body fat has been reported to be having good agreement (r= 0.82) in children 5-18 years old (Freedman & Sherry, 2009). Similar findings have been reported in other studies in children age 3-18 years (Mei et al., 2002). BMI z score with DXA trunk, DXA % fat, DXA fat, ranged between r=0.63-0.80. In addition it was reported that the Bland-Altman plots of the methods indicated good agreement (Boeke et al., 2013). The validity and reliability of this measure depends on the accuracy of both height and weight.
Bioelectrical Impedance %body fat	Bioelectrical impedance % body fat was correlated with DXA trunk, DXA % fat, DXA fat 0.82, 0.73 and 0.84 respectively in children (Boeke et al., 2013).
Waist Circumference	Waist circumference (WC) has been found to correlate with DXA trunk fat (r=0.79). The correlation between DXA trunk fat with WC has been found to be good for boys (0.79) and girls (r=0.87) (Boeke et al., 2013). Intrarater reliability (degree of agreement among repeated measures by a single rater) of waist circumference across BMI subgroups was reported as ICC>95 (Wang, Liu, & Chen, 2010). Intrarater technical error of measurement did not exceed 1.14 cm (Moreno et al., 2002).
Waist-to-height ratio (WHtR)	WHtR strongly relates to children's DXA-trunk fat mass index (r = 0.93). Umbilical waist-to-height ratio and trunk fat mass index (DXA) are markers of central adiposity and insulin resistance in children (Guntsche et al., 2010).
Dietary Intake (ASA 24)	The Automated Self-Administered 24-Hour Recall has been validated it against interviewer-administered Automated Multiple-Pass Method (AMPM) recalls and also against weighed actual food intake at meals. Data reported in ASA24 and AMPM were highly comparable (Kipnis et al., 2003; Kirkpatrick et al., 2014; Moshfegh et al., 2008). This validation was done in adults, not children. However, the ASA24-Kids method is used with a proxy reporter, such as parent or guardian (National Cancer Instititue, 2012).
Child Temperament	The child temperament scale was validated against the Reactions to Food Scale of the Colorado Childhood Temperament Inventory as well as EAS Temperament Survey for Adults (Pliner & Loewen, 1997).
Food Neophobia Scale (FNS)	The FNS assesses willingness to eat foods. The test-retest correlation in this scale was assessed in two samples r = 0.91 and r = 0.87, p < 0.01, and then tested 15 weeks later for all subjects, and the correlation was r=0.82, p<0.01(Pliner & Hobden, 1992). This measure has been validated against behavioral observations of children's willingness to taste foods (r = 0.38, p <0.001) and parent predictions of their child's willingness to try foods (r = 0.34, p < 0.001) (Pliner, 1994). The internal reliability of the short scale has been reported with a Cronbach's alpha coefficient of 0.92 (Cooke et al., 2006).

Study Variables

Several outcomes were measured in the children recruited for this study. **Table 7** summarizes some of the variables and their methods of measurement.

Table 6. *Variables and Methods*

Variable	Method of measurement	Operational variable
Demographics		
Gender	Reported by mother	Male or female
Age (at time of testing)	Reported date of birth by mother	Age in years on date of testing
Psychophysics		
Sucrose detection threshold Lowest sucrose concentration detected	Measured with forced choice paired comparison tracking procedure	mM of sucrose
Genetics		
TAS1R1, TAS1R2, TAS2R38, and GNAT3 polymorphisms	Genotyping	Genotype
Adiposity measures		
Body mass index	Measured height and weight	kg/m^2
BMI percentile and categories	CDC BMI for age and sex standards	Underweight, ≤5% Normal weight, 6-84.9%, Overweight, 85-94.9% Obese, 95-100%
BMI z-score	Calculated with EpiInfo 3.5	z-score
Waist circumference	Measured	Waist circumference in centimeters
Body fat	Measured by bioelectrical impedance analysis	Percent (%)
Waist to height ratio (WHtR)	Measured waist circumference and height	
Diet related food behaviors		
Dietary caloric intake	From ASA24	kcal/day
Total added sugars per day	From ASA24	g, g/kg, % of total kcal
Personal Characteristics		
Child temperament and Food Neophobia Scale (FNS).	Reported by mother	Emotionality, shyness, activity, sociability, negative reaction to foods and food neophobia

Importance of the Knowledge Gained

Although there is a body of literature describing the variability of taste preference and taste thresholds in humans little has been described in regards to sucrose detection in

children and little attention has been paid to how children differ in their sense of taste and how these differences might affect their health and behavior. Our study was able to make a contribution to the growing body of knowledge related to obesity in children and addresses an important gap in the field of chemosensory testing and obesity. The findings described herein could enhance our ability to identify children at higher risk for obesity and enable us to develop individualized interventions and treatment strategies. In addition, these findings can ultimately provide greater insight on how to intervene to generate sustainable long-term results for prevention of obesity and its related comorbidities, therefore helping to decrease health care costs associated with this condition.

Data Analysis Plan

Data Management

Data collection and coding occurred in the original studies; all subsequent analyses refer to these coded data in such a manner that individual identities cannot be traced. To maintain the safety and confidentiality of the subjects, all records were stored in a locked filing cabinet in a room dedicated solely for this purpose. Computer-based files were made available only to personnel involved in the study through the use of access privileges and passwords. Prior to access to any study-related information, personnel were required to sign statements agreeing to protect the security and confidentiality of identifiable information.

Each subject tested received a unique identity number, which was used to label cheek cell and saliva samples, questionnaires, and data collection forms. All hard copy

documentation obtained during the study was locked in the laboratory of Dr. Julie Mennella. All data were maintained in a database (Microsoft Access), and subject ID to facilitate data manipulation related the tables. The Mennella lab had a RAID 5 network-attached storage device that was backed up nightly to ensure database integrity. Several hard drives in her laboratory also maintained multiple backup copies of the database should any primary drive fail. The coded data were stored and maintained in a secure server that only Dr. Mennella and her staff can access. All biological samples were stored as coded samples in Dr. Reed's lab, kept in a -80 °C freezer. Solely Dr. Reed and her staff control this laboratory. Genotyping was done with coded samples, and no identifying information was available to Dr. Reed or her laboratory members.

Screening for Missing Data, Normality, Linearity and Multi-collinearity

Prior to data analysis, the data were screened for missing data. The amount to which the missing data were a problem was evaluated by exploring the pattern of the missing data within and across variables. Data were also tested for normality using the Kolmogorov-Smirnov test for normality. If violations in the normality assumption were detected, values were transformed to approximate a normal distribution and non-parametric data analysis options were considered.

Assessing for Selection Bias

We first established that there were no significant differences between the data obtained in both original taste studies (studies 1 and 2) for children from whom we had valid sucrose thresholds data for variables for which we had data for both groups. Study 2 refers to those children that were considered a subset in this dissertation. We determined

these differences using Student t-test and Chi square test (χ^2 test). We did this to ensure that findings with the subgroup of children were still representative of the overall group.

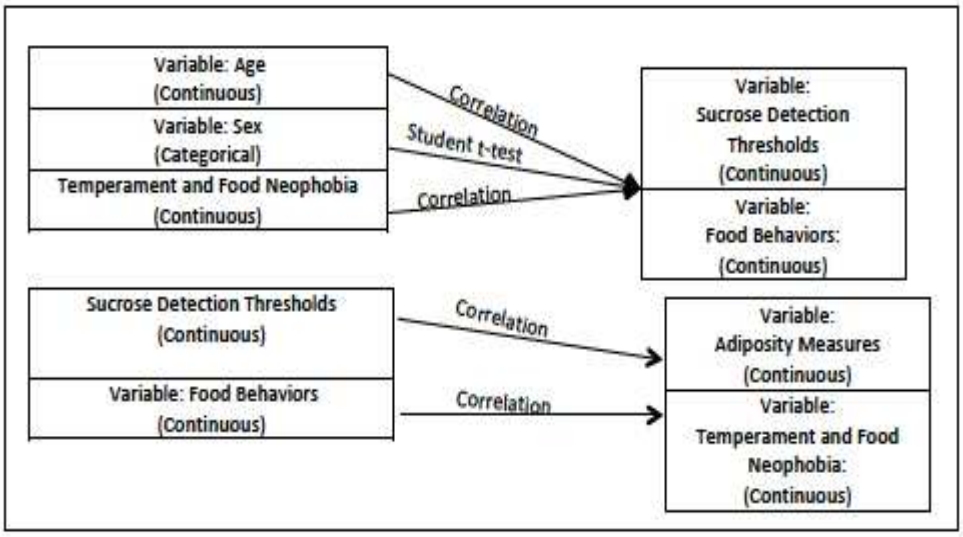
Statistical Analysis for Sample Description

For demographic characteristics and all summary statistics of primary and secondary measures, we computed means and standard deviations (means ± SD) for continuous variables and counts and percentages for categorical variables. The primary outcome measures for this study were sucrose detection thresholds, taste receptor genotype, adiposity measures, temperament and diet related food behaviors.

Statistical Analysis of Aim 1

For aim 1, student's t-tests were used to examine sex differences for sucrose detection thresholds, personal characteristics (temperament, food neophobia), and diet related food behaviors (**Figure 10**). Pearson's correlations were used to examine the relationship between sucrose detection thresholds, personal characteristics and diet related behaviors with age. In addition, Pearson's correlation was also used to explore the relationship of sucrose detection thresholds with personal characteristics (negative reactions to foods and food neophobia), diet related behaviors and adiposity. Correlation results were reported as follows: correlation (r) (degrees of freedom) = p value. We used the Colton's rule for interpreting the size of the correlations (Colton, 1974). Those findings that had a fair [r 0.25 to 0.50 (-0.25 to -0.50)], moderate to good [r 0.50 to 0.75(-0.50 to -0.75)] or very good [r >0.75 -0.75] relationship with sucrose thresholds were included in subsequent analysis for GLM in Aim 2 and Aim 3.

Figure 10. *Bivariate Data Analysis Plan for Aim 1*

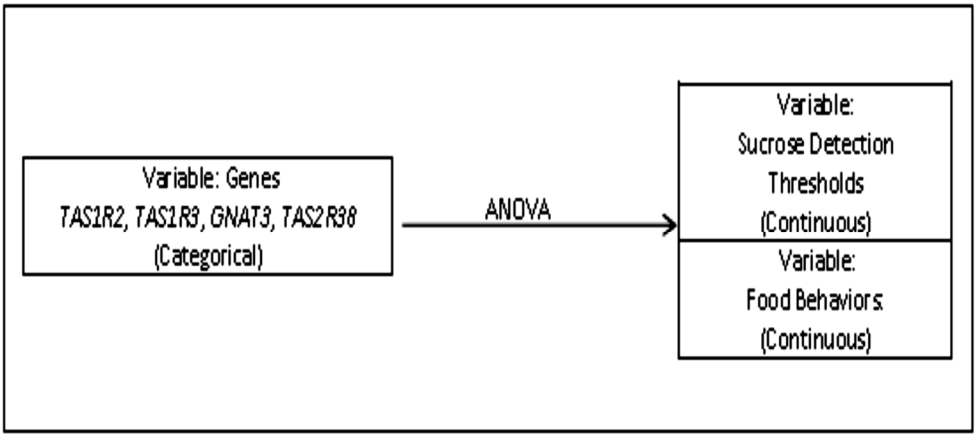


Statistical Analysis for Aim 2

For aim 2, only subjects for whom valid sucrose thresholds were obtained were included. Genotype–phenotype association studies require that subjects be genetically unrelated (N=175) (Khoury, Beaty, & Cohen, 1993), so in instances where more than one child from a family was tested, only one child was selected at random to be included in the genetic analysis (Mennella et al., 2012). Departure from the expected genotypic frequencies in HWE was tested using the Hardy-Weinberg Calculator by Michael H. Court by comparing the observed and expected genotype frequencies for cases and controls (Court, 2005). Separate one-way ANOVAs using genotype (*TAS1R2*, one variant site; *TAS1R3*, one variant site; *GNAT3*, one variant site; and *TAS2R38*, three variant sites) were conducted (Mennella et al., 2012). Genotypes that were associated with sucrose threshold were evaluated for their relationship with measures of diet related food behaviors (child's dietary intake), we did this to explore whether there was a difference in

dietary consumption based on child's genotype. **Figure 11** shows a depiction of the bivariate analysis plan for aim 2.

Figure 11. *Bivariate Data Analysis for Aim 2*



Analysis Plan for Multivariable Model Aim 2

Next drawing on the conclusions from the separate from the bivariate analyses described above, we used general linear models (GLM) with those outcome measures significantly related to sucrose detection thresholds ($p<0.05$). These variables were included in a multivariable model to establish the effect size of each and examine independent determinants of sucrose detection thresholds. The first model included sucrose thresholds, genotype, age and sex. This first model was run in the overall sample and then a second model with the same variables was run in the subset of children to assess whether the results remained consistent. Lastly, a model included sucrose threshold adjusted by age, sex, genotype and one adiposity measure. Only one adiposity measure was added to the model to avoid multi-collinearity. The variables chosen met significance in the univariate analysis. The analyses followed methods established in

earlier studies of genotype in children and taste-related measures and were conducted separately for each gene. Variants that were significant with a $p<0.05$ were included in subsequent genetic analysis.

Multivariable modeling is appropriate as a strategy because many of the measures were potentially interrelated (Rosner, 2006). The criterion for statistical significance for the omnibus statistical tests and Fisher's least squares difference tests were conducted on significant results to compare specific group means (post hoc tests was alpha=0.05). The parameter of interest for GLM was sucrose detection threshold.

Statistical Analysis for Aim 3

For aim 3, we further explored the relationship between personal characteristics with adiposity measures using Pearson's correlations. Correlation results were reported as follows: correlation (r) (degrees of freedom) = p value. Here, we used the Colton's rule for interpreting the size of the correlations (Colton, 1974) as previously described.

Separate one-way ANOVAs were used to look at whether gene variants that were related to sucrose thresholds from aim 2 were also related to adiposity measures.

Analysis Plan for Multivariable Model Aim 3

GLMs were used to construct a multivariable model to examine independent predictors of the adiposity measures. This model had the adiposity measures that were significantly related with sucrose thresholds as the dependent variables. Age, sex, sucrose thresholds, diet related food behaviors and personal characteristics were considered covariates.

All analyses for this study were conducted with Statistica, version 12 (StatSoft, Tulsa, OK), with the level of significance set at alpha = 0.05. HWE was calculated with Court's Hardy-Weinberg Calculator (Court, 2005). Graphs were generated using GraphPad Prism, version 6.01 (GraphPad Software, La Jolla, CA).

CHAPTER 4

Results

Normality test using Kolmogorov-Smirnov (K-S) test

The distributions of sucrose detection threshold values were not normally distributed as evaluated by the Kolmogorov-Smirnov (K-S) test for normality (Chakravarti, Laha, & Roy, 1967) ($d = 0.187$, $p < 0.01$). Prior to the main statistical analyses these values were square root transformed to approximate a normal distribution K-S $d = 0.105$, $p < 0.05$. The distribution was normal for age, adiposity measures, temperament scale and food behavior measures (**Table 8**).

Table 8. *Normality Test using Kolmogorov-Smirnov (K-S) Test*

Variable	K-S (*d*)	P value (*p*)
Demographic		
Age	0.077	<0.15
Sucrose Threshold		
Sucrose detection thresholds	0.187	<0.01
Anthropometric/Adiposity Measures		
BMIz	0.055	>0.20
WHtR	0.106	>0.20
%Body Fat	0.703	>0.20
Personal characteristics:		
Temperament Scale and Food Neophobia		
Shyness	0.064	>0.20
Emotionality	0.073	>0.20
Sociability	0.096	>0.20
Negative Reaction to foods	0.108	>0.20
Activity	0.076	>0.20
Food Neophobia	0.124	<0.15
Diet related food behaviors		
Total calories	0.125	<0.10
kCal/kg	0.129	<0.10
Added sugars as percent of kcal	0.118	>0.20
Added sugars g/kg	0.119	>0.20

Results from Assessing for Selection Bias

Prior to analyzing our aims, we established that there was no statistically significant difference between the children from whom data were collected in the original study #1 and study #2. There was no significant difference in sucrose thresholds t (df 214) = -1.28, p = 0.201, age t (df 214) = +0.03, p = 0.971, or sex (χ^2 = 0.71, p = 0.399) between the two studies (**Table 9**). We acknowledge that there were significant differences between the studies based on race, income and education. Parents in study 2 had higher income and levels of education compared to study 1. There were also significant racial differences between the groups. This could have been because study 1 had a larger percent of participants who selected other, more than one race or Hispanic. Although these findings were significant differences between groups, these variables were not part of the aims in this study. In our important predictors and outcome variables, there were no significant differences between study 1 and study 2. **Table 9** describes the comparison of variables in the two studies.

Table 9. *T- test or Chi- Square (χ^2) Comparison for Sucrose Thresholds, Demographics and Socioeconomic Characteristics for the Original Studies*

Characteristic			T-tests or Chi Square		
	Study #1	Study #2	t-value or χ^2	df	p-value
Demographics					
Age (years) [mean± *SD* (*n*)]	10.3±2.0 (130)	10. 4±1.8 (86)	+0.036	214	0.971
Sex [% (*n*)] (female)	52.3% (68)	58.1%(50)	+0.710	214	0.399
Race [% (*n*)]					
Black	60.8% (79)	51.2% (44)			
White	23.1% (30)	16.3% (14)			
Asian	0% (0)	2.3% (2)	+9.751	3	0.021
Other/more than one race	16.2% (21)	30.2% (26)			
Sucrose Detection Threshold					
Sucrose Thresholds (mM) [mean± *SD* (*n*)]	10.62±15.2 (130)	12.9± 8.5 (86)	-1.282	214	0.201
Anthropometric/Adiposity Measures					
Weight-for-age z scores [mean± *SD* (*n*)]	0.78±1.08 (128)	0.93±1.24 (86)	+0.959	212	0.339
Height-for-age z scores [mean± *SD* (*n*)]	0.50±1.12 (128)	0.58±1.08 (86)	+0.489	212	0.434
BMI z scores [mean± *SD* (*n*)]	0.72±0.98 (128)	0.84±1.21 (86)	+0.783	212	
BMI categories [% (*n*)]					
Underweight: BMI percentile <5%	0.8%(1)	3.5%(3)	+6.205	3	0.102
Healthy weight: BMI percentile 5-85%	58.6%(75)	46.5%(40)			
Overweight: BMI percentile 85-95%	23.4%(30)	22.1%(19)			
Obese: BMI percentile >95%	17.2%(22)	27.9%(24)			
Ethnicity [% (*n*)]					
Not Hispanic	96.9%(126)	87.2%(75)	+7.588	1	0.006
Hispanic	3.1%(4)	12.8%(11)			

Socioeconomic Characteristics					
Family income [% (*n*)]					
<$49,999	56.2%(73)	75.6% (65)	+15.251	3	0.002
$50,000-99,000	23.1%(30)	20.9%(18)			
>$100,000	10.8%(14)	3.5%(3)			
NO DATA	10% (13)	0%(0)			
Highest Level of Education, Mother [% (*n*)]					
Grade school	0%(0)	5.8%(5)	+25.003	4	<0.001
High School	36%(45)	44.2%(38)			
Trade	0%(0)	9.3%(8)			
College	56.8%(71)	37.2%(32)			
Graduate	7.2%(9)	3.5%(3)			

Subject Characteristics

As shown in **Table 10**, the study population consisted of 235 children whose race/ethnicity, family income, and maternal education levels reflected the diversity of the city of Philadelphia (Pew Charitable Trust, 2011). More than half (57.8%) of the children were Black (*n*=136), 19.6% were White (*n*=46), and 21.7% were of more than one race. The vast majority (93.2%, *n*=219) were non-Hispanic (**Figure 12**). This increased representation of under-represented minorities in the subject population is reflective of the greater Philadelphia region (Pew Charitable Trust, 2013), and has been achieved, in part, through outreach efforts advertising in local newspapers throughout the city. The mean age of the sample was 10.4 ± 1.9 years. Female subjects made up 52.8% (*n*=124), and males accounted for 47.2% (*n* = 111) of the study population. Most children were unrelated (*n* = 122), but the sample included 46 two-sibling pairs (*n* = 92) and 7 families of three siblings (*n* = 21). More than half (66.4%, *n* = 156) of the participants' mothers reported a family income of less than $49,999, with 47.7% (*n* = 112) having a college degree.

Regarding adiposity measures, 21.6% of children were obese, 22.4% were overweight, 53.4% were normal weight, and 2.2% were underweight. The overweight and obese children in the sample were higher than most current statistics for children in Philadelphia by the CDC and lower than the reported data from the Pew Report for adults

in Philadelphia (**Figure 13**). We further show the breakdown of the sample in regards to the BMI category by sex (**Figure 14**).

Demographic Characteristic of the Subset of Children

The subset of children in this study for whom we had additional data did not differ from the overall sample for sucrose detection thresholds. The demographic characteristics for the subset of children (**Table 10)** reflected the diversity of the city of Philadelphia (Pew Charitable Trust, 2011). The mean age of children in the subgroup was 10.4±1.7 years and the distribution of female subjects made up 55.2% (*n*=53), and males accounted for 44.8% (*n*=43) of the subset group. In this group, 28.1% of children were obese, 20.9% were overweight, 47.9% were normal weight, and 3.1% were underweight. In addition, in the subset of children who had data on percent body fat and WHtR (*n*=96; **Table 10**), percent body fat averaged 32.9% (range, 9.8-60.8%) and 38.5% were classified as having central obesity.

Table 10. *Child, Maternal and Household Characteristics of Participants*

Characteristic	All Children Study 1+2 *N*=235	Subset[a] Study 2 *n*=96
Demographics		
Age (Years) [mean± *SD* (*n*)]	10.4±1.9 (235)	10.4±1.7 (96)
Sex [% (*n*)] (Female)	52.8% (124)	55.2% (53)
Race [% (*n*)]		
Black	57.8% (136)	51.0% (49)
White	19.6% (46)	16.7% (16)
Asian	0.9% (2)	2.1% (2)
Other/more than one race	21.7% (51)	30.2% (29)
Ethnicity [% (*n*)]		
Not Hispanic	93.2% (219)	87.5% (84)
Hispanic	6.8% (16)	12.5% (12)
Sucrose Detection Threshold		
Children who completed the task [% (*n*)]	91.9% (216)	89.6% (86)
Detection thresholds [mM; mean ± *SD* (*n*)]	12.0±12.9 (216)	10.6±8.4 (86)
Child Adiposity Measures		
Weight-for-age z scores [mean ± *SD* (*n*)]	98.7% (232)	100% (96)
Height-for-age z scores [mean ± *SD* (*n*)]	0.84±1.14 (232)	0.94±1.21 (96)
BMI z-scores [mean ± *SD* (*n)*]	0.54±1.09 (232)	0.63±1.07 (96)
BMI categories [% (*n*)]		
Underweight: BMI percentile <5%	2.2% (5)	3.1% (3)
Healthy weight: BMI percentile 5-85%	53.8% (125)	47.9% (46)
Overweight: BMI percentile 85-95%	22.4% (52)	20.9% (20)
Obese: BMI percentile >95%	21.6% (50)	28.1% (27)
Percent body fat [mean ± *SD* (*n*)]	-	32.9±11.6 (95)
Waist-to-hip ratio [mean ± *SD* (*n*)]	-	0.87±0.14 (96)
Waist-for-height ratio (WHtR) [mean ± *SD* (*n*)]	-	0.49±0.07 (96)
Diet-related Food Behaviors[b]		
Daily calories	-	2,284±794 (73)
Total (kcal)	-	57±28 (73)
Relative to body weight (kcal/kg)	-	
Daily added sugars [mean ± *SD* (*n*)]	-	81.43±48.19 (73)
Total (g)	-	14±7 (73)
Percent total calories	-	19.7±1.19 (73)
Relative to body weight (g/kg)	-	2,284±794 (73)
Socioeconomic Characteristics		
Family income [% (*n*)]		

<$49,999	66.4% (156)	77.1% (74)
$50,000-99,000	20.9% (49)	19.8% (19)
>$100,000	7.2% (17)	3.1% (3)
NO DATA	5.5% (13)	0.0% (0)
Highest Level of Education, Mother [% (*n*)]		
Grade school	2.1% (5)	5.2% (5)
High School	38.7% (91)	44.8% (43)
Trade	3.9% (9)	9.4% (9)
College	47.7% (112)	36.5% (35)
Graduate	5.5% (13)	4.1% (4)
Not known/not reported	2.1% (5)	0% (0)

[a]In this subset of children further anthropomorphic and dietary measures were measured.
[b]Dietary records for 19 of the 96 children were excluded based on the Goldberg cutoff. 4 children did not provide data for added sugars

Figure 12. *Race Demographics: Comparison of Pew Reports for Philadelphia and Current Study*

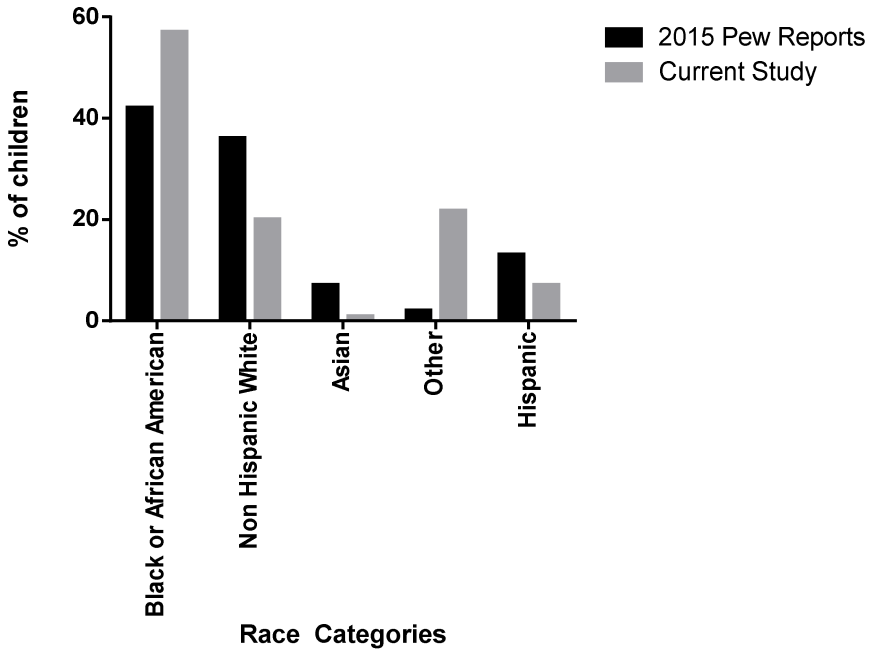


Figure 13. Overweight *and Obese: Comparison of Philadelphia Pew Reports, CDC Data for Pennsylvania* (CDC, 2015), *Philadelphia Department of Public Health and children in current study

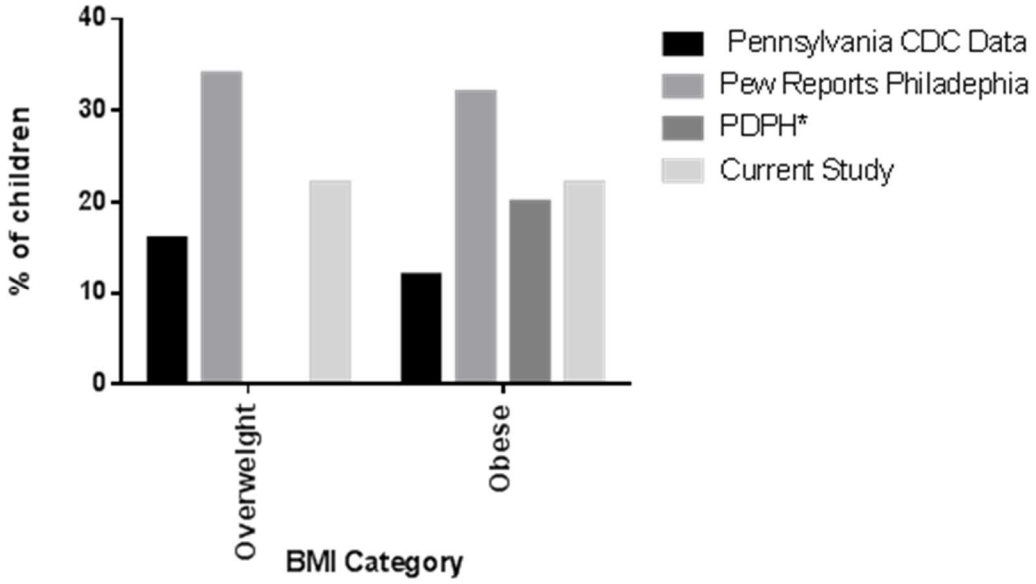
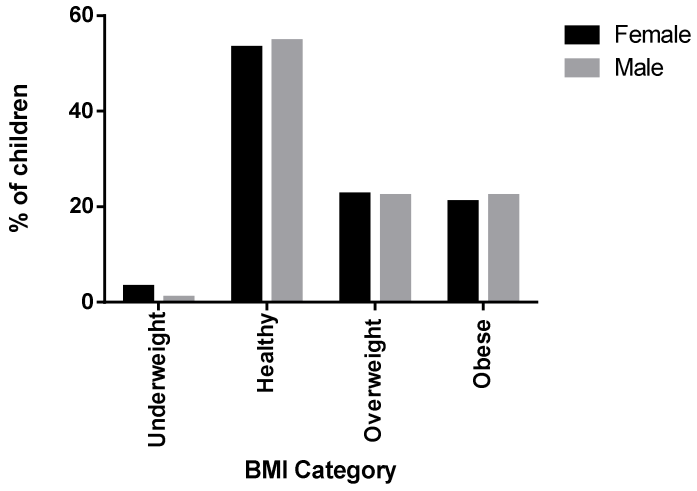


Figure 14. *Children's BMI by Sex*

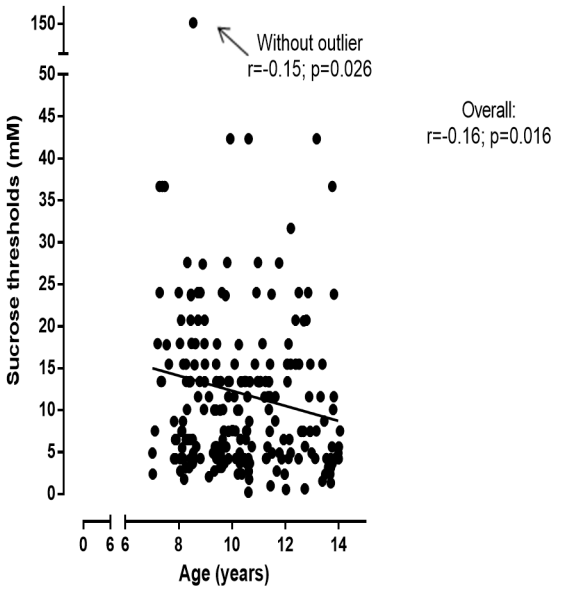


Results for Aim 1

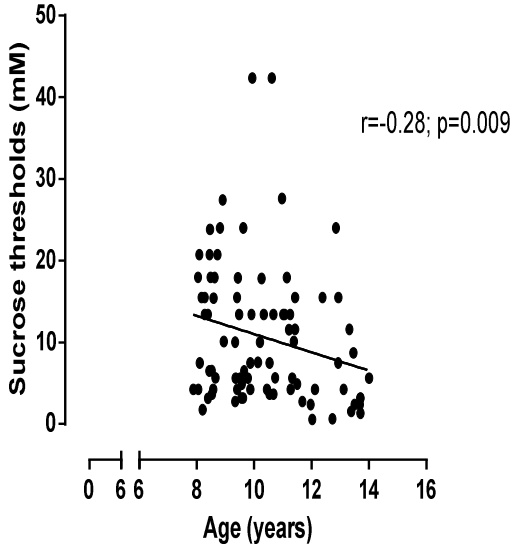
Relationship of Sucrose Detection Thresholds with Age and Sex

Most children (91.9%, n = 216/235) completed the psychophysical task; 19 children did not comply with the procedures, became tired, or refused to continue to participate. Only data from subjects with valid sucrose detection thresholds were used (n = 216). The mean detection threshold was 12.0 mM (±12.9 *SD*) and range was 0.23-153.8 mM sucrose (**Table 10**). In the whole sample, as age increased children had significantly lower sucrose detection thresholds (were more sensitive) [r (214) = -0.16, p = 0.016] **(Table 11, Figure 15A)**, and girls had significantly lower thresholds than did boys [10.5 ± 8.6 mM vs. 13.9 ± 16.6 mM; t (214) = 2.0, p = 0.047] **(Table 11, Figure 16**). We confirmed the negative correlation between age and sucrose detection thresholds in the subset of children [r (84) = -0.28, p = 0.009] **(Table 11)**.

Figure 15. *Sucrose Taste Detection Thresholds and Age*

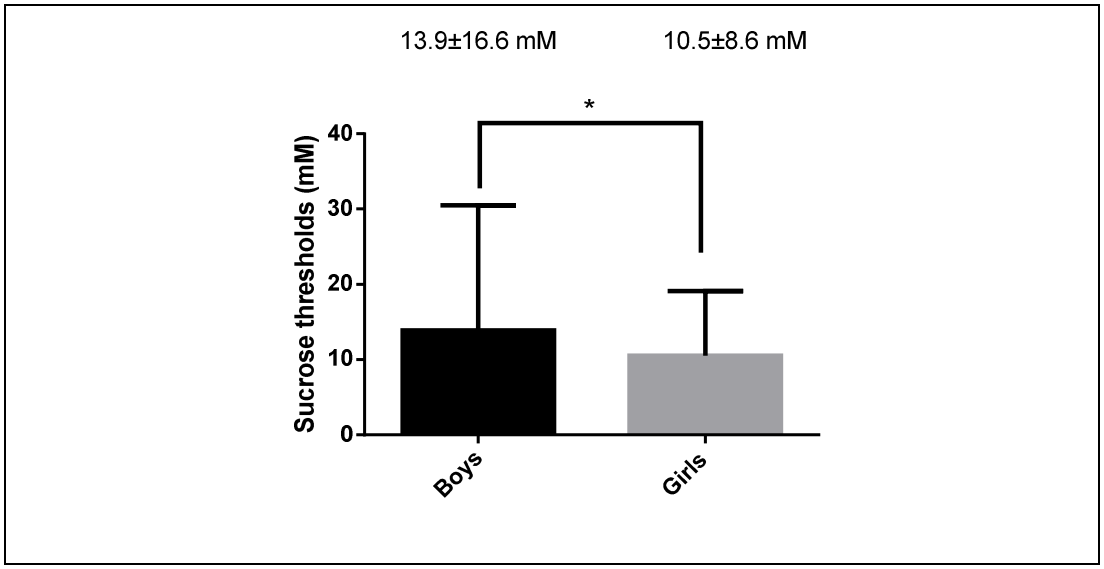


A- All Children (N=216) with outlier



B- Subset of children (N=86)

Figure 16. *Sucrose Taste Detection Thresholds and Gender*

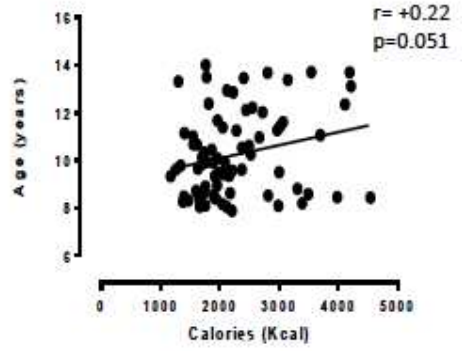


Relationship of Diet Related Food Behaviors with Age and Sex

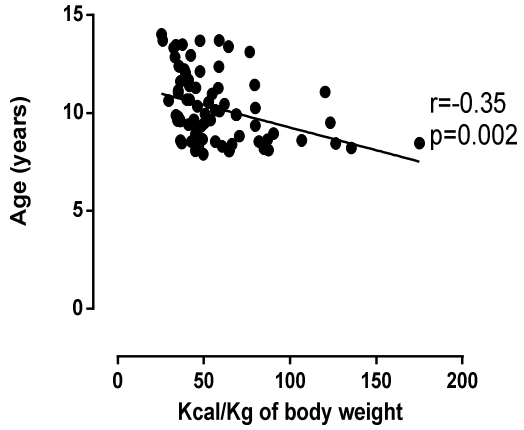
Diet-related food behaviors were associated with age (**Table 11, Figure 17**). While caloric intake was not related to age [r (71) = +0.22, p = 0.051] (**Figure 17**), caloric intake relative to body weight (kcal/ kg) was associated with age [r (71) = -0.35, p = 0.002] (**Figure 17B**). In addition, the percent total calories from added sugars [r (71) = +0.27, p = 0.020] (**Figure 17C**) were associated with age, but not added sugars relative to their body weight [r (71) = -0.08, p = 0.506] (**Figure 17D**).

There were no significant sex differences for any of the measures of diet related food behaviors (**Table 12**). Girls and boys ate the same percentage of their daily calories as added sugar [14 ± 7% vs. 14 ± 7%; t (71)=0.17, p = 0.865] and the same amount of added sugars relative to body weight [girls: 1.96 ± 1.15 g/ kg; boys: 1.99 ± 1.25 g/kg; t (71) = 0.07, p = 0.943] (**Table 12**).

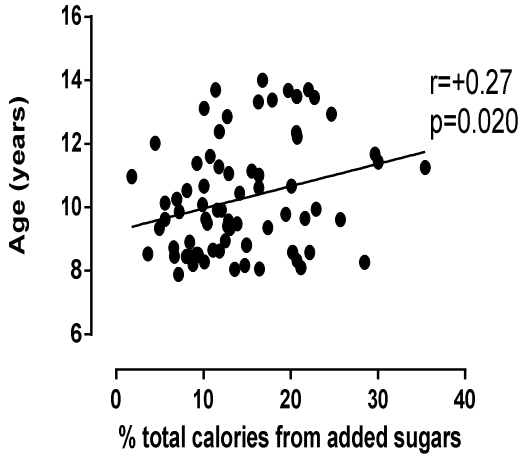
Figure 17. *Relationship of Diet Related Food Behaviors with Age*



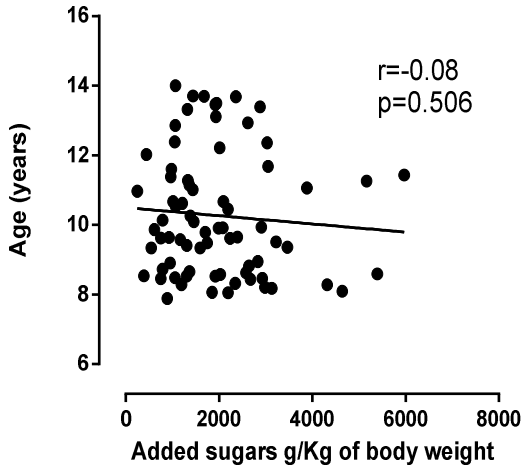
A. Total calories consumed and Age



B. Total calories consumed relative to body weight and Age



C. Percent total calories as added sugars consumed and Age

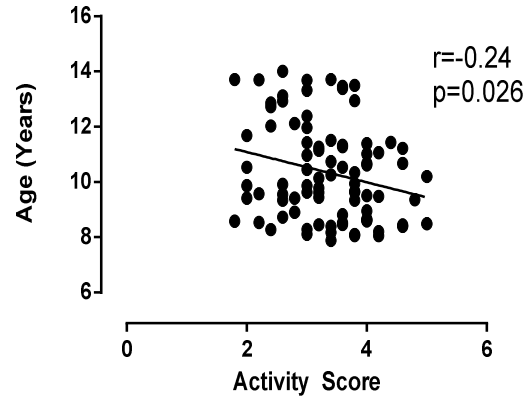


D. Added sugars as g/Kg of body weight and Age

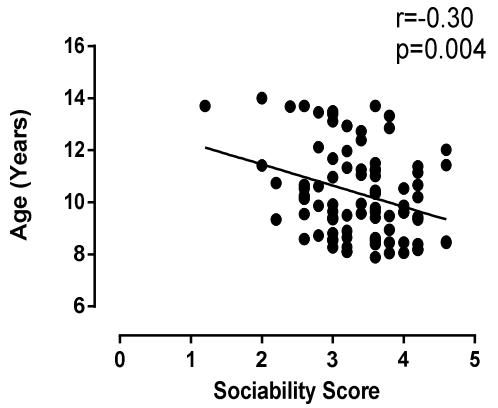
Relationships of Personal Characteristics with Age and Sex

We also looked at whether any dimensions of a child's personality characteristics were related to age. Both activity [r (84) = -0.24, p = 0.026] and sociability [r (84) = -0.30, p = 0.004] were significantly negatively correlated with age (**Table 11, Figure 18A & B**). This means that older children are less active and less sociable than younger children. However, we found no significant relationships between shyness, emotionality, negative reaction to foods and food neophobia and age (**Table 11).** There were also no significant sex differences for any of the measures of personal characteristics (**Table 12**).

Figure 18. *Relationship of Personal Characteristics with Age*



A. Activity Score and Age

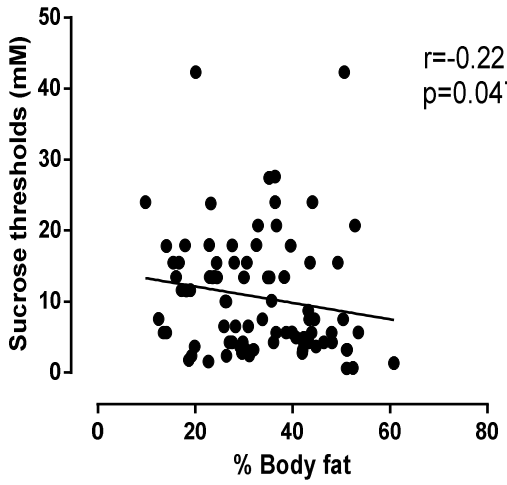


B. Sociability Score and Age

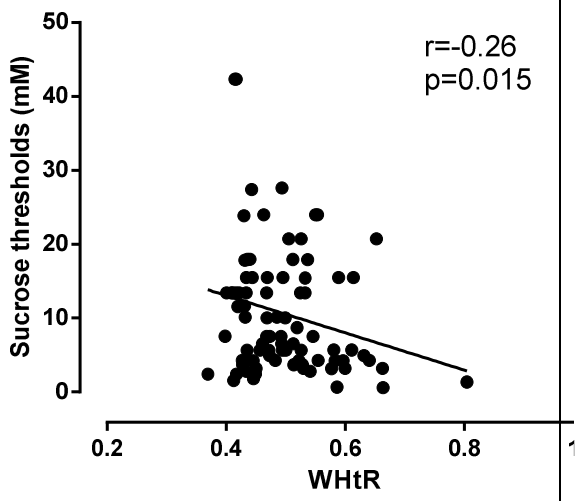
Relationship of Sucrose Detection Thresholds with Adiposity

We found no significant relationships between sucrose taste detection thresholds and z-scores for height-for-age [r (214) = +0.08, p = 0.257], weight-for-age z-score [r (214) = -0.03, p = 0.702], or BMI z-score [r (214) = -0.07, p = 0.319]. However, the greater the percent body fat (as measured by bioelectrical impedance) or WHtR, the lower the sucrose thresholds [r (84) = -0.22, p = 0.047; and r (84) = -0.26, p = 0.015, respectively] (**Table 13, Figure 19A & B)**. This means that children with more central adiposity and overall adiposity had lower sucrose detection thresholds, were more sensitive.

Figure 19. *Relationship of Sucrose Detection Thresholds with Adiposity*



A. Sucrose detection thresholds and %Body Fat

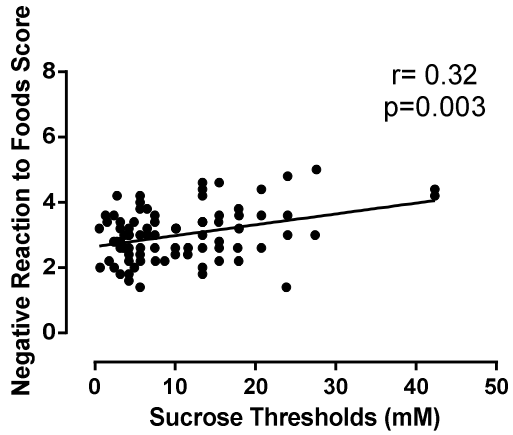


B. Sucrose detection thresholds and WHtR

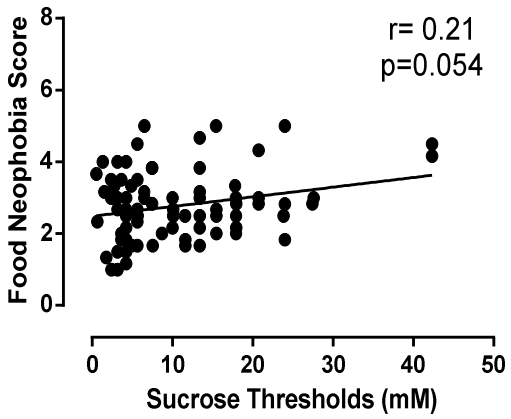
Relationship of Sucrose Detection Thresholds with Personal Characteristics

We found a positive correlation between sucrose thresholds and negative reaction to foods [r (84) = +0.32, p = 0.003]. This means that those with more negative reactions to foods tended to have higher sucrose thresholds levels (less sensitive) (**Table 13)**. There was no significant relationship between sucrose taste detection thresholds and food neophobia [r (84) = +0.21, p = 0.054]. Although not statistically significant, the positive correlation could suggest that children with higher sucrose detection thresholds may be less likely to try new foods (**Figure 20**). There were no differences between girls and boys for negative reaction to foods or food neophobia. We next probed to see whether negative food reactions were associated with diet of the child. Negative food behaviors were associated with kcal/kg [F (1,62) = 4.22, p = 0.007] even when sucrose detection thresholds were added to the analysis. This could suggest that sucrose detection thresholds is acting as mediator between the negative reactions to food and the calories consumed per body weight with picky eaters having altered consumption of calories. The findings were specific to the kcal/kg measure and not added sugars as percent kcal [F (1,63) = 0.90, p =0.346], or added sugars g/kg [F (1,63) = 0.33, p = 0.567] or total calories [F (1,63) = 0.07, p =0.788].

Figure 20. *Relationship of sucrose detection thresholds with personal characteristics (negative reaction to foods and food neophobia)*



A. Sucrose detection thresholds and Negative reaction to foods score



B. Sucrose detection thresholds and Food neophobia score

Relationship of Sucrose Detection Thresholds with Diet Related Food Behaviors

We next looked at whether sucrose taste detection thresholds were associated with diet-related food behaviors. We found that sucrose thresholds were not significantly related to total caloric intake [r (63) = -0.14, p =0.268], total calories relative to body weight [r (63) = +0.09, p =0.439] or added sugar as percentage of total calories [r (63) = -0.05, p = 0.720] or relative to body weight [r (63) = +0.07, p = 0.577] (**Table 13**).

Table 11. *Correlations (r) between Sucrose Detection Thresholds, Personality Characteristics and Diet Related Food Behaviors Measures and Age*

Trait	Age of child (years)					
	All Children (Study 1 & 2)			Subset (Study 2)		
	r	*n*	*p-Value*	*r*	*n*	*p-Value*
Psychophysical test						
Sucrose Thresholds (mM)	-0.16	216	0.016*	-0.28	86	0.009*
Personality characteristics: Temperament[a] & Food Neophobia[b]						
Shyness	NK	NK	NK	-0.06	86	0.557
Emotionality	NK	NK	NK	+0.01	86	0.919
Sociability	NK	NK	NK	-0.30	86	0.004*
Negative reaction to foods	NK	NK	NK	+0.07	86	0.531
Activity	NK	NK	NK	-0.24	86	0.026*
Food Neophobia	NK	NK	NK	-0.14	86	0.201
Food Neophobia	NK	NK	NK	-0.14	86	0.201
Diet related food behaviors						
Daily calories						
Total calories (kcal)	NK	NK	NK	+0.22	73	0.051
Relative to body weight (kcal/kg)	NK	NK	NK	-0.35	73	0.002*
Added sugar						
Percent total calories	NK	NK	NK	+0.27	73	0.020*
Relative to body weight (g/kg)	NK	NK	NK	-0.08	73	0.506

Note: r=Pearson's correlation coefficient, n=sample size. NK; not known since these measures were obtained only for the subset of children.
[a]Temperament measures from Pliner and Loewen (1997).
[b]Food Neophobia measures from Pliner and Hobden (1992). *=$p<0.05$. See text for other details.

Table 12. *T- Test for Sucrose Thresholds, Personality Characteristics and Diet Related Food Behaviors by Sex*

Variable	Mean Boys	Mean Girls	T-tests *t*-value	*df*	p-value	Valid *N* Boys	Valid *N* Girls	*SD* Boys	*SD* Girls
			All children						
Psychophysical test									
Sucrose detection thresholds (mM)	13.88	10.45	1.951	214	0.047*	98	118	8.58	16.61
Subset									
Personality characteristics: Temperament[a] & Food Neophobia[b]									
Shyness	2.48	2.66	-0.978	84	0.313	36	50	0.93	0.78
Emotionality	2.68	2.84	-0.748	84	0.456	36	50	0.81	1.10
Sociability	3.41	3.33	0.539	84	0.591	36	50	0.73	0.60
Activity	3.49	3.23	1.571	84	0.120	36	50	0.79	0.73
Negative reaction to foods	2.97	3.02	-0.269	84	0.788	36	50	0.87	0.77
Food Neophobia	2.94	2.66	1.393	84	0.167	36	50	0.97	0.87
Diet related food behaviors									
Daily calories	2357.59	2221.02	0.7304	71	0.468	34	39	721.99	829.30
Relative to body weight (kcal/kg)	55.75	58.81	-0.462	71	0.865	34	39	30.34	1.63
Added sugar									
Percent total calories	14.44	14.16	0.170	71	0.865	34	39	7.32	1.29
Relative to body weight (g/kg)	1.98	1.96	0.072	71	0.943	34	39	1.15	0.00

[a]Temperament measures from Pliner and Loewen (1997). [b] Food Neophobia measures from Pliner and Hobden (1992). *=p<0.05.

Table 13. *Correlations (r) Sucrose Detection Thresholds with Adiposity Measures, Personal Characteristics and Diet Related Food Behaviors*

	Sucrose Threshold (mM)					
	All Children (Study 1 & 2)			Subset (Study 2)		
Trait	*r*	*n*	*p-Value*	*r*	*n*	*p-Value*
Height[a]	+0.08	214	0.257	+0.00	86	0.991
Weight[a]	-0.03	214	0.702	-0.15	86	0.181
BMI[a]	-0.07	214	0.319	-0.15	86	0.165
Waist-to-height ratio (WHtR)	NK	NK	NK	-0.26	86	0.015*
Percent body fat	NK	NK	NK	-0.22	86	0.047*
Personality characteristics:						
Temperament[b]& Food Neophobia[c]						
Negative reaction to foods	NK	NK	NK	+0.32	86	0.003*
Food Neophobia	NK	NK	NK	+0.21	86	0.054
Diet related food behaviors						
Daily calories						
Total calories (kcal)	NK	NK	NK	-0.14	65	0.268
Relative to body weight (kcal/kg)	NK	NK	NK	+0.09	65	0.439
Added sugar						
Percent total calories	NK	NK	NK	-0.05	65	0.720
Relative to body weight (g/kg)	NK	NK	NK	+0.07	65	0.577

Note: r=Pearson's correlation coefficient, n=sample size. NK; not known since these measures were obtained only for the subset of children.
[a]Values are z-scores adjusted for the child's age and sex. [b]Temperament measures from Pliner and Loewen (1997).
[c] Food Neophobia measures from Pliner and Hobden (1992)
*=p<0.05. See text for other details.

Results for Aim 2

Of the 175 unrelated children, 168 had valid sucrose taste detection thresholds and were included in the genotype analyses. **Table 14** shows the allele frequencies and genotype frequencies of the SNPs used in this study. A few genomic DNA samples were refractory to genotyping: 6 for *TAS1R3* and 10 for *GNAT3*. All alleles were in HWE ($p>0.05$).

Table 14. *Hardy-Weinberg Equilibrium Calculations*

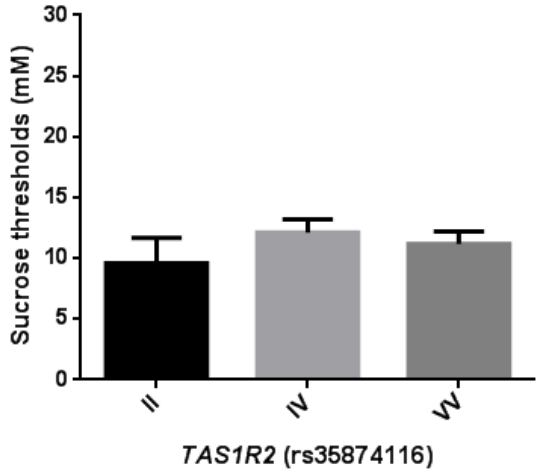
Genotype	Observed	Genotype Frequency #observed/ total observed	Expected	*N* alleles	Allele frequency p+q=1	Genotype frequency $p^2+2pq+q^2=1$	χ^2 Square (p-value)
			Sweet Receptor Genes				
TAS1R2; rs35874116							
II	16	0.103225806	14	310	0.293548387	0.086170656	0.79
IV	59	0.380645161	64		0.706451613	0.414755463	(0.373)
VV	80	0.516129032	77			0.499073881	
TAS1R3; rs35744813							
CC	52	0.320987654	47	324	0.537037037	0.288408779	2.49
CT	70	0.432098765	80		0.462962963	0.497256516	(0.114)
TT	40	0.24691358	35			0.214334705	
GNAT3; rs7792845							
CC	82	0.518987342	82	316	0.721518987	0.520589649	0.00
CT	64	0.405063291	64		0.278481013	0.401858676	(1)
TT	12	0.075949367	12			0.077551674	
			Bitter Taste Receptor Genes				
TAS2R38; rs713598							
AA	52	0.30952381	54	336	0.56547619	0.319763322	0.21
AP	86	0.511904762	83		0.43452381	0.491425737	(0.643)
PP	30	0.178571429	31			0.188810941	
TAS2R38; rs1726866							
VV	34	0.202380952	30	336	0.419642857	0.176100128	1.80
AV	73	0.43452381	82		0.580357143	0.487085459	(0.179)
AA	61	0.363095238	57			0.336814413	
TAS2R38; rs10246939							
II	49	0.291666667	50	336	0.544642857	0.296635842	0.09
IV	85	0.505952381	83		0.455357143	0.496014031	(0.7557)
VV	34	0.202380952	35			0.207350128	

Note: If $P < 0.05$- not consistent with HWE

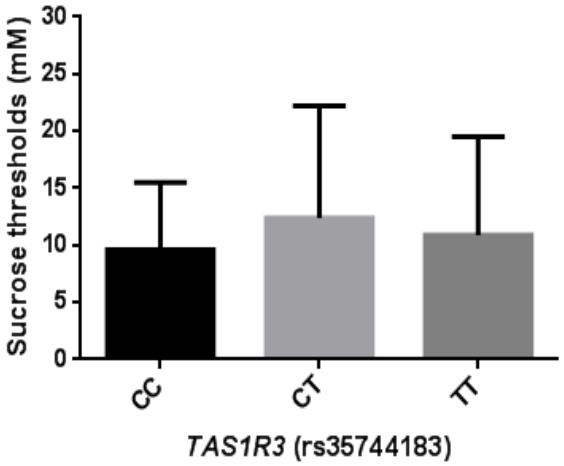
Relationship of Sucrose Detection Thresholds to Genotype

As shown in **Table 15**, sucrose detection thresholds were not significantly related to genotype of sweet taste receptor genes *TAS1R2* [F (2,152) = 0.54, p = 0.581], *TAS1R3* [F (2,159) =1.01, p = 0.364] and *GNAT3* [F (2,155) = 1.04, p = 0.357] (**Figure 21 A-C**) or to the bitter receptor gene *TAS2R38* variant rs713598 [F (2,165)=2.45, p=0.09] (**Figure 22 A**). However, *TAS2R38* bitter taste receptor gene variants rs1726866 and rs10246939 were related to sucrose detection threshold [F (2,165) = 4.55, p = 0.012; F (2,165) = 3.14, p = 0.046]. Children with one or two bitter-sensitive alleles (the A allele of rs1726866 V262A and/or the V allele of rs10246939 I296V) had lower sucrose detection thresholds (i.e., were more sensitive to the taste of sucrose) (**Figure 22C&D**). In addition, after adjustment for age and sex, those children with the *TAS2R38* variant rs713598 A49P were significantly more sensitive to sucrose [F (2,163) = 3.18, p = 0.044], with those having a P allele having lower sucrose detection thresholds (**Table 15, Figure 22B**).

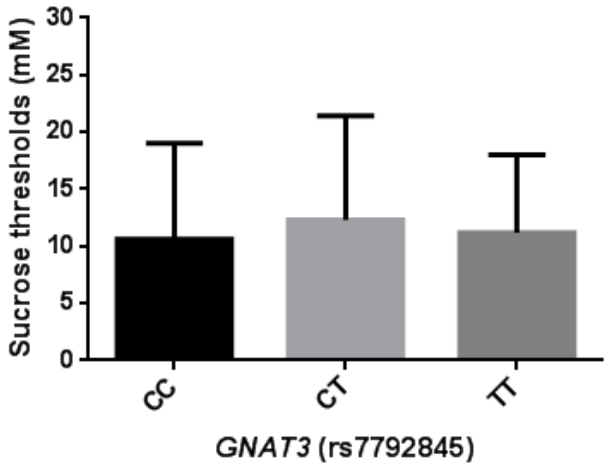
Figure 21. *Sucrose Detection Thresholds and TAS1R2, TAS1R3 and GNAT3 Genotypes*



A. A. Sucrose detection thresholds and *TAS1R2* genotype for rs35874116

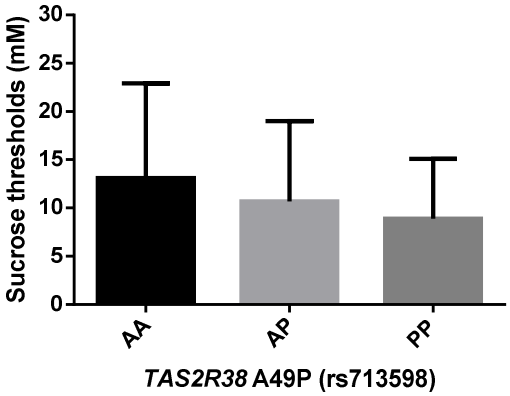


B. Sucrose detection thresholds and *TAS1R3* genotype for

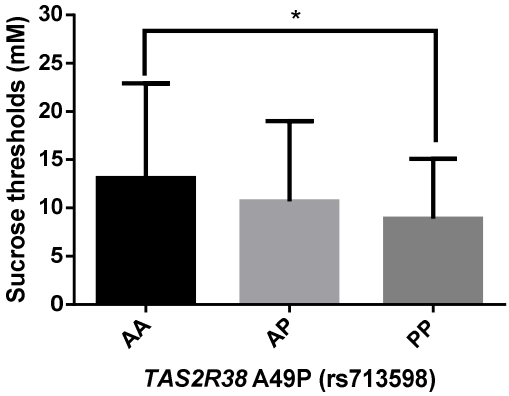


C. Sucrose detection thresholds and *GNAT3* genotype for rs77992485

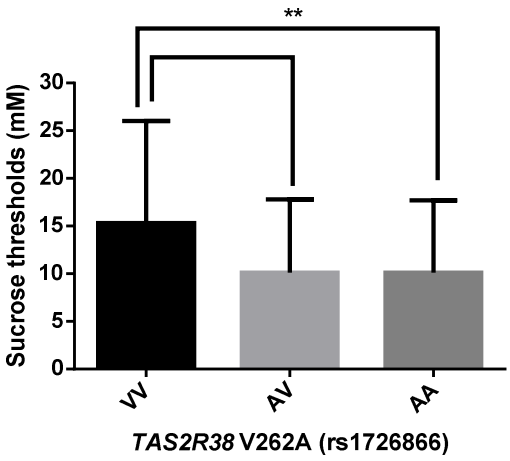
Figure 22. *Sucrose Detection Thresholds and TAS2R38 Genotypes*



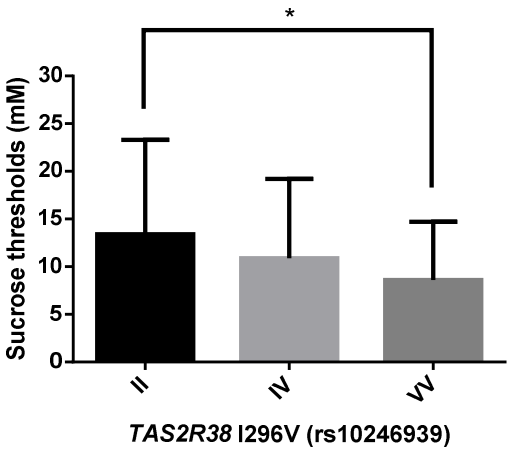
A. Sucrose detection thresholds and *TAS2R38* genotype for rs713598



B. Sucrose detection thresholds and *TAS2R38* genotype for rs713598 post adjustment by age and sex-post hoc analysis



C. Sucrose detection thresholds and *TAS2R38* genotype for rs1726866



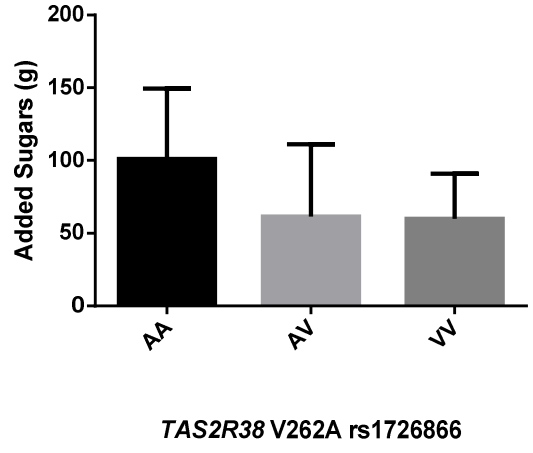
D. Sucrose detection thresholds and *TAS2R38* genotype for rs10246939

**Means differ by post-hoc testing

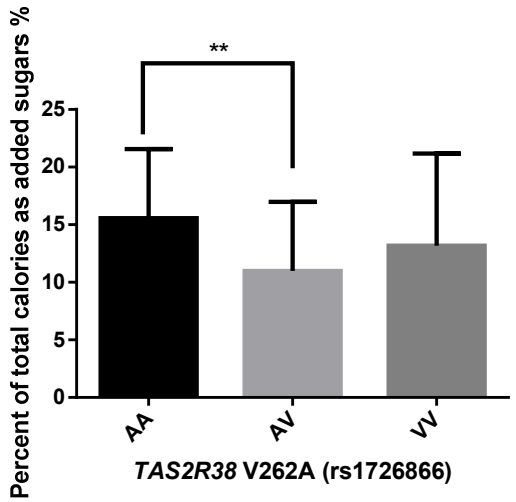
Relationship of Diet Related Food Behaviors with Genotype

We further assessed if there were differences in diet-related food behaviors based on an individual's genotype for the *TAS2R38* gene (**Figure 23**). There was no relationship of genotype with total calories [(F (2,65) = 0.80, p = 0.45], but the diet of children with the bitter-sensitive genotype (AA, rs1726866, V262A) contained more added sugar as a percentage of total kcal (16 ± 6% of kcal as added sugars) than the diet of children with the one or two copies of the other alleles (AV, 11±6 and VV, 13 ±8%; [F (2,62)=3.64, p = 0.032]). The results were specific to one *TAS2R38* variant (rs1726866), and this effect was not apparent for the remaining two variants (rs713598 [F (2,62) = 0.40, p = 0.67]; rs10246939 [F (2,62) = 0.85, p = 0.43]).

Figure 23. *Diet Related Food Behaviors Measures and TAS2R38 Genotypes*



A. Added sugars (g) and *TAS2R38* genotype for rs1726866



B. Percent total calories (%) as added sugars and *TAS2R38* genotype rs1726866

**Means differ by post-hoc testing

Table 15. *Sucrose Detection Thresholds in Children grouped by Taste Genotype*

Gene	Variant[a]	Genotype[b]	N[c]	Sucrose Threshold, [mM; mean ± *SD*]	*F* (*df*)	*p*-Value
			Unadjusted Thresholds			
TAS1R2	rs35874116	II	16	9.6±2.1	*F* (2,152)=0.54	0.581
		IV	59	12.1±1.1		
		VV	80	11.2±1.0		
TAS1R3	rs35744183	CC	52	9.6±5.9	*F* (2,159)=1.01	0.364
		CT	70	12.4±9.8		
		TT	40	10.9±8.6		
GNAT3	rs7792845	CC	82	10.6±8.4	*F* (2,155)=1.04	0.357
		CT	64	12.3±9.1		
		TT	12	11.2±6.8		
TAS2R38	rs713598	AA	52	13.1±9.8	*F* (2,165)=2.45	0.090
		AP	86	10.7±8.3		
		PP	30	8.9±6.2		
	rs1726866	VV	34	15.3±10.7[2]	*F* (2,165)=4.55	0.012*
		AV	73	10.1±7.7[1]		
		AA	61	10.1±7.6[1]		
	rs10246939	II	49	13.4±9.9[2]	*F* (2,165)=3.14	0.046*
		IV	85	10.9±8.3[1,2]		
		VV	34	8.6±6.1[1]		
			Thresholds Adjusted for Age and Sex			
TAS1R2	rs35874116	II	16	9.6±1.6	*F* (2,150)=0.61	0.543
		IV	59	12.1±1.2		
		VV	80	11.2±1.0		
TAS1R3	rs35744183	CC	52	9.6±5.9	*F* (2,157)=1.08	0.343
		CT	70	12.4±9.8		
		TT	40	10.9±8.6		
GNAT3	rs7792845	CC	82	10.6±8.4	*F* (2,153)=1.29	0.277
		CT	64	12.3±9.1		

Gene	Variant[a]	Genotype[b]	N[c]	Sucrose Threshold, [mM; mean ± *SD*]	*F* (*df*)	*p*-Value
			Thresholds Adjusted for Age and Sex			
TAS2R38	rs713598	AA	52	13.1±9.8[2]	*F* (2,163)=3.18	0.044*
		AP	86	10.7±8.3[1,2]		
		PP	30	8.9±6.2[1]		
	rs1726866	VV	34	15.3±10.7[2]	*F* (2,163)=5.07	0.007**
		AV	73	10.1±7.7[1]		
		AA	61	10.1±7.6[1]		
	rs10246939	II	49	13.4±9.9[2]	*F* (2,163)=3.92	0.022*
		IV	85	10.9±8.3[1,2]		
		VV	34	8.6±6.1[1]		

[a] rs=reference single nucleotide polymorphism; rs numbers are publicly cataloged in dbSNP (http://www.ncbi.nlm.nih.gov/SNP/).
[b]Variants are presented as nucleotides if they reside in regulatory regions (e.g., C or T); those in protein-coding regions are presented as amino acid substitutions (e.g., A or P, Alanine to Proline). N=number of children of each genotype.
[c]Alleles were tested for Hardy-Weinberg equilibrium; all *p*-values>0.05.
[1,2]Means that do not share a superscript differ by post-hoc testing.
*=$p<0.05$; **=$p<0.01$.

Multivariate ANOVA Models for Sucrose Thresholds

Drawing together the preceding results, we generated multivariate analysis of variance models to explore the independent determinants of sucrose detection thresholds with the variables age, sex, and *TAS2R38* genotype. In this model we used only the variant (rs1726866) for *TAS2R38*. This model explained 7% of the total variance among children in their sucrose detection threshold [F (2,163) = 3.9, p = 0.021]. Genotype accounted for the majority of the variance, and age and sex made more minor contributions (**Table 16**).

Table 16. *Age, Genotype, and Sex Effects on Sucrose Detection Thresholds for All Children*

Variable	*df*	*F*	*p-Value*	η^2
Age	1	3.3	0.072	0.02
Genotype	2	3.9	0.021*	0.05
Sex	1	2.4	0.122	0.01

Note: Genotype refers to rs1726866 (*TAS2R38*, V262A). $\eta 2$=effect size. *p<0.05

We confirmed the results in the subset of children who had measures of adiposity and food intake. The model in the subset explained 14% of the total variance among children in their sucrose detection threshold [F (2,64) = 3.6, p = 0.032]. Overall, the modeling results suggest that sucrose threshold is related to *TAS2R38* genotype and, to a lesser extent, to age (**Table 17**).

Table 17. *Age, Genotype, and Sex Effects on Sucrose Detection Thresholds for Subset of Children*

Variable	*df*	*F*	*p-Value*	η^2
Age	1	2.1	0.148	0.03
Genotype	2	3.6	0.032*	0.10
Sex	1	0.4	0.539	0.01

Note: Genotype refers to rs1726866 (*TAS2R38*, V262A). $\eta 2$=effect size. *p<0.05

In a final model we added a measure of adiposity (WHtR) to the variables age, sex and genotype. Although, percent body fat and WHtR were both significantly related to sucrose detection thresholds at the univariate level, we only used the strongest adiposity measure to avoid multi-collinearity for this model. Only variables with a moderate correlation (r = 0.25 or greater) were included *a priori*. This model explained 35% of the total variance in the subsample of children [F (1,62) = 4.8, p = 0.01] (**Table 18**), with age and genotype accounting for 29% of the variance. Overall, the modeling results suggest that sucrose threshold remains related to *TAS2R38* genotype adjusted for age and sex, but becomes a stronger predictor with the addition of WHtR.

Table 18. *Age, Sex, Genotype, and Adiposity Effects on Sucrose Detection Thresholds in the Subset of Children*

Variable	*df*	*F*	*p-Value*	η^2
Age	1	11.3	0.001*	0.15
Genotype	2	4.8	0.012*	0.13
Sex	1	1.6	0.211	0.03

Note: Genotype refers to rs1726866 (*TAS2R38*, V262A). $\eta 2$=effect size. *p<0.05

Analysis for Aim 3

Relationship of Sucrose Detection Thresholds with Adiposity Measures

The relationship of sucrose detection thresholds with adiposity measures was shown and discussed in aim 1, with WHtR and percent body fat being significantly associated with sucrose thresholds. Because of these findings, in the following analysis we explored these two measures of adiposity with the other variables of interest for aim 3.

Relationship of Diet-related Food Behaviors with Adiposity Measures

From the analysis conducted for aim 3, we observed that there was no significant relationship between percent body fat and total daily calories consumed (kcal) [r (70) = +0.18, p = 0.136] or percentage of calories from added sugars [r (70) = -0.04, p = 0.767]. However, percent body fat was significantly related to added sugar relative to body weight, with leaner children having higher daily sugar intake by weight (g/kg) [r (70) = -0.40, p = 0.001] and higher caloric intake (kcal/kg) [r (74) = -0.47, p<0.001].

WHtR was significantly related to total daily calories consumed (kcal) [r (71) = +0.26, p = 0.022], with children with more central adiposity consuming more calories per day. There was no relationship between WHtR and daily sugar consumption [r (71) = +0.12, p = 0.332] or between WHtR and percent total calories from added sugars [r (71) = -0.09, p = 0.452] or relative to body weight [g/kg: r (71) = -0.22, p = 0.056; kcal/kg: [r (71) = -0.22, p = 0.059]. Correlations among consumption of added sugar and obesity measures are shown in **Table 19**.

Table 19. *Correlations (r) between Diet -Related Food Behaviors and Adiposity Measures*

Caloric Intake and Added Sugars	Percent Body Fat		Waist-to-Height Ratio	
	r	*p-value*	*r*	*p-value*
Daily calories (kcal)[a]	+0.17	0.136	+0.26*	0.027
By calories (kcal/kg) [a]	-0.48*	0.000	-0.22	0.059
Added sugars				
Percent total calories[b]	-0.04	0.767	-0.09	0.452
Relative to body weight				
By amount (g/kg)[b]	-0.40*	0.001	-0.22	0.056

Note: r=Pearson's correlation coefficient, [a]*N*=72; [b]*N*=73 children. *$p<0.05$

Relationship of Personal Characteristics with Adiposity Measures

Shyness and activity were significantly related with WHtR and percent body fat suggesting that children who have both greater central adiposity [r (85) = +0.24, p = 0.025] and greater percent body fat [r (71) = +0.21, p = 0.049] tend to exhibit more shyness. Children who were less active by maternal report have a greater central adiposity [r (85) = -0.41, p = 0.000] and percent body fat [r (85) = -0.43, p = 0.000] (**Table 20**).

Table 20.*Correlations (r) between Personality Characteristics and Adiposity Measures*

Trait	WHtR			percent body fat		
	r	*n*	*p-Value*	*r*	*N*	*p-Value*
Personality characteristics: Temperament[a] & Food Neophobia[b]						
Shyness	+0.24	86	0.025*	+0.21	86	0.049*
Emotionality	-0.10	86	0.355	+0.08	86	0.493
Sociability	-0.16	86	0.146	-0.16	86	0.143
Negative reaction to foods	+0.12	86	0.275	+0.19	86	0.089
Activity	-0.41	86	0.000*	-0.43	86	0.000*
Food Neophobia	+0.05	86	0.616	+0.05	86	0.623

Note: r=Pearson's correlation coefficient, n=sample size, Waist-to-height ratio= WHtR.
[a]Temperament measures from Pliner and Loewen (1997).
[b] Food Neophobia measures from Pliner and Hobden (1992). *$p<0.05$. See text for other details.

Multivariate ANOVA Models for Adiposity Measures

For aim 3, we generated multivariate analysis of variance models to explore the independent determinants of adiposity measures (WHtR) with the variables age, sex, sweet food diet related behaviors and personal characteristics. Only the categorical variable of sex and continuous variables with a moderate correlation (r=0.25 or greater) were included in the models. This model explained 40% of the total variance among children in their adiposity for WHtR. Here activity had a big effect on that variance [F (1,59) = 13.3, p = 0.000] with sucrose detection thresholds [F (1,59) = 6.1, p =0.016] and age [F (1,59) = 5.5, p = 0.022] still being significant predictors (**Table 21**).

Table 21. *Age, Sex, Activity, Added sugars, and Sucrose Detection Thresholds Effects on Adiposity (WHtR)*

Variable	*df*	*F*	*p-Value*	η^2
Age	1	2.9	0.093	0.05
Sucrose detection thresholds	1	6.1	0.016*	0.09
Sex	1	5.5	0.022*	0.08
Activity	1	13.3	0.000*	0.18
Added sugars g/kg of body weight	1	0.44	0.509	0.00

Note: Genotype refers to rs1726866 (*TAS2R38*, V262A). $\eta 2$=effect size. *p<0.05

The second model explored independent determinants of adiposity measures (percent body fat) with the variables age, sex, sweet food diet related behaviors and personal characteristics. This model explained 29% of the total variance among children in their percent body fat. Activity had a large effect on that variance [F (1,58) = 7.9, p = 0.006], as did added sugars g/kg body weight [F (1,58) = 6.6, p = 0.013]. Here, sucrose

detection threshold was not a significant predictor and had a very small effect on the variance [F (1,58) =1.4, p =0.238] (**Table 22**).

Table 22. *Age, Sex, Activity, Added sugars and Sucrose Detection Thresholds Effects on Adiposity (% body fat)*

Variable	*df*	*F*	*p-Value*	η^2
Age	1	0.0	0.959	0.00
Sucrose detection thresholds	1	1.4	0.238	0.02
Sex	1	2.9	0.093	0.05
Activity	1	7.9	0.006*	0.12
Added sugars g/kg of body weight	1	6.6	0.013*	0.10

Note: Genotype refers to rs1726866 (*TAS2R38*, V262A). $\eta 2$=effect size. *p<0.05

CHAPTER 5

Synthesis and Discussion

The purpose of this study was to investigate the degree of variation in children's sucrose detection thresholds and whether sweet and bitter taste receptor-related genotypes partially accounted for variation in taste thresholds. We also examined the relationship of sucrose detection thresholds with adiposity and dietary measures as well as dimensions of child's temperament with both thresholds and adiposity.

The findings of this study showed that like adults, children 7 to14 years of age differed markedly in their ability to detect sucrose at low concentrations. Thresholds for sucrose detection ranged from 0.23 mM to 153.8 mM, with an average of 12.0 mM, which approximates thresholds previously reported both for adults (Pepino et al., 2010; Pepino & Mennella, 2007; Pribitkin et al., 2003) and for children (James et al., 1997; Overberg et al., 2012).

Age and sex were determinants of sucrose detection threshold, on a continuum even within this narrow age range, with younger children being less sensitive than older children and boys less sensitive than girls; the latter finding is consistent with prior work (James et al., 1997). To further establish the dynamics of changes with age, research is needed that assesses detection thresholds of children and adults of varying ages within the same study, using identical methodologies (Pepino et al., 2010; Pepino & Mennella, 2007).

The age- and sex-related effects during childhood and early adolescence may be specific to sweet taste, as suggested by a recent study that found no such relationships for

detection thresholds for example of two other basic tastes: NaCl (salty) and monosodium glutamate (umami) (Bobowski & Mennella, 2015). That study used the same the psychophysical method used here in children of the same age range, so we can conclude that the higher detection thresholds for sucrose we found among younger children and boys were not likely due to differences in cognition or the ability to complete the task. Both age and gender of a child reflect underlying hormonal and developmental processes that may shape this sensory system (Posner, Rothbart, Sheese, & Voelker, 2012). Sex-related differences may be due to girls undergoing puberty at earlier ages than boys.

Children are born with different taste genotypes, and our results suggest that some but not all genetic variants affect the sensory experience of the child. Unlike in adults (Fushan et al., 2009), we found no relationship between a variant in the *TAS1R3* gene and sucrose detection thresholds in these children. Similarly, a variant in *GNAT3* related to taste sensitivity in adults (Fushan et al., 2010) had no measurable effect in the children studied here. One explanation is that the psychophysical methods used to measure sucrose detection thresholds in the Fushan studies of *GNAT3* and *TAS1R3* (Fushan et al., 2010; Fushan et al., 2009) were slightly different than those we used here, which may account for the different results. Another explanation may be age-related effects: this particular genetic variant is in a regulatory region and may regulate gene expression more in adults than in children. This lack of genetic association is reminiscent of the gene's effect on sweet preference, detectable in adults but less apparent in children (Mennella et al., 2012; Mennella, Reed, Mathew, et al., 2014). We also found no relationship between a variant of *TAS1R2* gene and sucrose detection thresholds in this study. There may be a

weight effect since genetic variation was observed in the overweight group and not the normal weight individuals (Eny et al., 2010), however the investigators did not measure sucrose detection thresholds.

While we found no relationship among children between sucrose detection thresholds and sweet-taste genotypes, sucrose detection thresholds were related to variation in the bitter taste receptor gene *TAS2R38*. Children differ in their ability to perceive the bitter compound propylthiouracil, due in large part to *TAS2R38* alleles (Mennella, Pepino, Duke, et al., 2010; Mennella et al., 2005; Mennella, Reed, Roberts, Mathew, & Mansfield, 2014). As discussed earlier, *TAS2R38* alleles also partially explained individual differences in children's sweet preferences (Mennella et al., 2005). Moreover, studies in adults have linked the perception of the bitter ligands of this receptor to sweet thresholds (Chang et al., 2006; Hong et al., 2005). Taken together, these studies point to a role of this bitter taste receptor gene in sweet perception and suggest that the sweet and bitter taste systems are more tightly linked than previously understood (Mennella, Reed, Mathew, et al., 2014).

Four hypotheses, not mutually exclusive, might account for the observed relationship between variation in the *TAS2R38* gene and heightened sweet preferences and reduced sweet sensitivity among children. First, alleles of *TAS2R38* could lead to proteins with different capacities to bind sucrose directly. Other sweet substances like saccharin bind members of the bitter receptor family (Pronin et al., 2007), so this hypothesis has some experimental support. The results were most marked for one variant within the protein, which might point to the place in the receptor that binds sucrose

(V262A). Second, the *TAS2R38* gene and its alleles could be in linkage disequilibrium with nearby genes that might influence sweet taste perception and sensitivity ("Linkage disequilibrium" refers to the tendency for genes physically close on the chromosome to be co-inherited during meiosis). Third, *TAS2R38* allele frequency may be an especially sensitive genetic marker of racial ancestry (Guo & Reed, 2001), a variable with large and reliable effect on sweet preference (Mennella et al., 2005) and bitter taste thresholds (Guo & Reed, 2001; Mennella, Pepino, Duke, et al., 2010; Mennella, Pepino, Lehmann-Castor, et al., 2010). Fourth, differences in diet may affect sucrose threshold via changes in gene expression (Lipchock, Mennella, Spielman, & Reed, 2013).

In this study, children with a bitter-sensitive allele of *TAS2R38* also reported consuming more added sugars than did those with the less sensitive allele, a finding consistent with previous reports that children with a bitter sensitive allele preferred cereal and beverages with higher sugar content than those without the sensitive allele (Mennella et al., 2005). These results are similar to a recent study that measured added sugar (e.g., candy) consumption in children (Hoppu, Laitinen, Jaakkola, & Sandell, 2015). Children in the study herein consumed, on average, 14% of total calories as added sugar, almost three times the 5% recommendation of international experts in public health (World Health Organization, 2015). In fact, of the 73 children in this study that provided valid dietary data, only 4 did not exceed the dietary recommendation. These reports of added sugar in the present study, while not in compliance with public health recommendations, are typical, and remarkably consistent with intake data obtained from larger-scale epidemiological studies (Ervin et al., 2012).

This study also examined the relationships between sweet taste threshold and body weight, central adiposity, and percent body fat. We found that body mass index was not related to sucrose thresholds, but when more direct measures of obesity were examined, children who were fatter and those with larger waistlines relative to their height had lower thresholds. This result is consistent with results of a study that measured sucrose threshold in obese and lean adolescents (Pasquet, Frelut, Simmen, Hladik, & Monneuse, 2007) but differs from results of another study that found obese adolescents, as measured by body mass index, were less sensitive to low sucrose concentrations than were lean adolescents (Overberg et al., 2012). Differences in methods relying on less sensitive measures of childhood obesity such as body mass index (Demerath et al., 2006), rather than more direct measures like percent body fat or waist- to- height ratio may account for the inconsistencies across studies. New knowledge about the age-related and molecular bases of individual differences in taste and the use of methodologies that are validated and appropriate for children (Mennella, Spector, Reed, & Coldwell, 2013), a generation that will struggle with obesity and diabetes, may suggest strategies to overcome diet-induced disease.

In this study, we also examined the relationship between temperament measures (negative food behaviors and food neophobia) and sucrose detection thresholds. Our findings suggest that children who have more negative reactions to food have a higher threshold for sucrose. No previous study has reported an association between these two measures of temperament and sucrose thresholds but rather sweet taste preference (Liem & Mennella, 2002) and other food preferences (Cooke et al., 2006; Skinner, Carruth,

Wendy, & Ziegler, 2002; Wardle, Herrera, Cooke, & Gibson, 2003). Sucrose detection thresholds might be a mediator for diet with negative food behaviors. We also examined temperament and adiposity; we found that children who were shy and were less active had more adiposity. This finding was consistent with the literature since studies have reported that obese children may experience stigma and become withdrawn. There is also evidence that has associated lower physical activity with increased adiposity in children (Caspersen, Pereira, & Curran, 2000; Janssen et al., 2005; Trost, Kerr, Ward, & Pate, 2001).

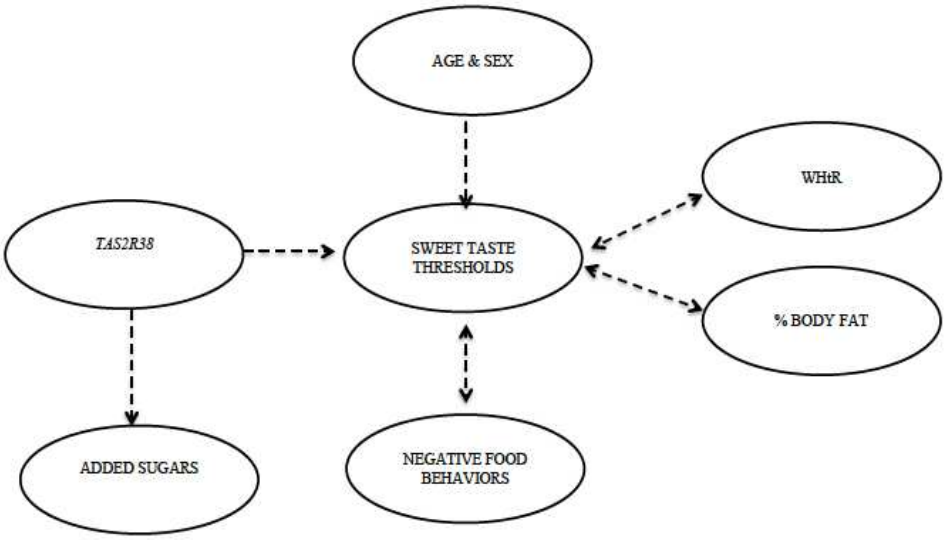
Collectively, the results of our study have expanded our knowledge of the association of bitter taste receptor genes with sucrose detection thresholds. To the best of our knowledge this study was the first to show that sweet taste receptors genes were not, but bitter taste receptor genes were associated with sucrose detection thresholds in children. It is also the first study to report a relationship between negative food reactions and sweet taste thresholds in children.

Conceptual Model- Revised

The conceptual model described earlier is revised here. The initial hypothesized linkages were proposed based on the literature on sucrose thresholds, food behaviors, personal characteristics and adiposity measures. Based on the results in this study, some changes are suggested to adjust that conceptual model (**Figure 24**). We expect that the modifications are not final since changes will be made as new findings emerge. The first modification includes *TAS2R38* as the gene related to sucrose detection thresholds in

children and to show that this gene is related to predicted percent of added sugar consumption as kilocalories. The second change is to alter the outcome measure of adiposity to reflect that of the direct obesity measures such as WHtR and % body fat were related to sucrose detection thresholds, but not BMIz scores. The relationship of sucrose detection thresholds with negative food behaviors has been depicted here as well.

Figure 24. *Revise Conceptual Model*



Limitations of the Study

The data used in this study were obtained cross-sectionally and a longitudinal approach would be needed to be able to make claims about change overtime. Although we combined data from study 1 and study 2, which was a possible strength to increase the number of subjects, there were some differences between the samples in the two studies for education, race and income. However, there were no differences for the main

variables used in this study (i.e., sucrose thresholds). The study was limited for some variables such as dietary reports, and percent body fat and waist- to- height ratio, which limits generalization of results to larger populations of children as expected since we had data only for a subset of children. Further, while the overall sample of children was ethnically diverse (which is useful for generalizing to US populations), most participants were African-American. Thus, we did not have enough power to conduct robust subgroup analysis based on race (e.g., African American vs. Asian vs. Caucasian). There was no measure of pubertal status in the participants, so consideration of hormonal influences on sweet taste threshold was not possible. Our measures of temperament and food intake were obtained by self-report, a method that carries the risk of recall bias. Furthermore, the temperament survey questions were answered by the mother about her child, an indirect approach. Stronger, more direct methods of assessing temperament are needed for future research. The ASA24, with its web-based interface had the added problem of no real-time human contact to resolve questions about food choices or portion size, variables that would greatly impact the accuracy of the data. While the Goldberg cutoff is a validated method to identify under-reporters, the fact that almost 25% of the food records were rejected for underreporting resulted in a significant loss of data and raises the question of whether the ASA24 method was limited in some way. Typical food intake is best represented by three 24-hour dietary recalls, including both weekdays and a weekend day. This study was limited to a single day's record. The BIA method of measuring body composition has the advantage of using portable equipment with no radiation exposure. However, the method can be less accurate in states of altered

hydration, but this would not have been expected in healthy children. The method also requires the choice of appropriate equation for the population being measured, since the equations used vary based on the population being studied (i.e., obese adults, people with HIV or end-stage renal disease, elderly). This study used an equation validated in children. Despite these limitations, the findings are promising and suggest that the use of a larger sample is warranted.

Implications

An important goal of this study was to make a novel contribution to the literature regarding sucrose detection thresholds, sweet and bitter taste receptors genotype and obesity measures in children. Children are becoming overweight and obese and consuming large amount of calories as added sugars, which place them at higher risk to develop obesity related comorbidities. This study has implications for the study of gustation and adiposity. The findings of this investigation lay some evidence for future clinical studies that will aid in recognizing children at the greatest at risk of obesity and over-consumption of calories and added sugars. Ultimately these children can be directed to early interventions aimed at decreasing environmental factors that may contribute to weight gain. Effective early intervention may have positive health outcomes by decreasing the probability of emerging comorbidities such as diabetes, and cardiovascular disease and avoid the use of surgical procedures like weight loss surgery when possible.

Future Directions

There are a few suggestions for future research in this area. Replication of this study entirely with measurements of body composition, waist circumference and food intake collected for all the subjects would be recommended. It would also be preferable that the study be designed as a prospective study with repeated measures over time to allow for analysis to identify causality. Adding measures of sucrose preference concurrently with sucrose thresholds measures may also help to assess relationships between sucrose detection thresholds and sweet taste preference and/or confirm whether the sweet taste receptor genes are specifically related to preference, but not sensitivity in children. Development of a developmentally appropriate short survey to assess the reward processes associated with added sugars might enable comparisons with sucrose detection thresholds. To obtain a more accurate assessment of typical food intake, research dietitians could collect three dietary recalls, one-week day and one weekend day. Lastly, gathering measures of puberty and hormonal changes may elucidate differences in sucrose detection thresholds by gender. A future study can also be designed measuring other variables from Contento's model of food choices to see how these variables relate to both diet and thresholds and outcomes of obesity.

Although the ultimate goal is to translate research findings into human interventions, based on the findings from this study a proposed study in animal models is necessary to further understand the mechanisms that contribute to an association between *TAS2R38* and sweet taste detection thresholds, to describe variations that may relate to susceptibility in gustatory function. As mentioned in the above discussion the etiology

behind this finding is unknown to date. In addition, an epigenetic study is needed to describe how external factors such as oversaturation of taste buds with sugars might activate and deactivate genes and how that affects sucrose detection thresholds.

Although sweet taste detection thresholds were not related to variation in sweet taste receptor genes in this study, it would be helpful to re-assess this relationship in a larger sample, to examine in more detail the effects of sweet taste genes variants, to determine which of these variants are found in regulatory regions in the DNA, and to examine their effects on gene expression and protein abundance in taste cells.

Results of recent studies have strengthened the body of evidence demonstrating that high intake of added sugars are associated with greater risk of obesity in children (Hu & Malik, 2010; Linardakis et al., 2008; Malik, Popkin, Bray, Després, & Hu, 2010; Malik, Willett, & Hu, 2009). But to date, nutritional interventions have been moderately successful in improving weight and decreasing excess calories and added sugars. Findings from this work show promise for developing nursing-driven interventions to advance human health and nutrition. The present findings also suggest that children with specific genotypes tend to consume a higher percentage of kcal as added sugars. Applying the knowledge collected from this research and future work will help us take into account the variability of the sensory world of the child. This evidence may help us target specific interventions for those children at higher risk for obesity related diseases, and provide efforts to minimize children's consumption of added sugars, which continues to exceed recommended limits. For example, to prevent the development of obesity in

susceptible children, a trial of substitution of added sugars by non-caloric sweetener might be considered.

Summary

In summary, this dissertation illustrated that bitter taste receptor genotypes were associated with sucrose detection thresholds. Sucrose detection thresholds were also associated with age and sex in a diverse sample of healthy children living in an urban US city. Children with the highest risk genotype also reported greater intake of added sugars, a behavior with potential to lead to obesity. The sweet taste receptor genotype, however, was not related to sweet taste thresholds. While these findings may suggest possible useful points for future intervention strategies to prevent pediatric obesity, further studies in a larger sample are needed to confirm and extend these findings.

Appendix A

Sucrose Dilution Instructions for Threshold

A. General Instructions

1. The instructions make 1 liter of the top 5 concentrations and 500 ml of the rest.

2. Maximum possible test= 15 subjects.

3. The solutions take about 2 hours to make (includes cleanup).

4. The following glassware is required:
    a. 2000-, 1000-, 100-, and 50-ml graduated cylinders (one of each)
    b. 4 2000-ml flasks (labeled 1-4)
    c. 10- and 50-ml pipettes
    d. 18 1-liter glass bottles

5. Taste stimuli Info:

Tastant	Taste	Concentration #'s	Molarities Formula Weight
Sucrose	Sweet	0-17	1 M to $5.6x10^{-5}$ M 342.30g

6. All the flasks and solution bottle are numbered with tape, along with the date made.

******** Solutions need to be remade every two weeks or more often if needed********

B. Step 1: Making 0 concentration

Conc. #	Amount Sucrose	Glassware used
0	684.6 g	2000 ml (2L) graduated cylinder with stopper

1. Using the large purple cup, weigh **684.6g** of sucrose to the nearest 1/100 using the 1500g scale.

2. Use a funnel to pour sucrose into 2 L graduated mixing cylinder.

3. Using dH_2O, rinse any sucrose remaining in weigh cup/dish and funnel into cylinder.

4. Fill the graduated cylinder to the 2000 ml mark with distilled water after the sucrose is in (Fill the last ½ inch or so with a pipette so you do not go over the mark), make sure the sucrose at the bottom of the cylinder is wet before measuring to the 2000ml.

5. Place stopper on flask and shake until the entire sample is dissolved (make sure to always hold stopper so it does not fall off or leak).

C. Step 2: Making concentration 1-4 using dilution steps

1. In 2000ml flask labeled 1-4 put the following measured amounts of **dH_2O**:

Conc. #	**Amount dH_2O in 1000 ml flask** (using graduated cylinders)
1	440ml
2	680ml
3	820ml
4	900ml

2. Fill each 2000 ml flask to mark with **0 concentration** of solution.

Conc. #	**Amount of 0 concentration**
1	560ml of 0 concentration
2	320ml of 0 concentration
3	180ml of 0 concentration
4	100ml of 0 concentration

3. Put stoppers in the flask and shake (overturn) 4 times to mix solution.

4. Pour solution into 1-liter bottles using a funnel.

5. To clean glassware soak in soapy water in sink for an hour and rinse out with hot water 5 times and 5 times with distilled water. Let dry before saving.

D. **Step 3: Making concentrations 5-17 using dilution steps** (*** NOTE: To make 1000 ml of each solution, just double the amount of water and sucrose solution, ex. 450 ml of dH_2O = 900 ml dH_2O and 50 ml of sucrose = 100 ml of sucrose)

1. Set up bottles as follows:

13 ↑	14	15	16
9 ↑	10 ↑	11 ↑	12 ↑
5 ↑	6 ↑	7 ↑	8 ↑
1 ↑	2 ↑	3 ↑	4 ↑

2. Fill 1L bottles number 5-17 with 450 ml dH_2O.

3. The rest of the series is a simple dilution from the four bases (bottles 1-4). Starting with concentration #4, pipette 50ml of 4 and put it into #8. Then pipette 50ml of #3 into #7, 50ml of #2 into #6 and 50ml of #1 into #5 as shown below:

Conc. #	Amount of dH_2O in 1L bottle	Amount of conc.
8	450ml dH_2O	50ml Bottle #4
7	450ml dH_2O	50ml Bottle #3
6	450ml dH_2O	50ml Bottle #2
5	450ml dH_2O	50ml Bottle #1

Note: The pipette does not need to be rinsed out between each set of four dilutions as long as you are going from weaker concentration (#8) toward the stronger (#5).

4. Shake Bottles #5-8 and throw out pipette.

5. Repeat step #3, pipetting 8 thru 5 into 12 thru 9 as follows:

Conc. #	Amount of dH_2O in 1L bottle	Amount of conc.
12	450ml dH_2O	50ml Bottle #8
11	450ml dH_2O	50ml Bottle #7
10	450ml dH_2O	50ml Bottle #6
9	450ml dH_2O	50 ml Bottle #5

6. Shake bottle #9-12 to mix and throw out pipette.
7. Repeat step #3 pipetting 12 thru 9 into 16 thru 13 as follows:

Conc. #	Amount of dH_2O in 1L bottle	Amount of conc.
16	450ml dH_2O	50ml Bottle #12
15	450ml dH_2O	50ml Bottle #11
14	450ml dH_2O	50ml Bottle #10
13	450ml dH_2O	50 ml Bottle #9

8. Shake #13-16 to mix and throw out pipette.

9. Repeat step #3, pipetting 13 into 17 as follows:

Conc. #	Amount of dH_2O in 1L bottle	Amount of conc.

17	450ml dH_2O	50ml Bottle #13

10. Shake #17 to mix and throw out pipette
11. Pour solutions into small 120ml bottles for testing.
12. Store bottles in cold room on second floor.

Appendix B

Taste Threshold Training Protocol

Training trials or familiarizing trials
Have child rinse mouth 4 times with water before test

Instructions to child:

Show the 2 medicine cups to the child and say, "Now we are going to play our first game. This is a game with things to taste. We are going to play detective to see which cup has a taste in it. I will place two cups on the table. You will taste the first cup and swish it around your mouth, (but do not shallow it) and spit it out in the sink. You will then rinse once with water and spit it out. You will then tell me in which cup you can taste something different than water. Remember, we are playing detectives here. Even if you are not sure you can taste something, say which cup you think has a taste to it even if you have to guess. You will then rinse your mouth 2 times with water.

Trial 1: pair of plastic cups containing **water and step 17** will be presented and the child will be instructed to proceed with instructions presented above
For the tester: The first condition is given because it was important to give the children experience with a pair of solutions where they could not detect a difference because it was likely that most would not detect difference with the first 3-4 pairs of taste solutions.

Instructions to child:

Show the 2 medicine cups to the child and say, "Now we are going to play our first game. This is a game with things to taste. We are going to play detective to see which cup has a taste in it. I will place two cups on the table. You will taste the first cup and swish it around your mouth, (but do not shallow it) and spit it out in the sink. You will then rinse once with water and spit it out. You will then tell me in which cup you can taste something different than water. Remember, we are playing detectives here. Even if you are not sure you can taste something, say which cup you think has a taste to it even if you have to guess. You will then rinse your mouth 2 times with water.

TRIAL 2: pair of plastic cups containing **water and step 7** of sucrose solution. Same instructions as above
For the tester: Equally, it was important to provide them with an example of a pair where one solution was easily discernible as the stronger because a number of the test pairs would fall into this category.

Appendix C

Protocol for Testing Sweet Sensitivity

1. Start off with 120-ml bottles of the sucrose concentrations 0-17* and four 120-ml bottles of dH_2O. ** (All bottles labeled accordingly; you will have an extra bottle each of concentrations 7, 8, 9, 10, & 11)

   *Bottles of sucrose concentrations must be stored in the COLD ROOM on the 3rd floor.
   **Distilled water is abbreviated "dH_2O" throughout the instructions.

2. Every series is started with 2 medicine cups, 1 containing 10 ml dH_2O and the other containing 10 ml of 0.0032 M sucrose (bottle 10).

3. With each trial, every subject receives 10 ml of water and 10 ml of corresponding sucrose solution (order depends on grid numbers and concentration depends on previous correct/incorrect answer).

4. In room 326, set up the 21 bottles. Have a tray of medicine cups ready to use. (Each tray will be labeled in the order of the grid. Sucrose cups will be placed in the tray cup marked 2, and water cups will be in the tray cups left blank.)

5. Pour 10 ml of water into the cups in the blank cups and pour 10 ml of the sucrose according to concentration step (concentration step = bottle #).

6. Present order of cups according to the order of the grid one at a time.

7. Place the 2 cups on respective number on table. The subject will swish each cup around in the mouth for at least 5 seconds and then spit it out in the sink, rinsing once between each cup and twice after each set. After the second cup, the subject will say which cup had a taste to it. Even if the subject did not taste anything, a guess must be made.

8. Continue steps 5-7 until the subject has 4 reversals that meet the criteria, or until the subject reaches his/her maximum threshold.

Appendix D

Protocol to use Threshold Grids for Taste

1. The order numbers (the 1's and 2's running across the top and bottom of the grid) determine the order of presentation of solution (1's= water first, 2's=sucrose first) for each trial.

2. Have a piece of tape labeled 1 or 2 on the table for you to use throughout the testing. You will put the first cup on the #1 and the second cup on the #2 on the table. After the subject tastes the first cup they will rinse their mouth once with water and then taste the second cup. The subject will swish the liquids around in their mouth for 5 seconds before spitting them out in the sink. The subject will then rinse 2 times between each trial. (The subject cannot go back and re-taste any cups.)

3. Testing is a forced choice paradigm. After the subject has tasted both cups (a sucrose concentration and water), they must pick the cup they think has a taste to it. Even if the subject cannot taste anything, the subject must pick a cup even if a guess must be made.

4. The threshold testing starts at Step 10 (0.032M) of the 18 step sucrose concentration. (Step 0= 1M solution (most concentrated), Step 17=0.000056M solution (least concentrated).)

5. To record on the grid, a plus (+) is put in the square if the subject picks the sucrose cup, which is a correct response, and a minus (-) is put in the square if the water cup is picked, which is an incorrect response.

6. If the subject is incorrect after the first trial (picks water), you then proceed up the chart to the next concentration (Step 9). If the subject is correct after the first trial, you then retest Step 10. If the subject is correct on the second trial, you then proceed down the grid to the next sucrose concentration (Step 11). Each change in direction on the grid is called a **reversal.**

   If you get:

   Minus sign (-): proceed up the grid to the next concentration (ex. Step 10→ Step 9)

   Plus sign (+): retest same concentration

   +, + on same concentration: proceed down the grid to the next concentration (ex. Step 10→ Step 11)

   +, - on same concentration: proceed up the grid to the next concentration (ex. Step 10→ Step 9)

7. Total score for the threshold is based on the last 4 reversals and when the following criteria are met: (The score of the threshold is the average of the last 4 reversal concentration)
    a. When 4 reversals are grouped in such a way that there are no more than 2 dilution steps between any two successive reversals (see example 1). If the subject skips 3 or more dilution steps between 2 reversals (after having obtained a second reversal), the tester must continue until 4 reversals are obtained in a group (see example 2).
    b. When at least 4 correct answers (any two sets of 2) have been obtained on the same dilution level (see example 1). If the subject never guesses the same dilution level correctly 4 times and successive reversals form an ascending pattern, the tester must continue until either the reversals stabilize or until the subject reaches step 0 (see example 3).

8. If the subject reaches the bottom of the series (step 18) and guesses correctly twice at step 17, the threshold score is 17.

9. If the subject reaches the top of the series (Step 0) and guesses incorrectly, the score is 0 (see examples 5 and 6).

Appendix E

Threshold Grid Score Sheet

Reversal #	Threshold Step #	mM Concentration
1		
2		
3		
4		

Average mM Threshold: _____

Total # of positive (+) reversals: _____

Total # of negative (-) reversals: _____

# of reversals used for threshold: _____

Note: If you need to go for 5 reversals, score the last four reversals

STEP #	**Mol Concentration**
5	**.056**
6	**.032**
7	**.018**
8	**.010**
9	**.0056**
10	**.0032**
11	**.0018**
12	**.0010**
13	**.00056**
14	**.00032**

Appendix F

Approaches to Calculating Goldberg Cutoff

Approach 1: a very conservative approach (will identify least number of under-reporters)

1. Calculate the BMR (Basal Metabolic Rate) for each subject.
   Equation for boys
   Ages 3–10 BMR = 22.706 × W + 504.3
   Ages 10–18 BMR = 17.686 × W + 658.2

   Equation for girls
   Ages 3 – 10 BMR = 20.315 x W + 485.9
   Ages 10-18 BMR = 13.384 x W + 692.9

2. Compare reported energy intake to BMR for each subject (reported energy intake/BMR)
3. Anyone whose reported energy intake/BMR is < 1.0 is an under-reporter

Approach 2: less conservative, recommended in one article (will identify more subjects as under-reporters)

1. Calculate the BMR (Basal Metabolic Rate) for each subject.
   Equation for boys
   Ages 3–10 BMR = 22.706 × W + 504.3
   Ages 10–18 BMR = 17.686 × W + 658.2

   Equation for girls
   Ages 3 – 10 BMR = 20.315 x W + 485.9
   Ages 10-18 BMR = 13.384 x W + 692.9

2. Multiply each child's BMR by the appropriate age/gender PAL to determine estimated energy expenditure

Girls PAL	Age	Boys PAL
1.30	6 – 7 yo	1.30
1.35	7 – 8 yo	1.35
1.40	8 – 9 yo	1.40
1.40	9– 10 yo	1.40
1.45	10–11 yo	1.45

1.50	11-12 yo	1.50
1.55	12-13 yo	1.55

3. Compare reported energy intake to estimated energy expenditure for each subject (reported energy intake/estimated energy requirement)
4. Anyone whose reported energy intake/estimated energy expenditure is < 1.0 is an under-reporter.

Appendix G

Child's Temperament Questionnaire and Food Neophobia Scale (FNS)

Please rate, on a scale of 1-5 whether the following statements are true for your child that participates in the study. Circle the appropriate number: 1-Completely disagree, 2-slightky disagree, 3-neither agree or disagree, 4- slightly agree, 5-completely agree						
1	Once my child decides s/he doesn't like something, there is no way of getting him/her to like it	1	2	3	4	5
2	My child makes friends easily	1	2	3	4	5
3	My child likes to be with people	1	2	3	4	5
4	My child has strong likes and dislikes in food	1	2	3	4	5
5	My child tends to be shy	1	2	3	4	5
6	My child is afraid to try new foods	1	2	3	4	5
7	My child often fusses and cries	1	2	3	4	5
8	My child makes faces at new foods	1	2	3	4	5
9	My child is off and running as soon as s/he wakes up in the morning	1	2	3	4	5
10	My child rarely takes a new food without fussing	1	2	3	4	5
11	My child is very friendly with strangers	1	2	3	4	5
12	My child is always on the go	1	2	3	4	5
13	My child reacts intensely when upset	1	2	3	4	5
14	When my child moves about, s/he usually moves slowly	1	2	3	4	5
15	My child likes foods from different countries	1	2	3	4	5
16	My child consistently dislikes many kind of foods	1	2	3	4	5
17	My child will eat almost anything	1	2	3	4	5
18	My child takes a long time to warm up to strangers	1	2	3	4	5
19	My child cries easily	1	2	3	4	5
20	My child tends to be somewhat emotional	1	2	3	4	5
21	My child constantly wants to try new foods	1	2	3	4	5
22	My child finds people more stimulating than anything else	1	2	3	4	5
23	My child gets upset easily	1	2	3	4	5
24	My child does not trust new foods	1	2	3	4	5
25	My child prefers quite, inactive games to more active ones	1	2	3	4	5
26	When alone, my child feels isolated	1	2	3	4	5
27	My child prefers playing with others rather than alone	1	2	3	4	5
28	My child is very sociable	1	2	3	4	5
29	If my child does not know the food, he/she won't try it	1	2	3	4	5
30	My child is very energetic	1	2	3	4	5
31	My child is something of a loner	1	2	3	4	5

Appendix H

Child's Temperament Questionnaire and Food Neophobia Scale (FNS) Scoring Sheet

From Pliner, P., and Loewen. (1997). Temperament and Neophobia in Children (5-11years) and their mothers. Appetite, 28, 239-254

Reversed Items: 2, 11, 14,15,17,21,25,28,31
Add score for each item

Shyness: Items 2, 5,11,18,28
Shyness Scores:_____

Emotionality: Items 7, 13,19,20,23
Emotionality Scores:____

Sociability: Items 3, 22,26,27,31
Sociability Scores: _____

(Negative) reactions to food: Items 1,4,8,10,16
(Negative) reactions to food Scores: _____

Activity: Items 9, 12,14,25,30
Activity Scores: ____

From Pliner, P., & Hobden, K. (1992) Development of a scale to measure the trait of food neophobia in humans. Appetite, 19, 105-120

Food Neophobia: Items 6, 15, 17,21,24,29
Food Neophobia Scores: _____

Appendix I

BuccalAmp™ DNA Quick Extract DNA Extraction Protocol for Collection Swabs

epicentre
an Illumina company

BuccalAmp™ DNA Extraction Kit

QuickExtract™ DNA Extraction Solution 1.0

Catch-All™ Sample Collection Swabs

Cat. Nos. BQ0901S (CR, SC, RB, BS), BQ0908S (CR, SC, RB, BS), BQ0916S (CR, SC, RB, BS), QE09050, QEC091H, QEC89100, MB100BR, and MB100SP

STOP Please store the QuickExtract™ solution at -20°C.

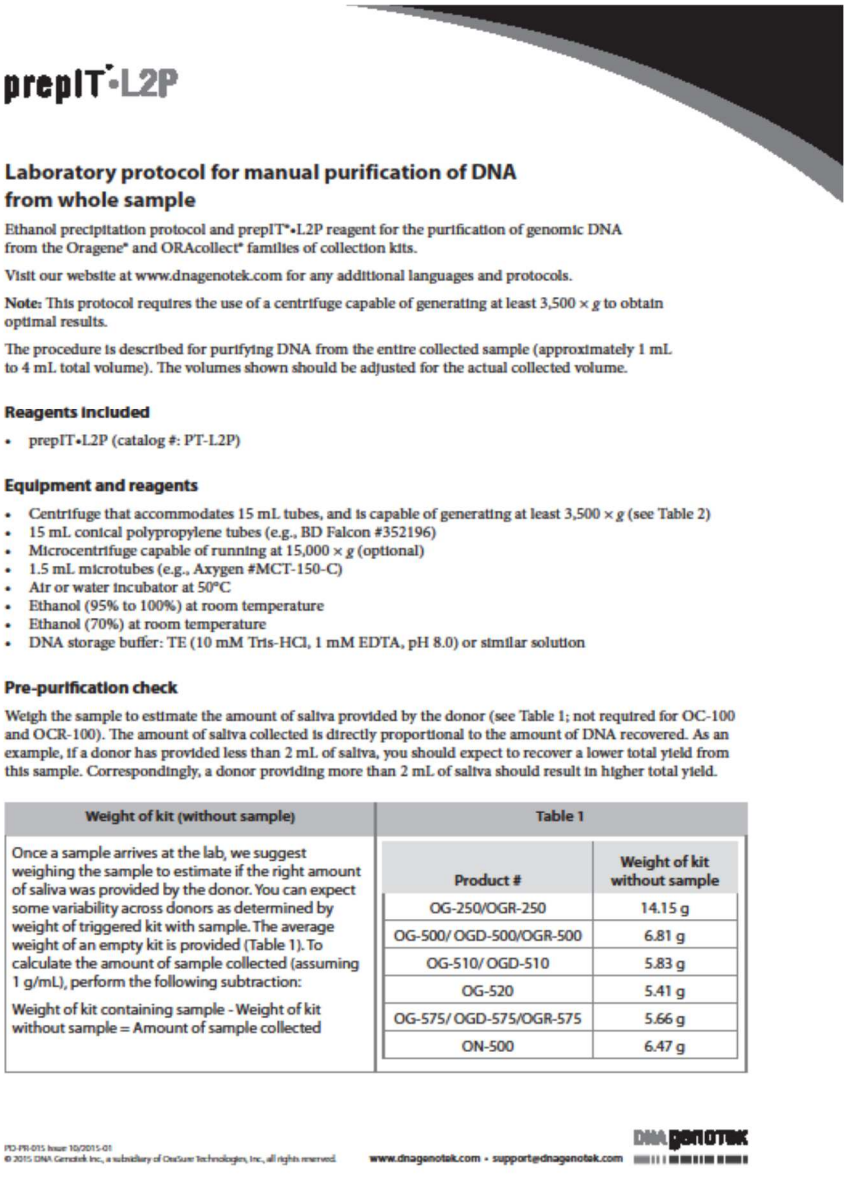
Connect with Epicentre on our blog (epicentral.blogspot.com), Facebook (facebook.com/EpicentreBio), and Twitter (@EpicentreBio).

PDF

buccalamp-dna-extra
ction-kit-quickextract-c

Appendix J

Orangene® DNA Laboratory Protocol for Manual Purification of DNA from 4.0 mL of Oragene®•DNA/saliva



prepIT•L2P

Laboratory protocol for manual purification of DNA from whole sample

Ethanol precipitation protocol and prepIT®•L2P reagent for the purification of genomic DNA from the Oragene® and ORAcollect® families of collection kits.

Visit our website at www.dnagenotek.com for any additional languages and protocols.

Note: This protocol requires the use of a centrifuge capable of generating at least 3,500 × *g* to obtain optimal results.

The procedure is described for purifying DNA from the entire collected sample (approximately 1 mL to 4 mL total volume). The volumes shown should be adjusted for the actual collected volume.

Reagents included

- prepIT•L2P (catalog #: PT-L2P)

Equipment and reagents

- Centrifuge that accommodates 15 mL tubes, and is capable of generating at least 3,500 × *g* (see Table 2)
- 15 mL conical polypropylene tubes (e.g., BD Falcon #352196)
- Microcentrifuge capable of running at 15,000 × *g* (optional)
- 1.5 mL microtubes (e.g., Axygen #MCT-150-C)
- Air or water incubator at 50°C
- Ethanol (95% to 100%) at room temperature
- Ethanol (70%) at room temperature
- DNA storage buffer: TE (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) or similar solution

Pre-purification check

Weigh the sample to estimate the amount of saliva provided by the donor (see Table 1; not required for OC-100 and OCR-100). The amount of saliva collected is directly proportional to the amount of DNA recovered. As an example, if a donor has provided less than 2 mL of saliva, you should expect to recover a lower total yield from this sample. Correspondingly, a donor providing more than 2 mL of saliva should result in higher total yield.

Weight of kit (without sample)	Table 1	
Once a sample arrives at the lab, we suggest weighing the sample to estimate if the right amount of saliva was provided by the donor. You can expect some variability across donors as determined by weight of triggered kit with sample. The average weight of an empty kit is provided (Table 1). To calculate the amount of sample collected (assuming 1 g/mL), perform the following subtraction:	Product #	Weight of kit without sample
	OG-250/OGR-250	14.15 g
	OG-500/ OGD-500/OGR-500	6.81 g
	OG-510/ OGD-510	5.83 g
	OG-520	5.41 g
	OG-575/ OGD-575/OGR-575	5.66 g
	ON-500	6.47 g
Weight of kit containing sample - Weight of kit without sample = Amount of sample collected		

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Note: DNAgenotek changed the name of the product to PrepIT® L2P but protocol remained the same. Protocol was adjusted for 1mL extraction.

Laboratory Protocol for Manual Purification

Appendix K

TaqMan® GTXpress™ Master Mix Protocol

TaqMan® GTXpress™ Master Mix

Protocol

Applied Biosystems

TaqMan GTXpress Protocol.pdf

Appendix L

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