SWEETENERS, SWEET ANTAGONISTS, AND METABOLISM

By

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ABSTRACT OF THE DISSERTATION

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Sugars and sweeteners are proposed to stimulate human sweet taste via the receptor, T1R2-T1R3. T1R2-T1R3 is a heterodimeric GPCR expressed in oral taste tissue. T1R2-T1R3 binds sugars, non-nutritive sweeteners, and sweet taste blockers. It was recently discovered that T1R2-T1R3 is also expressed in extra-oral tissues including the intestine, hypothalamus, pancreas, and adipose. This finding is striking because non-nutritive sweeteners are believed to be metabolically inert. Hence, it raises the question of whether T1R2-T1R3 plays a role not only sweet taste perception but also in regulatory and metabolic physiology.

The purpose of this research project is to investigate the perceptual and physiological functions of T1R2-T1R3 using a pharmacological approach in human participants. In the first aim, I determined whether glucose and fructose behave as partial agonists of the sweet taste receptor and can enhance or suppress each other in mixture. In the second aim, I sought to assess and improve the sweetness of glucose and its metabolic profile relative to fructose and sucrose. In

the third aim, I conducted psychophysical studies to determine whether metabolically active drugs act on the sweet taste receptor. In the fourth aim, I conducted glucose tolerance studies to determine whether sweet taste stimuli influence glucose metabolism. And in the fifth aim, I conducted glucose tolerance tests to determine whether sweet taste inhibitors influence glucose metabolism.

I found that sweeteners and antagonists had both perceptual and physiological functions. In the first aim, I demonstrated that glucose is a poor sweetener relative to fructose because it is not a full agonist of the sweet receptor. In the second aim, I demonstrated that glucose can be rendered almost indistinguishable from sucrose at the same caloric level with the addition of a non-nutritive sweetener. Thus, the difference in glucose and fructose sweetness can be overcome when adding stevioside to glucose. In the third aim, I demonstrated that clofibric acid, a lipid lowering prescription drug, inhibits sweet taste perception. Since it has been shown to inhibit T1R3 *in vitro*, I conclude from our data that it is also a T1R3 inhibitor *in vivo*. In the fourth aim, I demonstrated that high potency sweeteners (HPS), which are thought to be metabolically inert, enhance insulin and glucose responses relative to a standard OGTT. And in the fifth aim, I found that sweet taste blockers caused an opposite reaction and slowed glucose rise in the blood relative to a standard OGTT.

As a sugar receptor, T1R2-T1R3 imparts a powerful influence on human health by guiding food choice and metabolism. My findings are of public health relevance because excessive intake of sweet tasting compounds such as sugars and other sweeteners are a major long-term health concern. Overconsumption of dietary sugars, particularly in the form of sweetened beverages, is thought to promote obesity, diabetes, fatty liver disease, and metabolic syndrome. Despite efforts to curb intake of sweet beverages, the typical American consumes 50 liters of caloric and non-caloric soft drinks per year and even more in sugar-added foods and confections. As the prevalence of metabolic diseases grows, there is a greater need to understand the perceptual and physiological mechanisms, drives, and responses for sweet tasting compounds.

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Dedications

To Mom, Dad, Eddie, and Kim.

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List of abbreviations

3-OMG	3-O-methyl glucose
ANOVA	Analysis of variance
AUC	Area under the curve
BMI	Body mass index
CPIR	Cephalic phase insulin release
CPPP	Cephalic phase pancreatic polypeptide
DIO	Diet induced obesity
DPPIV	Dipeptidyl peptidase-IV
GIP	Gastric inhibitory peptide
gLMS	General labeled magnitude scale
GLP1	Glucagon-like peptide 1
GLP2	Glucagon-like peptide 2
GLUT2	Glucose transporter 2
GLUT4	Glucose transporter 4
GPCR	G protein coupled receptor
GSIS	Glucose stimulated insulin secretion
HFCS	High fructose corn syrup
HPS	High potency sweetener

K_{ATP} channel	ATP-sensitive potassium channel
MODY2	Maturity onset diabetes of the young 2
OGTT	Oral glucose tolerance test
PPAR	Peroxisome proliferator-activated receptor
RXR	Retinoid X receptor
SGLT1	Sodium-glucose linked transporter 1
T1r2-T1r3	Sweet taste receptor (murine)
T1R2-T1R3	Sweet taste receptor (human)
T2DM	Type 2 diabetes mellitus

Chapter 1.

Introduction and literature review

Introduction

The sweet taste receptor, T1r2-T1r3, signals the presence of carbohydrates and other sweet tasting compounds in mice and is hypothesized to play the same role in humans. The ability to detect carbohydrates and ingest them underlies a critical set of psychological and physiological processes that ensure our survival and well-being. First, T1R2-T1R3 is needed for the conscious perception of sweet taste, which guides food intake and eating behavior. Second, T1R2/T1R3 regulates metabolic processes which promote efficient digestion and assimilation of the food we eat.

Taste perception allows us to evaluate the chemical makeup of foods in order to determine whether they contain nutrients and/or toxins. Sweet taste perception guides food intake towards foods that provide carbohydrates, chiefly sugars. The ability to identify sugars is paramount because carbohydrates are an important energy source for all mammalian, non-obligate carnivores, including humans. Glucose is the primary fuel for the brain and it cannot be directly substituted with amino acids or fatty acids. When the level of glucose in the blood falls below a narrow range of concentrations, the effects can be dizziness, fainting, and even death, short of ketone bodies coming to replace glucose as a fuel for the brain. In light of such dire consequences, infants and children are both innately and heavily attracted to sweet tasting compounds [1].

Anticipatory responses to food ingestion enhance our ability to assimilate foods into nutrients and clear them from the blood. Just as low blood glucose levels can be harmful, so too can high blood glucose levels. High levels of glucose in the blood promote damage to blood vessels and other tissues. High blood glucose is a hallmark of diabetes and metabolic syndrome, which increases risk of chronic vascular disease, heart disease, and death. Anticipatory responses, which enhance the clearance of glucose from the blood and promote plasma glucose homeostasis are, therefore, highly adaptive.

The sweet taste receptor has also been implicated in taste responses and anticipatory physiological responses to sugars, but its functions are not clear. By clarifying the functions of the sweet taste receptor, we can better understand the perception and metabolism of sugars and other carbohydrates. Given that sugar consumption has reached historic highs in the US and abroad, the sweet taste receptor may play a key part in our understanding of nutrition related diseases.

Literature review

Sweet taste transduction

T1R2-T1R3 is primarily known as a putative sweet taste receptor. T1R2-T1R3 is a heteromeric receptor comprised of two subunits, T1R2 and T1R3. In rodents, *Tas1r2* and *Tas1r3* are co-expressed in taste receptor cells, and loss of function polymorphisms correspond with taste impairments [2, 3]. Functional expression assays show that in rodents and in humans, T1R2-T1R3 binds a host of sweet tasting compounds, and this binding triggers cellular responses *in vitro* [4, 5] Inhibition of the receptor with sweet blockers abolishes cellular responses. In rodents, T1R3 ablation drastically reduces neural and behavioral responses to sweet tasting compounds [6]. Interestingly, sweet taste is not entirely abolished in these animals, suggesting a separate sensor for sweetener detection. Candidates for this sensor include components of the sodium potassium pump (Na+/K+-ATPase), sodium-glucose linked transporter 1 (SGLT1), and several glucose transporters (GLUTs), all of which are expressed in taste cells [7].

The sweet receptor is expressed on taste cells, which are arranged in groups called taste buds. Taste buds are distributed in distinct loci throughout the oral cavity, each of which is innervated by branches of the 7^{th}, 9^{th}, and 10^{th} cranial nerves [8]. Taste buds are found on the fungiform papillae on the anterior tongue, the foliate and circumvallate papillae on the posterior tongue, and

the smooth epithelia of the soft palate and the pharynx [8]. An often cited, but inaccurate belief is that specific regions of the oral cavity are solely responsible for specific taste modalities [9]. Although certain regions of the oral cavity are particularly responsive to certain taste qualities, all taste modalities can be elicited in all regions. Sweet taste transduction begins when sweet tasting stimuli enter the taste bud pore and bind sweet receptors on taste cells, which are electrically active, specialized, epithelial cells. Receptor binding can activate alpha gustducin (an alpha component of a taste GTP-binding protein), which begins the intracellular signaling cascade leading to taste cell depolarization and neurotransmitter (e.g. ATP, serotonin) release [10]. The signal is then carried to the brain by primary afferent taste neurons. The brain represents taste from unique patterns of activity across a large set of neurons, chiefly within the opercular, insular, and orbito-frontal cortices.

There are key differences in the detection of high potency sweeteners (HPS) and carbohydrates in the oral cavity. The detection of HPS in mice relies entirely on the sweet taste receptor. T1r3 ablation in mice abolishes neural and behavioral responses to HPS [6]. In contrast, T1r3 ablation reduces but does not abolish responses to glucose [6]. Therefore, the presence of glucose in the oral cavity is also signaled, to some extent, by a T1r3-independent mechanism. Taste cells express the monosaccharide transporters SGLT1 and GLUT2, which move glucose into cells [7, 11]. Glucose uptake into taste cells may contribute to activation of downstream signaling events and depolarization via K_{ATP} channels. This sensory mechanism could explain why T1r knockout animals retain cephalic phase reflexes to saccharides [12]. Like glucose, galactose binds glucose transporters [13]. Unlike glucose, galactose elicits aversive postingestive effects in rodents [14]. Free galactose is not a major component of the adult human diet. Galactose is a constituent of lactose (galactose + glucose), but it is not released by lactase in the oral cavity. It is not known whether galactose stimulates non-T1R mediated responses in the oral cavity.

Glucose polymers are also detected in a distinct manner. In rats, glucose polymers (Polycose) and sucrose elicit independent neural and behavioral responses [15]. In humans, oral rinsing with maltodextrin, but not HPS, elicits neural responses and ergogenic effects [16]. Humans can

also discriminate high concentrations of maltose (the disaccharide of two glucose molecules) from other saccharides [17]. It is unclear whether humans express a receptor for glucose polymers.

Sweet receptor structure

T1R2 and T1R3 are class C GPCRs, which are characterized by a 7 transmembrane domain (7TMD), cysteine rich domain (CRD), and an N-terminal venus flytrap domain (VFD). Other examples of class C GPCRs include mGluR, GABA type B receptor, and the extracellular calcium receptor [18]. T1R2-T1R3 couples with the G protein alpha gustducin, which transduces both sweet and bitter taste perception [19]. The sweet receptor shares a subunit in common with the umami receptor, T1R1/T1R3. The T1R2/T1R3 heteromer has multiple ligand binding sites. Binding sites have been identified in the T1R2 VFD, T1R3 VFD,T1R3 7TMD, and T1R3 CRD [20]. The structures of murine and human sweet taste receptors are similar, but there is a key structural difference in the T1R3 7TMD. As a result, sweeteners and sweet blockers which bind the hT1R3 7TMD do not elicit physiological effects in mouse models and vice versa [20].

Sweet receptor ligands

Human T1R2-T1R3 binds a structurally diverse set of agonists at multiple sites. Broadly speaking, T1R2/3 agonists include carbohydrates (glucose, fructose, galactose, maltose, lactose, and sucrose), peptides (aspartame, neotame), proteins (thaumatin, brazzein, and monellin), and synthetic sweeteners (sucralose, cyclamate, saccharin, acesulfame-K). Sucrose, fructose, sucralose, aspartame, neotame, saccharin, acesulfame-K bind the T1R2 VFD, cyclamate and neohesperidin dihydrochalcone bind the 7TMD of T1R3, and the sweet proteins thaumatin and brazzein bind the T1R3 CRD [21-27].

T1R2/T1R3 ligands vary broadly in terms of potency and maximal activity. A ligand's potency is proportional to its ability to bind and activate its receptor [28]. Sweetener potency can be assessed in terms of its absolute detection threshold and recognition threshold. The detection threshold is the lowest concentration needed to be distinguishable from water, and the recognition threshold is the lowest concentration needed to elicit sweetness. Carbohydrate sweeteners have relatively low potency. Of the carbohydrate sweeteners, glucose has the lowest potency. Fructose is roughly twice as potent as glucose on a mole for mole basis [17]. Sucrose, a disaccharide of glucose and fructose, is three times as potent as glucose on a molar basis [17]. High potency sweeteners, as their name indicates, are much more potent than carbohydrate sweeteners. For instance, the detection threshold for sucralose is < 1 mM. Because the detection threshold for sucralose and other HPS can be several hundred fold lower than that of sucrose, HPS are somewhat misleadingly described as being several hundred times sweeter than sugar. This is accurate only in the sense that sucralose has much greater affinity for the sweet taste receptor than does sucrose. Potency is distinct, however, from maximal activity.

Maximal activity describes the degree to which a ligand activates its receptor and produces a response [29]. For a sweet receptor ligand, maximal activity is synonymous with maximal sweetness. Maximal sweetness can be assessed in suprathreshold concentration-intensity functions. Generally, as the concentration of HPS increases, sweetness intensity reaches an asymptote past which greater concentrations do not increase sweetness [30]. Carbohydrate sweeteners, despite relatively low potency, elicit greater asymptotic sweetness intensity [30, 31]. In contrast, high potency sweeteners elicit lower levels of sweetness at asymptote [31]. In fact, high concentrations of HPS such as sucralose and acesulfame potassium elicit strong bitter and metallic tastes [31] and can be self-inhibitory at high concentrations [32].

T1R2/T1R3 also binds sweet taste antagonists. The primary examples of human T1R2/3 inhibitors are gymnemic acid and sodium lactisole. Both inhibitors are believed to bind the hT1R3 7TMD, but their inhibitory properties differ [33, 34]. Gymnemic acid inhibits sweet receptor activity when presented prior to exposure to a sweet compound [35]. Sweetness inhibition

continues after gymnemic acid is expectorated. Sodium lactisole, in contrast, inhibits sweet receptor activity only when presented in admixture with a sweet compound [36]. When washed off the receptor with water, sodium lactisole induces rebound activity which elicits sweet taste [32] The effect is sometimes called “sweet water-taste”. This difference is due to the fact that sodium lactisole is an inverse agonists of the sweet receptor [32]. The sweet receptor is constitutively active. When sodium lactisole binds, the receptor’s conformation changes to the inactive state and when lactisole is rinsed away, the receptor rebounds to the partially active state, thereby eliciting sweet taste [32].

Sweet taste informs nutrient selection

Taste is a chemical sense. We use our sense of taste to identify the chemical makeup of a potential food source in order to assess its nutrient content. Appetitive taste stimuli reinforce the consumption of needed nutrients. Aversive stimuli, on the other hand, discourage the consumption of potential toxins. Amino acids, specifically glutamate, elicit savory (umami) taste. Savory taste identifies foods rich in amino acids as well as protein, which is essential for survival. Similarly, salty tastes identify sodium and other ions. Sweet taste identifies sugar rich foods. Given that human ancestors were likely predominantly frugivorous (deduced from the observation that all species of living apes other than humans are largely frugivorous), the ability to identify sweet foodstuffs containing sugars was critical for survival. Sour taste indicates the presence of acid, which is aversive at high levels and appetitive at low levels, especially when mixed with sugar (such as in fruit) [37]. Bitter taste, which is aversive and can induce nausea, is adaptive because it deters us from consuming large quantities of toxins [38].

The importance of taste perception is highlighted in patients with taste disorders. Taste sensitivity can be partially lost (hypogeusia) or entirely lost (ageusia) due to various causes at the cellular and organ level resulting from aging, disease states, and medical therapies [39]. Taste is also lost in head and neck patients receiving radiotherapy. Loss of taste sensitivity is associated

with unintentional weight loss and reduced quality of life [40, 41]. Taste and flavor enhancement have been successfully employed as a means of increasing food intake and improving health status in elderly patients [42].

Sweet taste presents a particularly important problem to human health. Our innate attraction to sweet tasting foods, which served well our ancestors in the tropical forests, has since become a major public health concern. Carbohydrate rich foods are no longer scarce, thanks to advances in agriculture and technology. The amount of food energy available per capita has increased to the point that the major nutritional challenge for humans of industrialized nations has shifted from undernutrition to overnutrition [43]. The prevalence of conditions related to overnutrition such as obesity and type 2 diabetes mellitus have increased dramatically in the latter half of the 20th century [44]. The epidemic of nutrition-related diseases has been attributed to a long list of factors, but one of the most often cited causes is the overconsumption of added-sugar foods, including sugar-sweetened beverages.

For example, the consumption of sugar-sweetened beverages has more than doubled since the 1970s [45]. As of 2010, the average American youth (age 20 years and younger) consumes approximately 155 kilocalories per day from soft drinks [46]. According to NHANES 2005-2008, 25% of Americans consume at least 200 kilocalories per day from soft drinks [47]. Of the caloric sweeteners, high fructose corn syrup (HFCS) and sucrose are particularly maligned. Fructose is a preferred sweetener because it is roughly twice as sweet as glucose [17]. But, observational studies show that fructose consumption is associated with increased risk for metabolic syndrome and sucrose and HFCS are approximately 50% fructose [48-50]. Controlled trials show that excessive fructose consumption, more so than excessive glucose consumption, promotes metabolic dysfunction [51-55].

Diet soft drinks sweetened with HPS are meant to satisfy the desire for sweet foods without inflicting metabolic derangements. The first diet soft drink, No-Cal, was introduced in the early 1950s [56]. No-Cal and its successors were marketed toward health-conscious consumers. The impact of diet soft drink on public health is equivocal, however. This is likely due in part to the

fact that diet soft drinks are much less popular than regular soft drinks. According to NHANES 1999-2002, mean diet carbonated soft drink consumption in women and men age 20-39 years was 108.1 and 69.9 grams per day. For the same groups, regular carbonated soft drink consumption was 430.2 and 637.9 grams per day [57].

Diet soft drink consumption lags behind regular soft drink consumption for several reasons. First, although diet soft drinks are meant to mimic the taste profiles of regular drinks, they can easily be distinguished due to off-tastes and lingering aftertastes from HPS [58]. Soft drink manufacturers have attempted to address this problem with mid-calorie product lines that contain HPS and low amounts of added sugar. Second, some consumers avoid HPS on the *ad naturam* argument that artificial ingredients are unhealthy [59]. To appeal to such biased customers, naturally occurring high potency sweeteners such as stevia and monkfruit are used in diet product lines. In response to complaints of off tastes from rebaudioside A, manufacturers have developed other natural sweeteners with more desirable taste profiles, such as rebaudioside D [60]. The sheer amount of resources put into designing sweet drinks illustrates the importance of sweet taste to human nutrition. Despite these efforts, diet soft drinks fail to take the place of sugar sweetened soft drinks.

Oral carbohydrate detection primes regulatory physiology

When food is consumed, blood chemistry changes dramatically. Or to put it more colorfully, our 'internal Eden is suddenly and devastatingly altered' [61]. Understandably, the body makes a coordinated effort to moderate such an alteration. Homeostasis, a term coined by Walter Cannon, is a central tenet of regulatory physiology describing the coordinated activity of organ systems by which steady states tend to be maintained in the body [62]. Cannon drew on the schema of Claude Bernard, who postulated that the regulation of the internal environment (the *milieu interieur*) in the face of external changes is of critical importance for human life.

With regards to eating, homeostatic activity begins before food is ingested. As Ivan Pavlov demonstrated, cues such as the ringing of a bell or the perception of food in the oral cavity can trigger digestive responses [63]. These responses are entirely independent of food ingestion, as evidenced by the fact that Pavlov observed them in fistulated animals and with very small stimulus volumes. They are absent, however, in vagotomized animals. Pavlov termed these phenomena “psychic reflexes” because they are neurally mediated. Currently, these effects are called cephalic phase (head-stimulated) responses.

A carbohydrate rich meal poses a threat to the internal environment. When a healthy person consumes a carbohydrate rich meal, blood glucose concentration can increase by 50% or more. This increase is much more exaggerated in pre-diabetes and frank diabetes. Glycemia levels need to be defended because high levels of glucose in the blood are dangerous. Excessive glucose in the blood can damage blood vessels, glycosylate proteins, and promote the pathogenesis of chronic disease [64, 65]. High postprandial glucose is thought to exert a more deleterious effect on the vasculature than high fasting glucose [66].

Cephalic phase insulin response (CPIR) is particularly helpful in facilitating glucose homeostasis. CPIR is a small, transient increase in plasma insulin that occurs before exogenous glucose appears in the blood [67]. Plasma insulin increases from baseline within 5 minutes and returns to baseline within 10 minutes of oral stimulation with carbohydrate [67]. Relative to postprandial insulin levels, the magnitude of CPIR is remarkably low. The magnitude of CPIR is roughly 1% of postprandial insulin peak [68]. It is typically defined as a 25% increase from baseline insulin concentration [69]. Because the magnitude of CPIR is so small, cephalic phase responses are sometimes assessed by measuring pancreatic polypeptide. Cephalic phase pancreatic polypeptide (CPPP) is a useful measurement because it is vagally activated, it is independent of blood glucose, and its magnitude is much greater than that of CPIR [68, 70].

CPIR reduces postprandial glycemia. The effects of CPIR can be observed by infusing glucose with and without sham-feeding. When glucose is infused without sham feeding, CPIR is absent. When glucose is infused and paired with sham feeding, the plasma glucose AUC from infused

glucose is reduced by 30% [71, 72]. Considering that the magnitude of CPIR is relatively small, its effects on postprandial glucose are striking. CPIR likely exerts its actions through the liver. Insulin is released by the pancreas into the portal circulation, degraded partially by the liver, and then released into peripheral circulation. CPIR is assessed using peripheral concentrations of insulin, which may not be reflective of portal levels of insulin [68]. It is possible that CPIR so effectively lowers plasma glucose because it inhibits hepatic glucose output well before exogenous glucose appears in the blood.

The sensory mechanisms underlying CPIR are unclear. In animals, aqueous glucose and sucrose solutions elicit CPIR [12, 73]. In humans, sucrose elicits CPIR in some studies [74] , but not others [75]. To further complicate the issue, it appears that CPIR occurs more reliably in response to sham feeding with solids than liquids [67, 72, 75]. The discrepancies between studies are possibly due to the fact that CPIR is a notoriously small and transient effect [68]. Analyzing CPIR requires an extended baseline period in order to account for normal fluctuations in plasma insulin. It also requires frequent blood sampling (~2 minutes between samples) and precise insulin measurements.

HPS do not reliably elicit cephalic phase responses [76-78]. CPIR may be mediated by T1R independent carbohydrate detection in the oral cavity. In a recent study, oral stimulation with glucose stimulated CPIR in T1r KO animals [12]. In the same study, fructose did not stimulate CPIR. A key difference between glucose and fructose is that only glucose binds SGLT1. Both of these proteins are expressed in taste tissue [7].

Oral carbohydrate detection also influences exercise performance. This phenomenon was first observed in a study comparing the effects of exogenous glucose on exercise performance. Surprisingly, oral glucose consumption improved exercise performance [79] but I.V. glucose infusion did not [80]. Subsequent studies demonstrated that oral rinsing with carbohydrate improves exercise performance [81-83]. It is thought that oral carbohydrate rinsing improves performance because it stimulates reward centers in the brain, thereby motivating performance [16]. Although oral rinsing with carbohydrate stimulates insulin release, it is unlikely that this

effect can be attributed to insulin. First, studies of CPIR and exercise performance differ substantially in terms of experimental design. Second, insulin inhibits hepatic glucose output which would normally be an important means of stabilizing plasma glucose. Similar to CPIR, the ergogenic effect of carbohydrate rinsing may be triggered by a T1R independent mechanism. The effect is stimulated by rinsing with starch, but not with HPS alone [16].

Extra-oral nutrient detection facilitates nutrient assimilation

T1R2-3 and glucose absorption

Glucose is absorbed from the intestinal lumen by sodium glucose transporter 1 (SGLT1). SGLT1 is an active transporter that uses the sodium gradient to move glucose across the apical membrane of the enterocyte. Glucose is transported out of the enterocyte and into the circulation through glucose transporter 2 (GLUT2) via facilitated diffusion. When luminal glucose concentration is high, GLUT2 translocates to the apical membrane to enhance glucose absorption [84]. SGLT1 expression also increases in response to high carbohydrate feeding [85] and SGLT1 expression is upregulated by glucagon like peptide 2 (GLP-2) [86]. In order to respond to changing levels of glucose, the intestine needs sensors to detect them.

Several lines of reasoning suggest that T1R2-T1R3 senses sugars in the gut and stimulates absorptive responses. First, T1R2-T1R3 is expressed in the apical membrane of endocrine cells in the gut, which secrete chemical signals. More specifically, T1R2-T1R3 is expressed on the surface of enteroendocrine L-cells, which secrete GLP-1 upon exposure to HPS [87, 88]. This effect is blocked by lactisole, a T1R2-T1R3 antagonist [88]. In *ex vivo* mouse models, intestinal perfusion with sucralose stimulates GLP-1 and GLP-2 secretion [89]. In knockout mouse models, T1r3 ablation abolishes the GLP-1 response to the presence of glucose in the intestine [87].

Second, HPS upregulate glucose transporters in animal models. The addition of HPS to a low-carbohydrate diet increases luminal SGLT1 expression in mice [90]. This effect is dependent on

T1r3 expression [90]. Also, intestinal perfusion with HPS has been shown to increase apical translocation of GLUT2 in mice [91].

It is thought that T1R2-T1R3 activation in L-cells stimulates the secretion of incretins, like GLP-1, which upregulate glucose transporter expression elsewhere in the lumen via paracrine signaling and ultimately increase glucose absorption [90]. This hypothesis is supported by a study in humans which found that elevated duodenal T1R2 expression is associated with increased absorption of 3-O-methylglucose (3-OMG) [92].

T1R2-3 and glucose clearance

After a meal, glucose is primarily cleared from the blood by insulin. Insulin binds insulin receptors on target tissues, where it increases the translocation of GLUT4 to the apical membrane. This enhances glucose uptake in adipose and skeletal muscle.

Insulin is released by pancreatic beta cells, which sense and respond to changes in blood glucose. Beta cells release insulin in response to cellular depolarization. Glucose oxidation in the beta cell is a key driver of depolarization and insulin release. When blood glucose increases, glucose uptake increases in the beta cell through GLUT2 [93]. Intracellular glucose concentration then increases, which increases flux through glycolysis. Glucose oxidation increases intracellular ATP, which inhibits K_{ATP} channels and opens voltage dependent calcium channels, which ultimately elicits cell depolarization [93]. The importance of glucose oxidation in insulin secretion is made clear in patients with maturity onset diabetes of the young 2 (MODY 2). MODY 2 is an inborn error of metabolism resulting in a loss of function mutation in pancreatic glucokinase [94]. In these patients, glucokinase is much less sensitive to glucose concentration, so glucose oxidation is impaired in the beta cell. As a result, insulin is only released at relatively high blood glucose values. This results in fasting glucose levels well above the “normal” range (~150 mg/dl) [94]. For this reason, glucokinase is considered a key ‘glucostat’ in the pancreas.

Beta cells also respond to incretin hormones, such as GLP-1. GLP-1 is thought to enhance glucose stimulated insulin secretion (GSIS) by inhibiting (closing) K_{ATP} channels and enhancing

calcium channel activity [95]. Together, these actions increase the excitability of the beta cell in response to glucose. The term “incretin effect” describes the increased insulin response to oral glucose relative to IV glucose [96]. As a consequence of the enhanced insulin response, the incretin effect improves glucose clearance and results in lower postprandial glucose. The incretin effect is due primarily to GLP-1 and gastric inhibitory peptide (GIP) [97]. In addition to promoting acute insulin responses, the incretin hormones also promote beta cell proliferation [95]. The incretin effect is impaired in type 2 diabetes mellitus (T2DM) [98]. In T2DM, GIP sensitivity is impaired [99]. GLP-1 sensitivity remains intact, but its abundance is reduced [99]. Because GLP-1 remains effective in T2DM, it is a particularly attractive candidate for pharmaceutical therapies. GLP-1 receptor mimetic drugs such as Liraglutide and Exendatide are effective diabetes treatments [100]. The incretin hormones are degraded by dipeptidyl peptidase-IV (DPP-IV). Presently, DPP-IV inhibitors are also prescribed to control glycemia in diabetics [101].

HPS may influence glucose clearance directly via binding pancreatic sweet receptors and indirectly via stimulation of GLP-1 in L-cells. Recent evidence suggests that pancreatic beta cells also respond to HPS *in vitro*. T1R2-T1R3 is expressed in pancreatic beta cells in humans and mice [102, 103]. *In vitro*, murine beta cells secrete insulin when exposed to HPS and this effect is blocked by sweet taste inhibitors [103, 104]. It is unclear whether dietary HPS stimulate pancreatic receptors *in vivo*. HPS are poorly absorbed by the intestine and the pancreas is exposed to little dietary HPS. Aspartame does not enter circulation because it is degraded to its amino acid constituents, phenylalanine and aspartate. Approximately 10% of ingested sucralose is absorbed in a paracellular manner. Diet Coke sweetened with Splenda contains 40 mg sucralose and it is unclear whether 4 mg sucralose would activate the sweet receptor when diluted in the circulation. Acesulfame K is absorbed in the intestine and is entirely excreted in the urine. Of the commonly consumed HPS, acesulfame K may be the most likely to interact with pancreatic T1R2-T1R3.

The notion that T1R2-T1R3 helps regulate glucose clearance is supported by data from T1R knockout animals. T1R3 knockout mice display drastically reduced GLP-1, delayed insulin, and

increased plasma glucose responses to glucose loads [105, 106]. Human studies, however, have yielded mixed results. HPS consumption in the absence of glucose has not been observed to affect GLP-1, insulin, and glucose [107-109]. But, HPS consumption 10-15 minutes prior to consumption of 75 g glucose appears to elicit significant, albeit mixed, effects. To date, 4 studies have examined the effect of an HPS preload on glucose tolerance [110-113]. In the first study, a mixture of sucralose + acesulfame K increased GLP-1 but did not affect insulin or glucose in healthy adults [111]. In the second, a mixture of sucralose + acesulfame K increased GLP-1 secretion in type 1 diabetics and healthy individuals, but not type 2 diabetics [112]. In the third, sucralose did not increase GLP-1, but did increase insulin and glucose in morbidly obese, non-diabetic adults [110]. In the fourth, sucralose enhanced GLP-1 release and lowered blood glucose in healthy subjects, but acesulfame K had no effect. In the same study, neither HPS affected blood glucose, incretin, or insulin responses in type 2 diabetic patients. In these studies, HPS appear to exert effects in non-diabetic participants, but not in type 2 diabetics. This key discrepancy remains to be explained, but it may be due to differences in T1R2-T1R3 expression in disease states. Furthermore, only one of the above studies reported enhanced glucose clearance from HPS.

T1R2-T1R3 and lipid metabolism

T1R2-T1R3 may also regulate fat metabolism, either indirectly through insulin or directly through adipocytes. T1R2-T1R3 is expressed in adipocytes and stimulation with HPS inhibits adipogenesis and lipolysis [114]. When fed an obesogenic diet, T1R2 and T1R3 knockout mice are partially protected against fat mass gain [115, 116]. T1R2 knockout animals are also protected against diet-induced hyperinsulinemia. Because T1Rs are knocked out whole body in these animals, it is unclear whether this effect is due primarily to T1Rs in the gut, adipose, or elsewhere.

The effects of T1R2-T1R3 on fat metabolism are intriguing because clofibric acid, a lipid lowering prescription drug, inhibits T1R2-T1R3 *in vitro*. Moreover, the physiological effects of T1R ablation appear to overlap with the physiological effects of clofibric acid treatment. Both reduce ectopic

lipid accumulation [116, 117] and improve insulinemia [116, 118]. Clofibric acid is thought to exert its effects through PPAR alpha activation [119]. But, its effects on T1R2-T1R3 have not been examined *in vivo*.

Specific Aims

Carbohydrate rich foods are a major component of Western diets and they are implicated in the increasing prevalence of metabolic dysfunctions. Growing evidence suggests that carbohydrate perception and assimilation are coordinated by the sweet receptor, but its role remains unclear. I sought to determine whether the sweet receptor could be manipulated using a pharmacological approach to influence food selection and glucose tolerance.

Specific Aim 1: To determine whether metabolically active drugs influence sweet taste

Fibrate drugs, which improve features of the metabolic syndrome, inhibit the T1R3 taste receptor *in vitro*. T1R3 forms a heteromer with T1R2 to form the sweet receptor. It is not known whether clofibric acid inhibit sweet perception. The purpose of this aim was to determine whether clofibric acid inhibits taste perception from T1R3 mediated pathways and is therefore a T1R inhibitor *in vivo*. I examined whether clofibric acid inhibits the perception of sweet taste.

Specific Aim 2: Analysis of glucose and fructose as partial receptor agonists.

Glucose may be preferred over fructose from a nutritional standpoint, but its potency as a sweetener is weak. The purpose of this aim was to examine why fructose is a privileged sweet receptor agonist relative to glucose. To do so, I examined the sweetness additivity of glucose and fructose on sweet taste perception using self-mixtures and binary-mixtures of each agonist.

Specific Aim 3: To assess and improve glucose sweetness relative to fructose

Excessive fructose intake, more so than excessive glucose intake, is thought to promote metabolic derangements. Sucrose and HFCS remain the most popular sweetening systems because glucose is weakly potent and diet soft drinks containing HPS are easily discriminated from regular soft drinks. I therefore sought to determine whether the sweetness of glucose could be matched to fructose using stevioside.

S**pecific Aim 4: To determine whether sweet taste stimuli influence metabolism**

The sweet taste receptor is expressed in the oral cavity, intestine, pancreas, liver, and adipose. It is thought to contribute to glucose homeostasis, but its functions are not clear. The purpose of this aim was to determine whether sucralose alters outcomes from a 75 gram oral glucose tolerance test. I examined whether sucralose, a T1R2-T1R3 agonist, enhances insulinemic and glycemic responses to oral glucose.

Specific Aim 5: To determine whether sweet taste inhibitors influence metabolism

Lactisole has been shown to inhibit the effects of HPS on hormone secretion from endocrine cells *in vitro*. And fibrate drugs, which yield metabolic benefits, have been shown to inhibit T1R3 *in vitro*. I therefore sought to determine whether the ingestion of lactisole, a T1R2-T1R3 inverse agonist, elicits metabolic effects. I examined whether lactisole impairs insulinemic and glycemic responses to oral glucose.

Chapter 2.

Glucose and Fructose as Sweet Receptor Partial Agonists

ABSTRACT

Glucose may be preferred over fructose from a nutritional standpoint, but its potency as a sweetener is much weaker both on calorie-for-calorie basis and a mole-for-mole basis. Glucose and fructose are thought to bind the same sites on T1R2-T1R3, the putative human sweet taste receptor. Despite this similarity, glucose is roughly half as sweet as fructose on a molar basis. We, therefore, sought to determine whether they behave as if they are partial agonists of the sweet receptor using a psychophysical approach in human participants. We hypothesized that if glucose is a partial agonist of the sweet receptor, then glucose would suppress the sweetness of fructose in a binary mixture relative to adding equally-sweet (hence equally activating) fructose to itself. We used a self-mixture approach to examine the behavior of each saccharide. We first prepared equisweet concentrations of glucose and fructose and confirmed their similarity using two-alternative forced-choice (2AFC) testing and sweetness scaling. Next, we determined separate dose-response functions for glucose neat and fructose neat across a broad range of concentrations. Last, we presented each agonist in self mixture and in binary mixture across the same range of concentrations. We found that the perceived sweetness intensity of fructose + glucose was significantly less than of fructose + fructose. Because the added levels of glucose and fructose were equisweet when tested in isolation, their behavior in mixture suggests that fructose and glucose sweetness are not interchangeable even when equi-sweet. Although we did not measure T1R2-T1R3 activity directly, these psychophysical data suggest that glucose may behave as a partial agonist of the sweet receptor, effectively interfering with the more activating fructose from binding when glucose occupies the binding site.

INTRODUCTION

Carbohydrate sweeteners, particularly those containing fructose, are used in food products because they elicit sweet taste, which is innately attractive [1]. Excessive sugar consumption is a global issue because it is thought to promote metabolic dysfunction [120, 121]. Clinical trials show that high glucose intake is less harmful than high fructose intake [53, 54]. Unfortunately, glucose is a poor sweetener relative to fructose [17] so it is required in higher amounts to provide similar sweetness. In order to develop interventions to curb sugar intake, it is important to understand the sensory properties of carbohydrate sweeteners. The difference in sweetness between glucose and fructose has not been explained on a molecular level.

In mice is known that sweet taste is transduced by T1R2/T1R3, a heteromeric g-protein coupled receptor expressed on taste cells in the oral cavity [4, 122]. Each subunit of the receptor is comprised of a venus flytrap domain, cysteine rich domain, and 7 transmembrane domain [20]. T1R2/T1R3 binds a diverse set of ligands which span wide ranges of molecular weight and structure including carbohydrate sweeteners and high potency sweeteners (HPS) [123]. Glucose and fructose are thought to bind the venus flytrap domains of T1r2 and T1r3 [123]. Although they bind the same active site on the sweet receptor, fructose is twice as sweet as glucose on a molar basis and on a calorie basis.

Because glucose is a particularly weak sweetener among the sugars, we considered whether glucose behaves as a partial agonist of the sweet receptor. A partial receptor agonist exhibits limited intrinsic activity relative to a full agonist [124]. When presented in a heterogeneous binary mixture, the partial agonist competes for a binding site with the full agonist. Because the partial agonist has lower intrinsic activity, the partial agonist, in effect, reduces the functional response of the agonist-receptor system by impeding the more activating stimulus from binding.

We investigated whether glucose and fructose have different intrinsic activity using an established additive model [125]. Under a simple additive model of a binary mixture:

$$e_{FXGY} = e_{FX} + e_{GY} \quad (1)$$

where e_{FXGY} is the effect the mixture; e_{FX} is the observed effect of concentration X of substance F; and e_{GY} is the observed effect of concentration Y of substance G.

This model is based on the assumption that G and F stimulate a common receptor and are functionally equivalent. This model can be used to investigate the interactions of binary sweetener mixtures. If the effect of the mixture matches the effect of its components ($e_{FXGY} = e_{FX} + e_{GY}$), then the two component sweeteners behave in an additive fashion. If $e_{FXGY} > e_{FX} + e_{GY}$, then the components are synergistic. If $e_{FXGY} < e_{FX} + e_{GY}$, then the components are suppressive.

We used a self-mixture approach to investigate whether glucose behaves as a partial agonist of fructose. We first determined concentrations of G and F that were functionally equivalent (equisweet). Next, we determined the concentration-response function for each agonist across a broad range of concentrations. Last, using the functionally equivalent concentrations of G and F, we presented each agonist in self mixture (Gs+G, Fs+F) and in binary mixture (Gs+F, Fs+G), where s denotes the concentration series stimulus, across the same range of concentrations. To express this in the additive model:

If

$$e_{FA} = e_{GB}$$

Then,

$$e_{FXFA} = e_{FX} + e_{GB} \quad (2)$$

where e_{FXFA} represents the effect of the mixture of F+F at concentrations X and A, respectively; e_{FX} represents the effect of F at concentration X; e_{FA} represents the effect of F at concentration A; and e_{GB} represents the observed sweetness of glucose at concentration B.

We hypothesized that if glucose is a partial agonist of the sweet receptor, then the observed sweetness of Fs+G would be less than the sweetness of Fs+F. Glucose would exert a subadditive effect on fructose sweetness, thus behaving as a partial agonist of the sweet

receptor. Considering the impact on the function, both sweeteners when added to the fructose function should left-shift the curve along the X-axis. But glucose should left-shift the fructose curve less than adding fructose to the fructose curve.

METHODS

Participants

Participants were recruited from the Rutgers University New Brunswick campus. Subjects were paid to participate and provided informed consent on an IRB approved form. This protocol complies with the Declaration of Helsinki for Medical Research involving Human Subjects and paid to participate after providing their informed consent. 4 participants took part in the preliminary sweetness matching study. 15 participants took part in the concentration-response study. Participants were asked not to eat, drink, or smoke one hour prior to each session.

Training

All subjects were trained in the use of a general Labeled Magnitude Scale (g LMS) following standard published procedures [126, 127]. The top of the scale was described as the strongest imaginable sensation of any kind. Participants were asked to identify the taste qualities elicited by each stimulus and to rate the perceived intensity along a vertical axis lined with the following adjectives: barely detectable, weak, moderate, strong, very strong, and strongest imaginable. The adjectives are spaced semi-logarithmically, based upon experimentally determined intervals to yield ratio quality data.

Taste discrimination methods

We used a two alternative forced choice protocol as well as a sweetness scaling protocol to verify the functional equivalence of 100 mM fructose and 200 mM glucose. In the 2AFC protocol, participants were presented with two coded samples, 100 mM fructose and 200 mM glucose.

Participants were asked to identify the sweeter sample in ten trials. In the sweetness scaling protocol, participants asked to rate the perceived sweetness intensity of 100 mM fructose and 200 mM glucose in 10 trials.

Concentration-response methods

We prepared separate concentration series of glucose and fructose. Each stimulus was prepared neat (G, F), in self-mixture (Gs+G, Fs+F), and in binary mixture (Gs+F, Fs+G). All series consisted of 6 concentrations ranging from 0.1 to 1.0 M. The concentration of G and F added to each mixture were 200 mM and 100 mM, respectively. In total, we prepared 6 concentration series: glucose neat (G), glucose + 200 mM glucose (Gs+G), glucose + 100 mM fructose (Gs+F), fructose neat (F), fructose + 200 mM glucose (Fs+G), and fructose + 100 mM fructose (Fs+F). Each series was presented to each subject in quadruplicate.

Sample presentation was randomized using a random integer generator (random.org). 10 ml of each solution was presented in 30 ml polyethylene medicine cups (Dynarex, Orangeburg, NY) on a numbered tray. Each session consisted of three series with an interstimulus interval of 60 seconds and a five-minute interval between trials. For each sample, subjects held 10 ml of solution in the mouth for 5 seconds and rated the taste qualities (sweet, bitter, salty, sour, savory) and intensity using the gLMS before expectorating. After expectorating, subjects rinsed with Millipore water four times during the interstimulus interval.

For all protocols, aqueous solutions of all stimuli were prepared with Millipore filtered water and stored in amber glass at 4°C. All solutions were allowed to rise to room temperature for at least one hour prior to testing. All solutions were fully dissolved and there were no visible signs of undissolved solids or precipitation from solutions.

Statistical Analysis

Taste discrimination

In the 2AFC studies, we tested the null hypothesis that the two stimuli were equally sweet. We tabulated the number of times that each participant identified 100 mM F as the sweeter sample and the number of times that each participant identified 200 mM G as the sweeter sample. We then used binomial distribution tables to determine whether the probability of either stimulus was significantly different from random chance at the $\alpha = 0.05$ level. In the sweetness scaling test, we compared the 200 mM glucose and 100 mM fructose intensity ratings in each participant using paired t-tests.

Concentration-response

Two-way repeated measures analysis of variance was used to analyze the effects of stimulus. We first compared across the neat, +G, and +F conditions. We then repeated the analysis using only +G and +F expressed as percentages of neat sweetness. Post-hoc Tukey's HSD tests were used to analyze differences at each concentration.

RESULTS

Taste discrimination

No participants in the discrimination study were able to differentiate 100 mM glucose from 200 mM fructose. In the 2AFC experiment, the probability of identifying 100 mM fructose or 200 mM glucose as sweeter than the other did not differ significantly from random chance (not shown). In the sweetness scaling experiment, the perceived sweetness intensity of 100 mM fructose did not differ from that of 200 mM glucose ($p > 0.4$ for each participant) (**Figure 2-1A**). The perceived sweetness intensity of each stimulus was weak.

Glucose Function

We first compared perceived sweetness intensity across Gs, Gs+G, and Gs+F (**Figure 2-1B**). There was a significant main effect of stimulus on sweetness intensity ($F_{2, 28} = 56.8$; $p < 0.001$).

There was a significant interaction of stimulus x concentration, which reflects the diminished effect of adding 200 mM glucose and 100 mM fructose to increasing concentrations of glucose. ($F_{8, 112} = 11.94$; $p < 0.001$). Tukey's HSD tests showed that Gs+F was significantly more sweet than Gs+G at glucose concentration 0.177 M ($p=0.01$).

When Gs+G and Gs+F were expressed as percentages of Gs sweetness, the main effect of stimulus remained significant ($F_{1, 14} = 5.5$; $p < 0.05$). There was also a significant interaction of stimulus x concentration ($F_{4, 56} = 7.49$; $p < 0.001$).

Fructose Function

We then compared sweetness across Fs, Fs+G, and Fs+F (**Figure 2-1C**). There was a main effect of stimulus ($F_{2, 28} = 50.4$; $p<0.001$). There was a significant interaction of stimulus x concentration, which again reflects the diminished effect of adding 200 mM glucose and 100 mM fructose to increasing concentrations of fructose ($F_{8, 112} = 11.91$; $p < 0.001$). Fs+F was significantly more sweet than Fs+G at fructose concentration 0.177 M ($p<0.001$).

When Fs+G and Fs+F were expressed as percentage increases from Fs, the main effect of stimulus was not significant ($F_{1, 14} = 2.307$; $p=0.15$). There was, however, a significant interaction effect of stimulus x concentration ($F_{4, 56} = 8.5$; $p < 0.001$).

DISCUSSION

In the current study, functionally equivalent concentrations of fructose and glucose elicited differing levels of sweetness when presented in mixtures. 100 mM fructose potentiated the sweetness of glucose (**Figure 2B**). 200 mM glucose did not potentiate the sweetness of fructose (**Figure 2C**). These observations are consistent with glucose and fructose behaving as partial agonists at the sweet taste receptor. When expressed in the additive model, we found that $e_{FXGY} > e_{FX} + e_{GY}$ when $e_{FX} = e_{GY}$. Therefore, glucose does not behave in an additive manner in binary mixture with fructose.

Differences in sweetness between the neat functions and the +F and +G functions were diminished for the higher concentrations of each sugar concentration-intensity series. This is likely due to diminished effects with increasing sweetness of the series as it approaches asymptote. This may also explain why the effect of stimulus was more pronounced in the glucose series relative to the fructose series. Further studies are needed to examine the interaction of high concentrations of equisweet glucose and fructose.

Soft drinks are commonly sweetened with 39 g HFCS55, which equates to approximately 344 mM fructose + 266 mM glucose. Because fructose is a more potent sweetener than glucose, sugar content could be reduced if soft drinks were sweetened with only fructose. But recent evidence shows that dietary fructose, more so than glucose, is associated with metabolic dysfunction. From a nutritional standpoint, a greater ratio of glucose to fructose is preferred. In the present study, 100 mM fructose potentiated the sweetness of 177 mM glucose, but 100 mM fructose did not potentiate 316 mM glucose sweetness. Further studies are needed to determine whether the sweetness of a >50% glucose solution could be matched to that of HFCS or sucrose.

Two different, full agonists will produce similar effects (similar perceived sweetness intensity) when agonist-receptor complex concentrations are equal. This is because the functional effect of an agonist-receptor system is proportional to the number of receptors occupied by the agonist. This can be expressed as:

$$e/e_{max} \ \boldsymbol{\alpha} \ [AR]/[R_o] \qquad (3)$$

where e_{max} represents the maximal functional effect, [AR] represents the concentration of agonist-receptor complex, and $[R_o]$ represents the concentration of unoccupied receptor.

A partial agonist competes with a more effective agonist for receptor occupancy. But the partial agonist has low intrinsic activity. Thus, the weaker partial agonist suppresses the functional effect of the stronger partial agonist or full agonist in a competitive manner.

It is possible that glucose binds multiple receptor proteins in the oral cavity. Taste cells express the glucose cotransporter SGLT1, which does not bind fructose. It is unclear whether glucose binding to transporters affects the dynamics of T1R mediated sweet taste. Further studies are needed to determine whether non-T1R proteins contribute to taste perception in humans.

In the present study, glucose suppressed the functional effect of fructose. According to (3), equisweet concentrations of glucose and fructose occupy similar numbers of receptors. Our findings indicate that glucose competes for receptor occupancy with fructose, but its intrinsic activity is less than that of fructose. In this sense, glucose behaves as a partial agonist.

Partial agonists vary in terms of agonist/antagonist activity. Weak partial agonists exhibit very little agonist activity, whereas strong partial agonists can exhibit activity near the levels of a full agonist [124]. Our results support the hypothesis that glucose is a modest partial agonist. Although its potency is poor, glucose elicits strong sweetness at high concentrations [30]. In the current study, the difference in sweetness between glucose neat and fructose neat decreased as concentration increased, suggesting a common asymptotic sweetness. Also, the sweetness limiting effect of glucose decreased with increasing fructose concentration. These data suggest that the fructose is a relatively strong agonist of the sweet taste receptor and the addition of fructose (or sucrose) to any sugar or carbohydrate mixture will strongly activate the T1R2-T1R3 receptor both in the oral cavity and in the gastrointestinal tract.

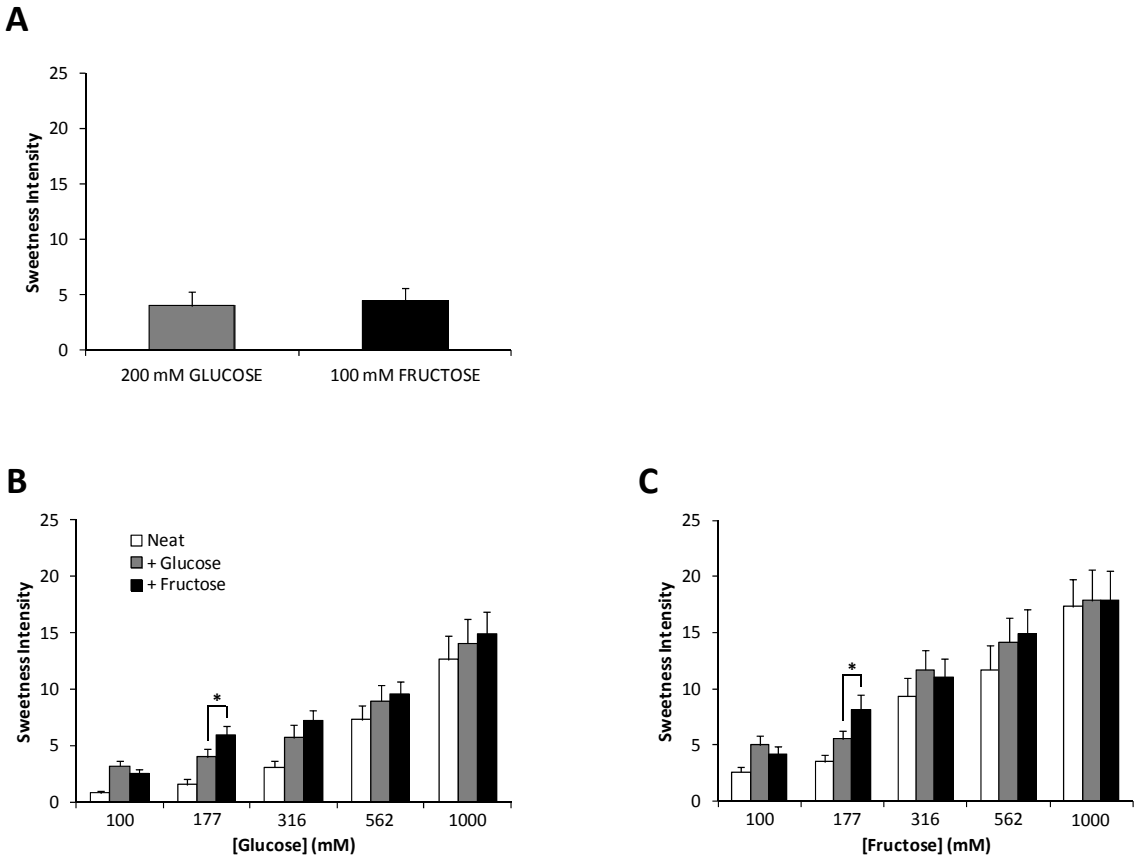

Figure 2-1. Glucose and fructose sweetness.

(A) Perceived sweetness intensity from 200 mM glucose and 100 mM fructose. Glucose and fructose were equisweet. n=4. **(B)** Effect of added 200 mM glucose and 100 mM fructose on glucose sweetness. There was a main effect of stimulus on sweetness intensity ($p < 0.001$). n=15. **(C)** Effect of added 200 mM glucose and 100 mM fructose on fructose sweetness. There was a main effect of stimulus on sweetness intensity ($p < 0.001$). n=15. * indicates significant difference between +Glucose and +Fructose.

Chapter 3.

Fructose-Free Sweetener Systems to Reduce Fructose intake

ABSTRACT

Excessive fructose intake, more so than excessive glucose intake, is thought to promote metabolic derangements. Sucrose and HFCS remain the most popular sweetening systems because glucose has weak sweet-eliciting potency and diet soft drinks containing HPS are easily discriminated from regular soft drinks. We, therefore, sought to determine whether it is feasible to develop a fructose-free beverage that is indistinguishable from a sucrose sweetened beverage. We first formulated a non-carbonated mixture of glucose + stevioside that were both equicaloric and equisweet to a sucrose-sweetened beverage. We used a duo-trio discrimination test to determine whether 12 participants could distinguish between sucrose and the glucose + stevioside beverages. Each panelist was tested in 60 repetitions. We then repeated the study using carbonated beverages and a panel of 10 participants. We found that in both the carbonated and non-carbonated conditions, our panels could not reliably discriminate glucose + stevioside from sucrose.

INTRODUCTION

The contribution of dietary fructose to the pathogenesis of chronic disease has come under close scrutiny. High fructose intake increases risk for the development of metabolic syndrome [48, 49, 128]. In controlled trials, high fructose consumption acutely elevates blood pressure [129], promotes dyslipidemia [130], reduces resting energy expenditure and fat oxidation [54], increases visceral adiposity [131], and promotes insulin resistance [132] to a significantly greater extent than does equi-caloric high glucose consumption.

The deleterious effects of excessive dietary fructose may be explained by key differences in glucose and fructose metabolism. Whereas ingested glucose passes through the liver to a large extent to be utilized by peripheral tissues, fructose is primarily metabolized in the liver [133]. The products of fructose metabolism, which bypasses the major regulatory enzyme in glycolysis, are substrates for *de novo* lipogenesis [134]. Thus, excessive fructose intake increases lipid synthesis and accumulation in the liver [134-136]. Hepatic lipid accumulation causes fatty liver disease and is thought to contribute significantly to the pathogenesis of metabolic diseases [135].

The replacement of fructose with starch ameliorates metabolic dysfunction [51, 52]. In a study of children with metabolic syndrome, the replacement of fructose with isocaloric starch lowered body weight, blood pressure, triglycerides, and small dense LDL and improved glucose tolerance and hyperinsulinemia [51, 52]. In this study, fructose intake was reduced from 12% to 4% of total calories and total energy intake and total carbohydrate intake were unchanged. These findings suggest that the replacement of fructose with glucose may help curb the prevalence of metabolic syndrome.

Soft drinks are a major source of dietary fructose and a potential point of nutritional intervention. Caloric soft drinks are typically sweetened with high fructose corn syrup (HFCS) in the US and sucrose elsewhere. HFCS 55 contains 55% fructose. The average American consumes 41.4 gallons of caloric soft drinks per year, which equates to 14.5 ounces per day [137]. A typical 12 oz soda contains 39 grams of HFCS. Thus, Americans consume 26 grams of fructose per soft

drink or 32 grams/per day [137]. Although fructose is found in other food sources such as fruit, fruit intake does not contribute a similar amount of fructose to the typical diet. For example, Americans consume 17.7 pounds of apples per capita per year (roughly 44 apples/year, assuming an average weight of 182 grams) [137]. One medium sized apple contains roughly 10 grams of fructose. To match the 26 grams of fructose from a soft drink, one needs to consume approximately 1 pound of apples (~2.5 apples). This equates to over 441 pounds of apples per capita per year, which exceeds average apple consumption in America almost 25-fold. Thus, the added fructose ingested in the diet from soft drinks would be difficult for typical Americans to ingest from fruit, requiring their fruit intake to increase 25 fold. This does not even take into account the added fructose in the diet from sucrose-added and HFCS-added cakes, cookies, doughnuts, ice-creams, and sundry confections.

Efforts to replace fructose or sugars in general in sweetened beverages have been largely unsuccessful. Per capita consumption of caloric soft drinks is 41.4 gallons per year, whereas the consumption of non-caloric soft drinks is 11.6 gallons per year [137]. Non-caloric sweeteners can match the sweetness intensity of the sugars in a soda, but they are easily distinguished because of off-tastes, lingering aftertastes, and possibly the absence of a caloric reward [138, 139]. The vast majority of sweetened beverages sold in the U.S. contain sugar, indicating an apparent reluctance among U.S. consumers to drink non-sugared beverages. We conclude that Americans, at least at the present time, prefer to drink sugared beverages. Therefore, any options to provide both a sugared beverage and a fructose free beverage will need to use glucose as the base of its sweetening system. Galactose could also be used but would not be practical.

Glucose is approximately half as sweet as fructose, making fructose (or any system containing fructose) a preferred sweetener [17]. Moreover, sucrose, which is 50% fructose, is three times sweeter than glucose on a molar basis. HFCS, which approximates the invert-sugar of sucrose (roughly equal parts glucose and fructose), is comparable in sweetness to sucrose on a calorie-for-calorie basis. To make a glucose-sweetened beverage that is free of fructose and is comparable in sweetness to a sucrose sweetened beverage, the glucose concentration must be

increased significantly. The glucose-only beverage would have approximately 150% more calories than a sucrose- or HFCS-sweetened beverage. However, a mixture of glucose and non-nutritive sweeteners may better match the taste profile of sucrose without the need to increase caloric content while still providing a caloric reward. The presence of glucose as the sweetener base allows for relatively little non-nutritive sweetener to be added to achieve desired sweetness levels. In effect, the glucose masks much of the off-tastes associated with non-nutritive sweeteners [139].

The purpose of this study was two-fold: 1) to determine whether a palatable, fructose-free beverage could be produced as proof-of-principle and 2) to determine if a fructose-free beverage could be comparable to a sucrose sweetened soda in overall taste. In the first study, we sought to determine whether a non-carbonated mixture of glucose and stevioside could be discriminated from an equicaloric solution of non-carbonated sucrose. In the second study, we sought to determine whether a carbonated glucose and stevioside sweetened beverage could be generated that was comparable in overall oral sensory profile to a sucrose sweetened beverage. If successful, this would serve as proof-of-principle that an acceptable fructose-free soda could be produced.

METHODS

Subjects

For the first study, 12 young adult subjects (six male and six female) were recruited from Rutgers University and the surrounding community. For the second study, 10 subjects were recruited in a similar fashion. We did not exclude participants on the basis of BMI, glucose tolerance, soft drink preference, or soft drink consumption frequency. Subjects were paid to participate and provided informed consent on an IRB approved form. This protocol complies with the Declaration of Helsinki for Medical Research involving Human Subjects. Subjects were asked to refrain from eating, drinking, and smoking for one hour prior to each session. Prior to enrollment in the

experiment, subjects were asked to rate the intensity of five concentrations of NaCl increasing in half logarithmic steps. Subjects whose ratings did not increase monotonically with NaCl concentration were excused, on the assumption that they had abnormal taste or were unable to follow instructions.

Stimuli

All sweeteners were reagent-grade compounds. Sweeteners were dissolved in water (Millipore) and maintained at 20 degrees Celsius. All solutions were prepared at least 24 hours prior to use to allow for complete muta-rotation of tautomers. Stimuli are described in **Table 3-1**. In experiment 1, the solutions used were 320 mM sucrose and 610 mM glucose + 0.235 mM stevioside. The concentration of sucrose reflects the concentration of sugar in popular sodas. The concentration of glucose was chosen to match the mass of sucrose used per liter, thus matching sucrose for calories. From pilot testing, the addition of 0.235 mM stevioside to glucose made the mixture equally sweet compared to the sucrose solution. In experiment 2, samples were carbonated using a commercial carbonation product (Sodastream) according to the product instructions. Carbonation exacerbated the difference in sweetness between glucose and sucrose, so the concentration of stevioside was increased to 0.750 mM. The solutions used were 320 mM sucrose and 610 mM glucose + 0.750 mM stevioside.

Procedure: Duo-Trio Taste Discrimination Protocol

Subjects participated in two test sessions per day and 6 sessions per week for a total of 12 sessions. Each session consisted of 5 discrimination trials. In total, each subject performed 60 trials. Subjects rinsed their whole mouth thoroughly with water prior to testing. 10 mL samples were presented in 1 oz medicine cups. In each trial, subjects were presented with a referent sample, followed by two coded samples. One of the two coded samples matched the referent. Subjects were asked to indicate which coded sample matched the referent. Solutions were tasted from left to right, with water rinsing between each cup. Subjects rinsed three times between stimuli. Answers were scored as correct or incorrect.

Statistical analysis

First, we determined whether each participant could consistently detect a difference between stimuli. We used binomial distribution tables because duo-trio trials are a type of two-alternative forced-choice (2AFC) trial. For each participant, at least 37 correct answers out of 60 repetitions (62% correct) were required to indicate that a participant could detect a difference between stimuli at the alpha = 0.05 level.

Next, we determined whether the panel as a sample population was able to distinguish between the beverages. We again used published binomial distribution tables. Participants who could consistently discriminate were considered discriminators. In experiment 1, at least ten discriminators out of twelve were required to indicate that a difference existed between stimuli for the panel at the α=0.05 level. In experiment 2, at least nine discriminators out of ten were required to indicate that a difference existed between stimuli for that panel at the α=0.05 level.

RESULTS

Non-carbonated stimuli

Data for each participant are presented in **Table 3-2**. 7 of 12 participants gave at least 37 correct answers and were thus able to discriminate between 320 mM sucrose and 610 mM glucose + 0.235 mM stevioside ($p<0.05$).

Carbonated stimuli

Data for each participant are presented in **Table 3-3**. In the study of carbonated beverages, 7 of 10 participants gave at least 37 correct answers and were thus able to discriminate between carbonated 320 mM sucrose and 610 mM glucose + 0.750 mM stevioside.

DISCUSSION

To help eliminate excess fructose intake from the diet, we demonstrate here that sucrose, a disaccharide of glucose and fructose, is indistinguishable from an equicaloric glucose solution mixed with the natural, non-caloric sweetener stevioside for about 40% of the sample population. For the remaining subjects the discrimination was difficult for half of them and the rest could discriminate the beverages relatively easily. This observation demonstrates that despite being considerably less sweet, glucose can serve as a fructose-free, equicaloric sweetener system for many people. Our results are supported by previous observations of model beverages sweetened with mixtures of sugars and high potency sweeteners [140].

In the first study, 5 of 12 participants were unable to distinguish a glucose + stevia sweetened beverage from a sucrose sweetened beverage matched for sweet intensity. Of the 7 participants who gave 37 or more correct answers, four gave no more than 41 correct answers (≤68% correct). The remaining three participants gave 42 or more correct answers. Thus, overall, nine of twelve subjects had difficulty discriminating between these two sweetener systems.

When the solutions were carbonated to better reflect the major sensory qualities of soda, 3 of 10 participants were unable to discriminate between the two sweetener systems. Of the 7 remaining participants who gave 37 or more correct answers, two gave fewer than 40 correct answers (<67%). Thus, 5 of the 10 subjects had difficulty discriminating between the glucose + stevia and the sucrose sweetener systems in a model soda.

These results provide proof-of-principle that it is feasible to produce a fructose free soda, which is similar to sodas made with sucrose (or high fructose corn syrup) and for 30 to 40% of individuals is indistinguishable from the sucrose sweetened versions. The strength of this study is limited by its sample size. But, it is important to note that the 2AFC techniques employed enable subjects to detect and discriminate with high sensitivity. Each subject had the opportunity to evaluate the stimuli 60 times. Therefore, for these subjects the two types of beverages were indistinguishable or extremely similar.

The glucose plus stevia sweetened beverage has the same total sugar content as the sucrose sweetened beverage and, therefore, the same number of calories. Substituting glucose sweetened beverages for those sweetened with sucrose or HFCS would not reduce caloric intake, but would reduce fructose intake by an average of 32 grams per day and 11.4 kilograms per year [137]. This reduction would relieve a major stress on the liver and could delay or prevent the onset of non-alcoholic fatty liver disease and metabolic syndrome associated with excessive fructose intake [51]. The risk factors associated with metabolic syndrome include elevated blood pressure, dyslipidemia, visceral adiposity, and insulin resistance, which are promoted by high fructose intake but not by comparable glucose intake [51, 129-132]. A recent clinical study [51] found that isocaloric substitution of starch for sugar in obese children with metabolic syndrome reduced their diastolic blood pressure, plasma triglycerides and HDL cholesterol, hyperinsulinemia and glucose intolerance, and body weight. All of this was accomplished simply by reducing daily fructose intake. It is worth noting that very high glucose intake can also cause fatty liver disease, but this level of glucose (or starch) intake is very high.

Water is an ideal replacement for both HFCS and sucrose sweetened sodas because it contains neither fructose nor calories. Yet, asking Americans to decrease ingestion of sugar sweetened beverages voluntarily is unlikely to succeed at a national level. Glucose, however, is a pragmatic replacement for HFCS and sucrose because feeding behavior is guided by taste and carbohydrate sweetened beverages are preferred over water. Bottled water and diet soda is widely available, yet caloric soda consumption predominates. Diet sodas were developed to satisfy the need for sweet while reducing energy density. Though non-nutritive sweeteners can match the sweetness intensity of sugars, diet soda is not as popular as caloric soda [137]. Theories as to why diet sodas are not as preferred as much as sugar sodas include: a) fear that artificial sweetener systems are less healthy than sugar and may cause diseases, b) dislike of high potency sweetener system taste profiles [139], c) fear of eating foods that are deemed unnatural, and d) the lack of caloric reward or reinforcement to drive future consumption

[141]. Some evidence suggests that non-caloric sodas do little to reduce appetite and may in fact promote it [142, 143]. Conversely, other evidence shows that diet soda is more effective than water in promoting weight loss [144]. Presently, diet sodas comprise only 10-30% of the soda market. Because diet sodas have failed to replace HFCS sweetened sodas in the United States, they have done little to curb rates of obesity or metabolic syndrome. Although an equicaloric, glucose sweetened beverage will not reduce energy intake, it can have much greater appeal than diet sodas and can feasibly replace a larger proportion of fructose in the American diet by removing HFCS and sucrose sweetened beverages. Moreover, an equicaloric glucose sweetened beverage holds the promise to reduce plasma dyslipidemias, HDL cholesterol, insulin insensitivity, and even to promote weight loss [51].

Beverage	Formulation	Glucose (g/L)	Fructose (g/L)	Energy (kcal/L)
Control (non-carbonated)	320 mM sucrose	55	55	440
Control (carbonated)	320 mM sucrose	55	55	440
Experimental (non-carbonated)	610 mM glucose + 0.235 mM stevioside	110	0	440
Experimental (carbonated)	610 mM glucose + 0.750 mM stevioside	110	0	440

Table 3-1. Control and experimental stimuli.

Noncarbonated stimuli were used in experiment 1. Carbonated stimuli were used in experiment 2.

Participant	# Correct	# Incorrect	% Correct
1	27	33	45
2	34	26	57
3	56	4	93*
4	45	15	75*
5	40	20	67*
6	35	25	58
7	29	31	48
8	41	19	68*
9	42	18	70*
10	41	19	68*
11	37	23	62*
12	30	30	60

Table 3-2. Results of duo-trio test comparison noncarbonated stimuli.

Stimuli were 320 mM sucrose and 610 mM glucose + 0.235 mM stevioside. 12 participants were each tested in 60 replicates. 7 of 12 participants consistently detected a difference between stimuli. * indicates participant detected difference between stimuli $\alpha=0.05$.

Participant	# Correct	# Incorrect	% Correct
1	36	24	60
2	48	12	80*
3	37	23	62*
4	42	18	70*
5	30	30	50
6	45	15	75*
7	44	16	73*
8	38	22	63*
9	33	27	55
10	47	13	78*

Table 3-3. Results of duo-trio test comparison carbonated stimuli.

Stimuli were 320 mM sucrose and 610 mM glucose + 0.750 mM stevioside. 10 participants were each tested in 60 replicates. 7 of 10 participants consistently detected a difference between stimuli. * indicates participant detected difference between stimuli at $\alpha=0.05$.

Chapter 4.

Lipid-Lowering Pharmaceutical Clofibrate Inhibits Human Sweet Taste

ABSTRACT

T1R2-T1R3 is a heteromeric receptor that binds sugars, high potency sweeteners, and sweet taste blockers. In rodents, T1R2-T1R3 is largely responsible for transducing sweet taste perception. T1R2-T1R3 is also expressed in non-taste tissues, and a growing body of evidence suggests that it helps regulate glucose and lipid metabolism. It was previously shown that clofibric acid, a blood lipid lowering drug, binds T1R2-T1R3 and inhibits its activity *in vitro*. The purpose of this study was to determine whether clofibric acid inhibits sweetness perception in humans and is, therefore, a T1R2-T1R3 antagonist *in vivo*. 14 participants rated the sweetness intensity of four sweeteners (sucrose, sucralose, Na cyclamate, acesulfame K) across a broad range of concentrations. Each sweetener was prepared in solution neat and in mixture with either clofibric acid or lactisole. Clofibric acid inhibited sweetness of every sweetener. Consistent with competitive binding, inhibition by clofibric acid was diminished with increasing sweetener concentration. This study provides *in vivo* evidence that the lipid lowering drug clofibric acid inhibits sweetness perception and is, therefore, a T1R carbohydrate receptor inhibitor. Our results are consistent with previous *in vitro* findings. Given that T1R2-T1R3 may in part regulate glucose and lipid metabolism, future studies should investigate the metabolic effects of T1R inhibition.

INTRODUCTION

In vitro functional expression and mouse knock-out data suggest that sweet taste perception is chiefly transduced by T1R2-T1R3, a heteromeric carbohydrate receptor of the seven transmembrane family, Class C [4, 5]. T1R2-T1R3 is activated by several mono- and di-saccharides as well as high potency sweeteners (HPSs) [20]. In humans it is inhibited by sodium lactisole, an inverse agonist which binds the transmembrane domain of human-T1R3 [34, 145]. A growing body of evidence shows that T1R2-T1R3 is expressed in tissues throughout the body and that it serves physiological roles in glucose metabolism, insulin secretion, lipid metabolism, adipocyte function, and reproductive health [90, 116, 146].

Several studies have established a role for T1R2-T1R3 in glucose metabolism. *In vitro*, high potency sweeteners induce the secretion of GLP-1 by intestinal L-cells [88, 90]. High potency sweeteners upregulate the expression of glucose transporters in the intestine [91, 147]. T1R2-T1R3 is also expressed in pancreatic beta cells and *in vitro* stimulation with a high potency sweetener induces insulin release [103, 148]. Several clinical studies have shown that a high potency sweetener preload alters the plasma insulin, glucose, and incretin responses to an oral glucose load, despite the fact that high potency sweeteners are not by themselves insulinogenic, neither do they contain glucose nor any calories of note [110-113].

T1R2-T1R3 is also expressed in adipocytes [114]. Stimulation with high potency sweeteners alters adipogenesis and lipolysis *in vitro* [114, 149]. When fed an obesogenic diet, T1R2 KO mice and T1R3 KO mice are protected against fat mass gain [115, 116]. This may be due to oral perceptual influences on nutrient utilization and metabolism [150], or to a reduced ability of adipocytes to sense and transport glucose internally [116].

Although the relationships between T1R2-T1R3 agonists and human physiology have come under scrutiny, the physiological effects of T1R2-T1R3 antagonists are less clearly understood. Genetic knock-out studies represent the ultimate loss of function of the T1R2-T1R3 receptors, but pharmacological inhibition may have similar, albeit less potent, effects on metabolism. It has

been shown that the T1R3 inhibitor sodium lactisole increases plasma glucose AUC response to a glucose load [151]. This effect is consistent with reduced stimulation of insulin by T1Rs [34, 104, 145]. Whether other sweet taste inhibitors have similar physiological effects on human physiology is unclear.

Lactisole is structurally a phenoxy propionic acid, and so has molecular similarity to other members of this class, including the phenoxy herbicides and the metabolic fibrate drugs. The fibrates are a class of "plasma lipid lowering" drug which are presumed to exert their effects by binding and inhibiting the nuclear receptor proteins, the peroxisome proliferator-activated receptors (PPARs), especially PPAR-α [152]. The PPARs form heterodimers with the retinoid X receptors (RXR) to regulate gene expression and modulate metabolism, among many other physiological functions [119, 152]. These heterodimers are the presumed mechanism for how fibrate drugs are able to lower plasma lipids in patients. In addition to binding PPARs, fibrate drugs behave pharmacologically *in vitro* like the T1R2-T1R3 inhibitor lactisole and bind the transmembrane domain of human T1R3 [153]. Curiously, fibrates bind PPARs and T1R3 with a similar affinity [153].

Fibrates have clinical physiological effects on metabolism and are known to bind the sweet taste receptor T1R2-T1R3. This raises the question of whether fibrates might exert some of their physiological effects through their actions on T1R2-T1R3. Thus, we wished to determine whether fibrates inhibit T1R2-T1R3 *in vivo* in humans. As one measure of this effect, we sought in the present study to determine whether the fibrate drug, clofibric acid, inhibits perception of sweetness in humans and is, therefore, a T1R2-T1R3 receptor antagonist in conscious behaving humans. We tested four sweeteners (sucrose, sucralose, acesulfame-K, and Na cyclamate) in mixture with either clofibric acid or the positive control sweet taste inhibitor, sodium lactisole.

METHODS

Subjects

Fourteen adult subjects (five male, nine female) aged between 18 and 29 years were paid to participate after providing informed consent on an IRB approved form. This protocol complies with the Declaration of Helsinki for Medical Research involving Human Subjects All participants were from Rutgers University and the surrounding community. Each subject participated in 6 sessions. They were asked not to eat, drink, or smoke one hour prior to each session.

Training

Subjects were trained in the use of a general Labeled Magnitude Scale (gLMS) following standard published procedures [127]. The top of the scale was described as the strongest imaginable sensation of any kind [126]. This gLMS required participants to rate the perceived intensity along a vertical axis lined with the following adjectives: barely detectable, weak, moderate, strong, very strong, and strongest imaginable. The adjectives are spaced semi-logarithmically, based upon experimentally determined intervals to yield ratio quality data. The subjects were shown both adjectives and numbers on the scale.

Stimuli

The sweet taste stimuli were: sucrose (ranging in concentration from 0.0292 to 1.64 M), sucralose (7.95x10-6 to 7.95x10-2 M), acesulfame potassium (1.57x10-5 to 0.15 M), and sodium cyclamate (4.97x10-4 to 0.496 M). Concentrations increased in quarter- and semi-logarithmic increments and captured asymptotic sweetness intensity. The sweet taste inhibitors used were 1.37 mM sodium lactisole and 1.37 mM clofibric acid. Each sweet compound was presented neat and in combination with each inhibitor. Clofibric acid was neutralized with sodium hydroxide to match the pH of the neat solution.

Aqueous solutions were prepared every other day with Millipore filtered water and stored in amber glass at 4°C. All solutions were removed from refrigerator and allowed to rise to room

temperature for at least one hour prior to tasting. All solutions were fully dissolved and there were no visible signs of undissolved solids or precipitation from solutions.

Stimulus Delivery

Sample presentation was randomized using a random integer generator (random.org) and 10mls of each solution was presented in 30 ml polyethylene medicine cups (Dynarex, Orangeburg, NY) on a numbered tray. Each session consisted of two trials with an interstimulus interval of 30 seconds and a five-minute interval between trials. For each sample, subjects held 10 ml of solution in the mouth for 5 seconds and rated the taste qualities (sweet, bitter, salty, sour, savory) and intensity on a gLMS before expectorating. Subjects separately rated each taste quality on a gLMS. After expectorating, subjects rinsed with Millipore water four times during the interstimulus interval.

Statistical Analysis

For each of the four sweeteners studied, two-way repeated measures analysis of variance (ANOVA) was used to analyze the effects of stimulus (sweetener neat, sweetener + lactisole, sweetener + clofibric acid). Post-hoc Tukey's honestly significant difference (HSD) tests were used to analyze differences among responses.

RESULTS

Clofibric acid inhibited the sweetness elicited by all of the sweeteners studied (**Figure 4-1**). The main effect of stimulus was significant for sucrose ($F_{2,26} = 6.4$; $p<0.01$), sucralose ($F_{2,26} = 8.4$; $p<0.01$), acesulfame K ($F_{2,26} = 9.1$; $p<0.01$), and Na cyclamate ($F_{2,26} = 50.2$; $p<0.01$). There was a significant interaction of stimulus and concentration for each sweetener, indicating that the efficacy of inhibitor was affected by concentration. Consistent with competitive binding, the inhibition by clofibrate was diminished with increasing concentration of sucrose and cyclamate.

However, in the case of sucralose and acesulfame K, inhibition was not completely diminished at maximum sweetener concentration.

Clofibric acid and lactisole were similar in terms of inhibitory potency. For sucrose, however, the effect of lactisole was greater than the effect of clofibric acid ($p<0.05$). There were no other significant differences in main effects between clofibric acid and lactisole, although clofibric acid tended to be a more potent inhibitor of sweet taste elicited by acesulfame K.

There were no significant differences in bitter, salty, sour, or savory qualities between neat and inhibited conditions (not shown). Sucralose and acesulfame K elicited weak bitterness at high concentrations in the neat and inhibited conditions in some subjects. There were no differences in bitterness ($p>0.05$). Sucrose elicited only sweetness in the neat and inhibited conditions.

DISCUSSION

These data show that clofibric acid inhibits sweet taste elicited by four different T1R2-T1R3 agonists: a sugar and three high potency sweeteners. This is consistent with previous findings that clofibric acid binds a transmembrane domain of T1R2-T1R3 [153]. These data also further support the hypothesis that T1R2-T1R3 is largely responsible for transducing sweet taste in humans.

Although clofibric acid and lactisole inhibited sweet taste, neither compound abolished sweet taste completely. Also, sweetness inhibition was overcome at higher concentrations of sucrose and cyclamate. This observation is consistent with competitive inhibition, similar to previously studied sweet taste inhibitors [24, 36]. The highest molar concentrations of sucrose and cyclamate used in this study were greater than those of acesulfame K and sucralose. It is unclear whether inhibition of acesulfame K and sucralose sweetness would be diminished at higher levels of these sweeteners or whether sweetness would return to uninhibited high levels.

As previously reported [31], high levels of sucralose (25 and 79 mM) and acesulfame K (50 and 150 mM) elicited bitter taste in some participants. In mixtures of sweet and bitter compounds, inhibition of sweetness has been shown to enhance bitterness [154]. However, despite reducing sweetness from sucralose and acesulfame K, neither inhibitor affected ratings for bitter, sour, salty, or savory taste qualities. It is possible that some participants conflated bitterness with metallic tastes or other off tastes that we did not measure.

Clofibric acid only modestly inhibited sweet taste from sucrose relative to the effects of lactisole. This finding could be explained by non-T1R3 mediated sweet taste [6]. K_{ATP} channels are part of a metabolic signaling pathway and are expressed in many taste cells and may contribute to depolarization of these cells and activation of downstream signaling events [7, 11]. In T1R3 knockout mice, nerve responses to glucose were diminished but not abolished [6], indicating residual signaling from sugars in mice.

Subjects in the present study reported sweet water taste after expectorating either lactisole or clofibric acid (not shown). Previous studies have shown that lactisole elicits a sweet water taste, most likely because it is a T1R2-T1R3 inverse agonist [32]. Our finding that clofibric acid elicits a similar rebound effect suggests that like lactisole, it too may be an inverse agonist of T1R2-T1R3.

Clofibric acid is used pharmacologically to lower blood cholesterol and triglycerides. Treatment with clofibric acid reduces hepatic lipid deposition [117], as well as fasting and postprandial glycemia [118, 155, 156]. Clofibric acid is hypothesized to act through peroxisome proliferator-activated receptor α (PPARα) activation [119]. PPARα is a transcription factor that upregulates genes responsible for fatty acid uptake, beta oxidation, lipolysis, and lipoprotein synthesis [152]. Although clofibric acid is known to bind PPARα, it is not known whether all of its physiological effects are due exclusively to PPAR inhibitory activity.

The effects of chronic treatment with clofibric acid share some overlap with the effects of T1R2 and T1R3 ablation. Similar to clofibric acid treatment, T1R ablation alters glucose and lipid metabolism [90, 115, 116, 150]. T1R2 knockout animals are protected against diet induced

obesity, hepatic lipid deposition, and hypersinsulinemia [115, 116]. Like the T1R2 knockout, T1R3 knockout animals are protected against diet induced weight gain and fat mass gain, independent of energy intake [150].

T1R knockouts may be protected against lipid accumulation because of impaired assimilation of dietary carbohydrate. T1R2-T1R3 is expressed in many tissues throughout the body, including the intestine [90], liver [102], pancreas [102], adipocytes [114], and brain [157]. T1R2-T1R3 agonists enhance intestinal glucose absorption [158], incretin response [111], insulin secretion [110], and adipocyte differentiation [114]. Sodium lactisole, a T1R2-T1R3 inhibitor, has been shown to lower insulin secretion and GLP-1 secretion *in vitro* [88, 159] and *in vivo* [151]. Given that clofibric acid inhibits (T1R2-T1R3), it is possible that its metabolic effects may be due, in part, to T1R2-T1R3 inhibition.

These data show that clofibric acid inhibits sweet taste perception and is thus not only a T1R2-T1R3 receptor inhibitor *in vitro* but appears to inhibit it *in vivo* as well. These perceptual data are supported by previous findings that clofibric acid binds selectively and inhibits T1R3 *in vitro*. Future studies should investigate the metabolic effects of T1R inhibition to determine the degree to which fibrates and other T1R inhibitors influence dyslipidemias and cholesterol.

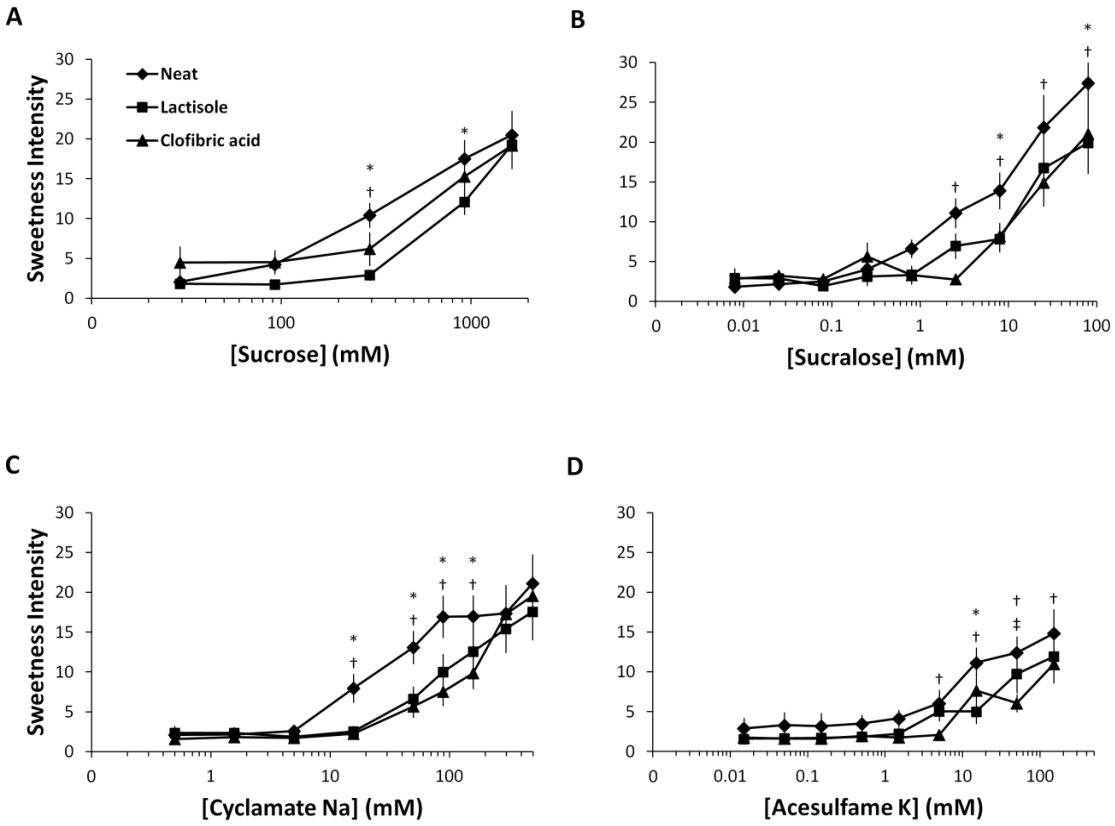

Figure 4-1. Effects of clofibric acid on perceived sweetness intensity.

Clofibric acid and lactisole inhibited the perceived sweetness elicited by **(A)** sucrose, **(B)** sucralose, **(C)** Na cyclamate, and **(D)** acesulfame K. Concentration-intensity functions were determined for each sweetener neat, in admixture with lactisole, and in admixture with clofibric acid. The main effect of stimulus was significant for every sweetener studied. Main effects of stimuli (sweetener neat, sweetener + lactisole, sweetener + clofibric acid) were determined using two-way repeated measures analysis of variance (ANOVA). Pairwise differences were determined using post-hoc Tukey tests. n=14. Each subject was tested in sextuplicate. Letters denote significant differences ($p<0.05$) between stimuli. ***** denotes significant difference between neat and lactisole. **†** denotes significant difference between neat and clofibric acid. **‡** denotes significant differences between lactisole and clofibric acid.

Chapter 5.

Sucralose Influences Glucose Metabolism: Role of T1R Receptors

ABSTRACT

Studies show that consuming sucralose, a non-caloric sweetener (NCS), 10-15 minutes prior to a glucose meal can alter insulinemic and glycemic outcomes, but the effect of consuming sucralose concomitantly with glucose has not been examined. Here we show that concomitant ingestion of sucralose enhances insulin responses to an oral glucose tolerance test. We conducted oral glucose tolerance tests in 12 human participants with and without added sucralose in a randomized, crossover design. We conducted psychophysical tests to assess relationships between perceived sweetness and metabolic responses to sucralose. Sucralose consumption significantly increased plasma insulin and tended to increase plasma glucose AUC. We found an association between insulin responses and perceived sweetness intensity of glucose. These data provide evidence that NCS are metabolically active and significantly modulate metabolic responses. Perhaps as a window into overall T1R sensitivities, there was a functional relationship between perceived sweetness intensity and the magnitude of regulatory responses to ingested sucralose and glucose.

INTRODUCTION

Despite containing neither glucose nor calories, high potency non-caloric sweeteners (NCS), such as sucralose and aspartame, may affect carbohydrate metabolism. The hypothesized mediators of this action are the T1R receptors, which respond to both sugars and high potency NCS. In the oral cavity high potency sweeteners are believed to bind to a sweet taste receptor, T1R2-T1R3, initiating a signaling cascade that elicits perceived sweet taste [5, 20]. The T1R2-T1R3 receptors are also expressed in other metabolically active tissues, including intestinal entero-endocrine L-cells and pancreatic β-islet cells [88, 103], neither of which elicit sweet taste when activated. Rather, intestinal L-cells co-express T1R2-T1R3 and glucagon-like peptide-1 (GLP-1), an insulinotropic hormone [88]. When exposed *in vitro* to high potency sweeteners, such as sucralose, human L cells secrete GLP-1. This effect is blocked *in vitro* by the presence of sodium lactisole and gurmarin, inhibitors of the human T1R2-T1R3 receptor [88, 90]. Additionally, high potency sweeteners promote the expression of the sugar transporter sodium-glucose linked transporter 1 (SGLT1), as well as the translocation of glucose transporter 2 (GLUT2) to the apical membrane of intestinal epithelia, thereby potentially enhancing glucose absorption in animals [90, 91]. Genetic “knock-out” mice unable to produce alpha gustducin (a metabotropic GTP-binding protein) or T1R3, both important to T1R signaling, secrete less GLP1 in response to a glucose load than do wild-type (WT) mice [88]. And in pancreatic β-islet cells, *in vitro* stimulation with NCS causes insulin secretion [103]. Importantly, some NCS, such as sucralose, are absorbed when ingested and likely reach the pancreas [160]. Taken together, these findings suggest that T1R signaling plays an important role in carbohydrate metabolism and, furthermore, that the high potency sweeteners in today’s diet may influence blood glucose regulation in humans via their action on T1Rs. The purpose of the present work was to determine in healthy humans whether sucralose, the T1R2/T1R3 agonist, taken with meals influences plasma glucose and insulin levels.

In contrast to the clarity of *in vitro* studies, human *in vivo* studies with T1R agonists have found varied and conflicting results. It has been clearly shown that when high potency sweeteners are

infused or fed in the absence of a carbohydrate load, they do not appear to affect blood concentrations of GLP-1, gastric inhibitory polypeptide (GIP), insulin, or glucose [107, 108, 161]. Yet when given as a pre-load to oral glucose in humans, high potency sweeteners appear to affect these parameters depending on circumstances. In obese, non-diabetic participants a sucralose preload reduced 2-hour blood glucose area under the curve (AUC) in an oral glucose tolerance test (OGTT) [113]. In morbidly obese, non-diabetic participants, a sucralose preload increased peak blood glucose, peak insulin, and 5 hour insulin AUC [110]. In both healthy participants and type 1 diabetic participants, drinking diet soda before a glucose load increased GLP-1 AUC [112]. In the same experiment, the preload had no effect in type 2 diabetic participants. Such discrepant findings could be due to differences in methodology or sample selection.

In the present study, we sought to assess insulin and glucose responses to sucralose mixed with glucose in order to determine whether NCS affect metabolic responses. We also sought to determine whether the physiological responses to sucralose were associated with perceived sweet taste intensity. There is substantial variability in the T1R family of receptors [162]. *TAS1R2*, the gene coding for the T1R2 subunit of the sweet taste receptor, is particularly diverse. And polymorphisms in the gustducin gene *GNAT3*, a GTP-binding protein associated with T1R2/3 transduction, are correlated with variation in sucrose perception [163]. These findings indicate that there is variability in the sweetener transduction proteins that influence NCS signaling, as indicated by related variation in sweet taste perception. Furthermore, it has been suggested that variation in the sweet taste transduction pathway may influence physiological responses because this pathway is expressed in the intestine, pancreas, and elsewhere [164].

When preceding a caloric load, high potency sweeteners tend to elicit an effect on glucose tolerance. But, this effect has not been consistently characterized in humans. Additionally, the majority of studies have been in obese or diabetic participants using a pre-load approach. The objective of the present study was to test the hypothesis that consuming sucralose mixed with glucose will significantly alter plasma glucose regulation in healthy participants compared to

meals where no additional T1R agonist is ingested. This combination would occur today with many beverages that combine the two sweeteners or when diet sodas are ingested during meals. We presented sucralose mixed with glucose in healthy, non-obese participants and compared the results to the OGTT without sucralose added. We also sought to examine the relationship between the taste psychophysical responses and the physiological responses to sucralose. To do so, we conducted concentration-intensity studies with a variety of taste stimuli, including sucralose, in each participant following the OGTT experiments.

METHODS

12 non-obese, non-diabetic participants participated in the study. BMI ranged from 19.5 to 29.7 kg/m2 and (mean 23.5 kg/m2). Age ranged from 20-32 years (mean 27 years). 4 males and 8 females participated. Of the participants, 7 were Caucasian, 2 were Asian, 2 were Latin, and 1 was African. Participants were screened with a medical and dietary questionnaire used in previous studies [110]. Participants who reported consuming more than one serving of artificially sweetened beverages or snacks per day were excluded. Participants with diseases and medications that affect digestion and absorption were also excluded. Based on these criteria, 6 participants were excluded. Of the twelve participants, two had high fasting glucose (fasting glucose between 100 and 110 mg/dl). Eleven of the twelve OGTT participants participated in the taste psychophysical tests. Subjects were paid to participate and provided informed consent on an IRB approved form. This protocol complies with the Declaration of Helsinki for Medical Research involving Human Subjects. In order to keep subjects blinded, subjects were informed of the compounds to be used before the study commenced, but not during the study.

Glucose tolerance tests

Participants were tested in a randomized, single blind, crossover design. Participants were tested on two occasions, each spaced one week apart. On one occasion, participants rinsed and consumed 75 grams of glucose (Sigma) dissolved in 300 mL water (Millipore). On the other

occasion, participants consumed 75 grams of glucose mixed with 0.6 grams of sucralose (Sigma) dissolved in 300 mL water. The concentrations of the stimuli were 1.39 M glucose and 1.39 M glucose + 5 mM sucralose. Some subjects could discriminate between stimuli during the glucose tolerance test, but the researchers did not identify the stimuli during the test.

Participants were instructed to fast after midnight before coming to the laboratory. Participants were also instructed to abstain from structured exercise for the 48 hours preceding each test. A single bout of exercise enhances insulin sensitivity for 48 hours and could therefore confound our results. Blood samples were collected using an indwelling catheter inserted into an antecubital vein. Samples were drawn at the following time points: -20, -15, 10, -5, 0, 3, 6, 9, 12, 15, 30, 45, 60, 75, and 90 minutes.

From -10 to 0 minutes, participants orally swished the stimulus (1.39 M glucose or 1.39 M glucose and 5 mM sucralose). Participants rinsed their mouths and gargled with 25 mls of the stimulus for 20 seconds. After 20 seconds, participants expectorated the sample and began rinsing with another 25 ml of stimulus. This was repeated for 10 minutes. At 0 minutes, participants drank 300 ml of the stimulus. Participants consumed the stimulus in less than 3 minutes.

Blood samples were collected in chilled EDTA plasma tubes. Samples were centrifuged (4 degrees, 15 minutes, 1300 RCF) and plasma was separated and stored at -80 degrees for later analysis. Plasma insulin and glucagon were measured using a double-antibody radioimmunoassay (EMD Millipore, Billerica MA). Glucose was measured spectrophotometrically using an auto-analyzer (Roche). These measurements were made by a core facility of the University of Pennsylvania Institute for Diabetes, Obesity, and Metabolism.

Calculations

Area under the curve

Area under the curve was determined for plasma glucose, insulin, and glucagon using the

trapezoidal method.

Psychophysical studies

Psychophysical studies were conducted one week after completion of the oral glucose tolerance studies. Concentration-intensity functions were determined for each participant using glucose, sucralose, and sodium chloride (Sigma). All stimuli were dissolved in de-ionized, filtered water (Millipore). Also, participants were also asked to rate the taste intensity of the glucose-sucralose mixture used in the OGTT experiment.

Participants were trained in the use of a general Labeled Magnitude Scale (LMS). Glucose, sucralose, and sodium chloride were each prepared in a concentration series increasing in half log steps. Glucose was presented in five concentrations ranging from 31.6 to 3160 mM. Sucralose was presented in six concentrations ranging from 0.016 to 5 mM. Sodium chloride was presented in six concentrations ranging from 4 to 1270 mM. The glucose-sucralose mixture was identical to the solution used in the OGTT experiment.

In each test session, samples were randomized and presented in 25 ml aliquots. Participants held each solution in the mouth for 5 seconds and rated the taste quality and intensity using a general labeled magnitude scale (gLMS). Participants then expectorated and rinsed their mouths with water four times (Millipore). The interstimulus interval was 2 minutes. Each solution was tested by each participant in quadruplicate.

Statistical analysis

Differences in OGTT outcomes were determined using repeated measures ANOVA with stimulus and time as within participant factors. Post-hoc Tukey's honestly significant difference (HSD) tests were used to assess differences between glucose, insulin, and glucagon concentrations at each time point. Differences in maximum concentration and incremental area under the curve (AUC) were compared across stimuli using paired t-tests. Analyses were conducted using Statistica (Statsoft, Tulsa, OK). Statistical significance for alpha (Type 1 errors) was set at $p <$

0.05.

The relationship between physiological and psychophysical responses to sucralose was assessed using Pearson's correlations.

RESULTS

Insulin

Sucralose elevated insulin responses (**Figure 1A**). Repeated measures analysis of variance showed a main effect of stimulus ($p = 0.06$) and an interaction of stimulus * time ($p<0.01$). Insulin peak was greater with sucralose ($p < 0.05$) and insulin AUC tended to be greater with sucralose ($p = 0.08$). Insulin rose from baseline more rapidly in the glucose + sucralose condition (**Figure 1A, inset**). In the glucose + sucralose condition, insulin was significantly elevated from baseline at 15 minutes. In glucose condition, insulin did not rise from baseline until 30 minutes.

Insulin elevations from sucralose corresponded with glucose elevations from sucralose (**Figure 1B**). There was a significant, positive association between the insulin change from sucralose and the glucose change from sucralose ($R^2 = 0.61$, $p < 0.05$). We found no correlations between sucralose effects and indicators of insulin sensitivity such as HOMA score, fasting glucose, or peak glucose. There was a significant correlation between BMI and the insulin change from sucralose ($R^2 = -0.66$, $p< 0.01$) (**Figure 1C**). The insulin elevation from sucralose increased with BMI.

Plasma glucose

Although insulin elevations from sucralose corresponded with glucose elevations on an individual basis, there was no effect on mean plasma glucose. Plasma glucose peak and AUC tended to be

greater with sucralose, but the differences were not significant (**Figure 2**).

Glucagon

We wished to know if the stability of plasma glucose in the face of elevated insulin might be due to increases in glucagon from sucralose. There were no differences in glucagon between the stimuli (**Figure 3**). There were no significant differences in glucagon peak, nadir, or AUC concentration between the stimuli.

Psychophysical Tests

Insulin responses to glucose neat were related to sweet taste perception. There was a positive association between insulin peak from glucose and the sweetness of the glucose + sucralose solution (R^2=0.65, $p< 0.05$) (**Figure 4**). There was no association between insulin peak from sucralose and sweet taste perception.

DISCUSSION

Effects of sucralose on oral glucose tolerance

In the current study, the addition of 5 mM sucralose (0.6 g per 300 ml) to an oral glucose tolerance test affected the metabolic response to ingested glucose. The addition of sucralose significantly enhanced the insulin response among subjects (**Figure 1A**).

Blood insulin was likely increased in response to elevated blood glucose. Although blood glucose was not significantly elevated in all participants (**Figure 2**), those with elevated glucose from sucralose showed corresponding insulin elevation (**Figure 1B**). It is also possible that blood

insulin was enhanced by the incretin response to NCS, since incretins promote insulin production and secretion. Although we did not measure GLP1 or GIP, previous studies have shown that NCS stimulate incretin release from the intestine *in vitro* [88, 90] and when given as a preload to oral glucose [111-113]. The addition of GLP1 or GIP to our analyses would not provide useful insights into the observed elevation in plasma glucose.

Plasma glucose can be elevated by increased intestinal absorption, increased hepatic glucose output, increased gastric emptying, and decreased clearance. Because sucralose by itself is inert, it is possible, even likely, that plasma glucose was elevated in sucralose-sensitive individuals because of rapidly increased expression of glucose transporters in the intestine. In animal models, acute exposure of the intestinal lumen to high potency sweeteners increases the expression of SGLT1 and GLUT2 and increases the rate of glucose absorption [90, 91, 165]. It is also possible that sucralose may have increased the rate of gastric emptying. Changes in gastric emptying rate can directly increase the postprandial plasma glucose response [166]. However, sucralose is not known to affect gastric emptying [107, 167]. It is therefore unlikely that our observations could be explained by differences in gastric emptying. It is also unlikely that sucralose increased blood glucose by stimulating hepatic glucose output in our experiment, since glucagon did not change in venous blood between conditions (**Figure 3**). Although it should be kept in mind that glucagon levels in peripheral circulation are not necessarily reflective of levels in the liver's portal vein [168]. It is also unlikely that plasma glucose increased via non-glucagon-related gluconeogenic pathways. Hepatic glucose output can be stimulated by catecholamines independent of glucagon release during exercise or hypoglycemia [169]. In the present study, participants were not exercising and blood glucose was elevated. Last, plasma glucose can be elevated by decreased clearance. This too is an unlikely explanation because insulin, which promotes clearance, increased accordingly with glucose. Our subjects were non-diabetic and there was no association between sucralose effects and indicators of insulin resistance. Although, data from knockout animal models suggests that sweet receptor activation slows glucose clearance independent of postprandial insulin. Glucose clearance tends to be enhanced in T1R2 knockouts [116]. It is possible that T1R2-T1R3 activation produces an opposite effect,

although the mechanism is unclear.

The enhanced plasma glucose from sucralose could not have come from the sucralose itself. Sucralose serves as a T1R agonist and has neither calories nor glucose within it. It has been repeatedly shown that administration of sucralose in the absence of glucose has no effect on blood glucose or hormonal responses [107, 161, 167]. Although we did not include a sucralose only condition in our experiment, there is little reason to believe that sucralose by itself would affect glycemia in our participants.

The effect of sucralose on insulin increased with BMI in our non-obese sample (**Figure 1C**). It is possible that intestinal T1R2-T1R3 activity is influenced by body weight, or vice versa. Recent evidence indicates genetic overlap between BMI and sweet taste perception in humans [170]. Further, intestinal T1R2-T1R3 expression is dysregulated in certain disease states. In healthy controls, luminal T1R2 expression is reciprocally regulated by plasma glucose [92]. In T2DM patients, however, hyperglycemia does not downregulate luminal T1R2 expression. In the same study, non-diabetic individuals with the greatest increases in duodenal T1R2 expression showed the highest plasma concentrations of 3-O-methyl-glucose (3-OMG), a nonmetabolized marker of SGLT1 activity [92]. This may in part explain our findings. Perhaps sucralose more effectively elevated insulin in participants with greater luminal T1R2 expression, which would allow for greater T1R2-T1R3 activation by sucralose. It is possible that luminal sweet receptor expression is similarly dysregulated in obesity and T2DM. This could explain why sucralose significantly elevated plasma glucose in a morbidly obese sample but not in the present study [110].

The present results are supported by previous studies [110, 113]. In two studies, consumption of a diet soda preload before an OGTT increased GLP-1 secretion but did not affect blood glucose in healthy participants [111, 112]. In one of these studies, blood insulin was also unaffected, despite increased GLP-1. In a later study of morbidly obese participants, consumption of a sucralose preload increased insulin AUC, but did not affect blood glucose AUC [110], similar to present mean findings. In yet another study, a sucralose preload increased GLP-1 secretion and reduced blood glucose AUC without affecting insulin AUC [113], in contrast to our observations.

The high magnitude of our insulin effects may be due, in part, to our use of high sucralose concentrations, which were selected to stimulate T1R receptors strongly and enable us to test the hypothesis that T1Rs participate in glucose metabolism in healthy adults.

It is unclear why the effects of artificial sweeteners have not been characterized consistently across studies. The differences in experimental designs could be responsible as sweeteners, dosages, the timing of ingestion, and the differences in health and obesity status of study participants all vary. In the current study, the test drink consisted of 0.6 g sucralose mixed with 75 g glucose in 300 ml water. In the studies by Brown [111, 112], the test drink was 2/3 of a can of commercial diet soda containing acesulfame potassium and sucralose, which was consumed 10 minutes prior to a glucose load. In the study by Pepino [110], the test drink was 0.048 g sucralose in 60 ml water consumed 10 minutes prior to a glucose load. In the Temizkan study [113], the test drink was 0.024 g sucralose or 72 mg aspartame consumed 15 minutes before a glucose load. Further, the participants in the studies by Pepino and Temizkan were obese, older participants, whereas we used normal weight, younger, healthy participants (mean BMI = 23.5, mean age = 27). Our finding that sucralose effects were associated with BMI may explain some discrepancies between our results and those from Pepino. It is also important to note that we did not measure two hour blood glucose or insulin. The two hour oral glucose tolerance test is a method used to diagnose diabetes mellitus per the criteria issued by the World Health Organization. The goal of our study was not to determine whether sucralose acutely affects diabetes diagnosis.

Whether these sucralose-induced effects are due to changes in glucose absorption, gastric emptying, the incretin effect, oral-pre-absorptive phase stimulation, stimulation of pancreatic beta cells, or a combination of these effects is unclear at this time. Furthermore, it has also been shown that ingestion of NCS alters the gut microbiome [171]. In our experiment, consumption of a single dose of 0.6 g sucralose immediately altered blood glucose and insulin responses. Thus, our effect was unrelated to substantial changes in gut flora in this time frame, although same day changes in gut microflora are possible [172]. Therefore, high potency sweeteners influence

glycemia through action on T1R receptors in the short term and also on the gut microbiome in the longer term. It should be noted, however, that although saccharin, sucralose, and other high potency sweeteners bind T1R2/T1R3, we cannot assume that their metabolic influences via T1Rs are all the same.

Relationships between metabolic and pyschophysical responses

The T1R2/T1R3 receptor signals the presence of sugars and high potency sweeteners in both the oral cavity and the intestinal lumen. We, therefore, sought to determine whether the perception of sucralose in the oral cavity was related to its metabolic activity overall. We hypothesized that if taste responses to sucralose (which binds and activates T1R2/T1R3) correlate with metabolic responses, then T1R2/T1R3 receptor activity is likely similar in multiple sites and is a strong candidate for mediating the metabolic responses observed.

We did not find a relationship between insulin responses to sucralose and taste responses to sucralose. We did, however, find significant associations between insulin responses to glucose and taste responses to glucose + sucralose (**Figure 4**). We found that individuals with the greatest sweet taste responses to glucose + sucralose also showed the greatest increases in insulin from glucose alone. Increased insulin peak from glucose correlated with greater perceived sweetness from the glucose + sucralose drink.

Although we did not directly measure T1R activity, our findings could be explained by enhanced oral and post-oral T1R activity. Intestinal T1R activity upregulates incretin release [88], and pancreatic T1R activity directly stimulates insulin release from Beta-islet cells *in vitro* [103]. Note that ingested sucralose is partially absorbed into blood (~10%), possibly by paracellular transport in the small intestine [160, 173]. It is also possible that all of sucralose's effects are mediated by the oral cavity alone. This seems unlikely given that oral stimulation with sugars is associated with decreases in postprandial insulin and glucose by means of cephalic phase insulin release. In our study, insulin and glucose were elevated. This finding is not consistent with cephalic phase

responses. Unless all of our results are orally mediated, they indicate that sucralose activity is similar in the oral cavity and elsewhere in the body. They also strengthen the argument that T1R2/T1R3 is a mediator of metabolic responses to sweet compounds.

Our findings are supported by a similar study of sucralose and glycemia [110], the detection threshold for sucralose was correlated with glycemic responses to sucralose. Greater changes in plasma glucose peak elicited by sucralose were correlated with low sucralose detection thresholds (greater sensitivity). It was speculated that this relationship could be due to enhanced sensitivity of T1R receptors. Intestinal T1R receptors are known to upregulate intestinal glucose transporters and enhance glucose absorption [90, 91]. Our results do not completely match those previously published (26), but this may be due to methodological differences.

Other psychophysical studies of diabetic patients show relationships between sweet taste perception and glucose metabolism [174-176]. Sucrose detection threshold may be correlated with fasting and postprandial blood glucose [176]. Compared with healthy controls, type 2 diabetics show deficits in threshold and suprathreshold sweet taste perception [175-177]. Sweet taste perception is also impaired in pre-diabetics, although not to the same extent as individuals with frank diabetes [175]. Taken together, these studies suggest that impaired sweet taste perception is associated with impaired glucose regulation. The underlying mechanism is unclear, but it has been suggested that sweet taste receptor activity may be sub-optimal in type 2 diabetic patients [178, 179]. Some evidence shows that T1R expression patterns are altered in type 2 diabetic patients and may contribute to glucose dysregulation [92, 179]. Our findings strongly support the hypothesis that T1R sweet taste receptors influence glucose metabolism. Further investigation is needed to clarify the relationship between taste sensitivity and metabolic responses to high potency sweetener T1R agonists.

In summary, sucralose, a non-caloric T1R agonist, affected the metabolic outcomes from an oral glucose load. We also found that the perceived sweetness intensity of glucose + sucralose was positively associated with the insulin response to an oral glucose tolerance test. Although we did not directly measure T1R activity, these data collectively point to the critical role of T1R receptors

in regulating human carbohydrate metabolism and indicate that consuming sweeteners in conjunction with meals has unintended consequences. This study was not intended to assess sucralose's safety nor its effects on long-term blood glucose control. Further studies are needed to clarify the relationship between oral and intestinal sweet taste receptors (T1Rs) and to explain the variability in metabolic reactions to high potency sweeteners.

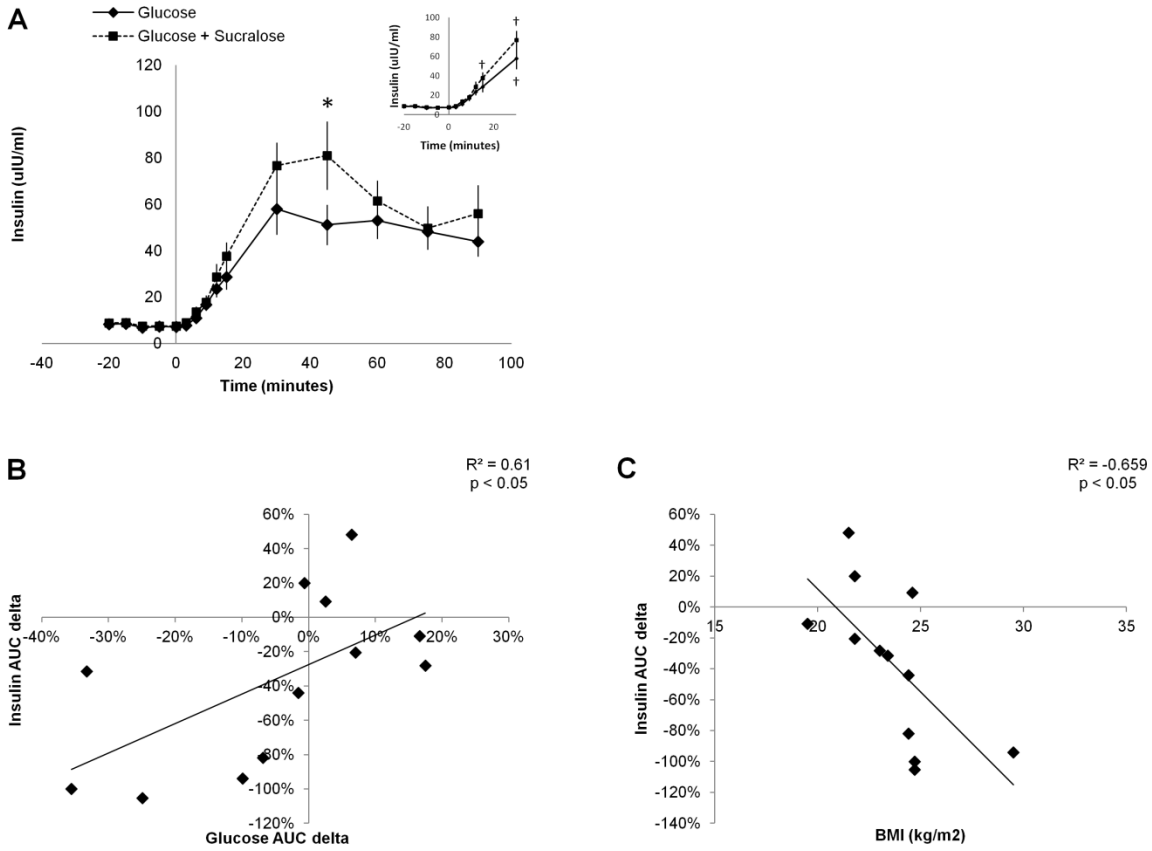

Figure 5-1. Effect of sucralose on plasma insulin.

(A) Plasma insulin responses to oral glucose tolerance tests with and without added sucralose. Plasma insulin response was elevated in the glucose + sucralose condition. There was a main effect of stimulus ($p=0.06$) and an interaction effect of stimulus x time ($p<0.01$). Data are means +/- standard error. * indicates a significant difference between stimuli ($p<0.05$). **(Inset)** Plasma insulin responses for the first 30 minutes after consuming stimuli. Insulin rose significantly from baseline at 30 minutes in the glucose condition and at 15 minutes in the glucose + sucralose condition. † indicates significant elevation from baseline. **(B)** Scatterplot showing changes in glucose and insulin elicited by sucralose for each participant. There was a significant correlation between insulin and glucose responses to sucralose ($R=0.61$, $p=0.03$). **(C)** Scatterplot showing the relationship between BMI and changes in insulin elicited by sucralose. There was a significant correlation between the change in plasma insulin elicited by sucralose and BMI ($R = -0.66$, $p < 0.05$). n=12.

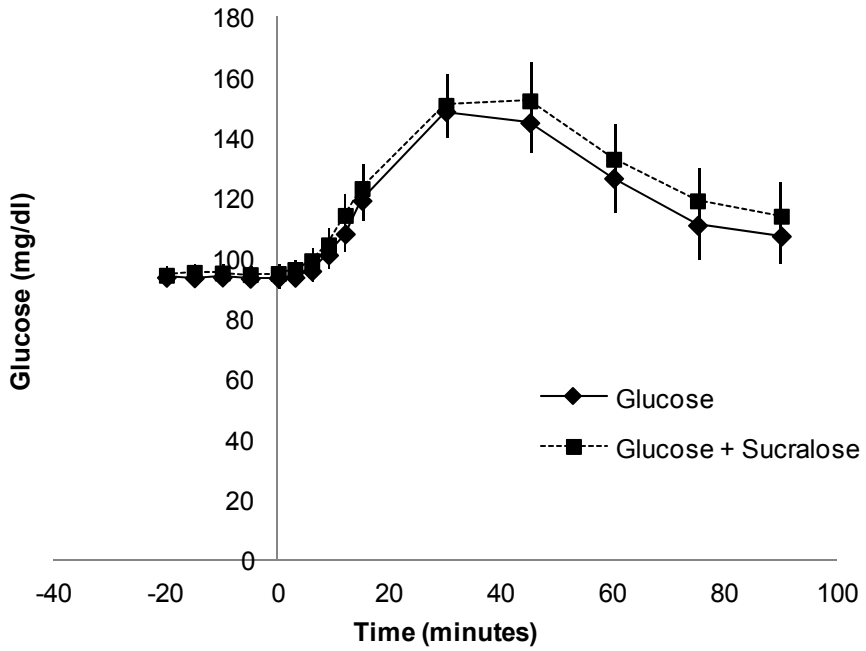

Figure 5-2. Effect of sucralose on plasma glucose.

Sucralose did not affect plasma glucose response to a glucose load. There were no main effects or interactions effects of stimulus. There were no differences in glucose peak or AUC between stimuli. Data are means +/- standard error.

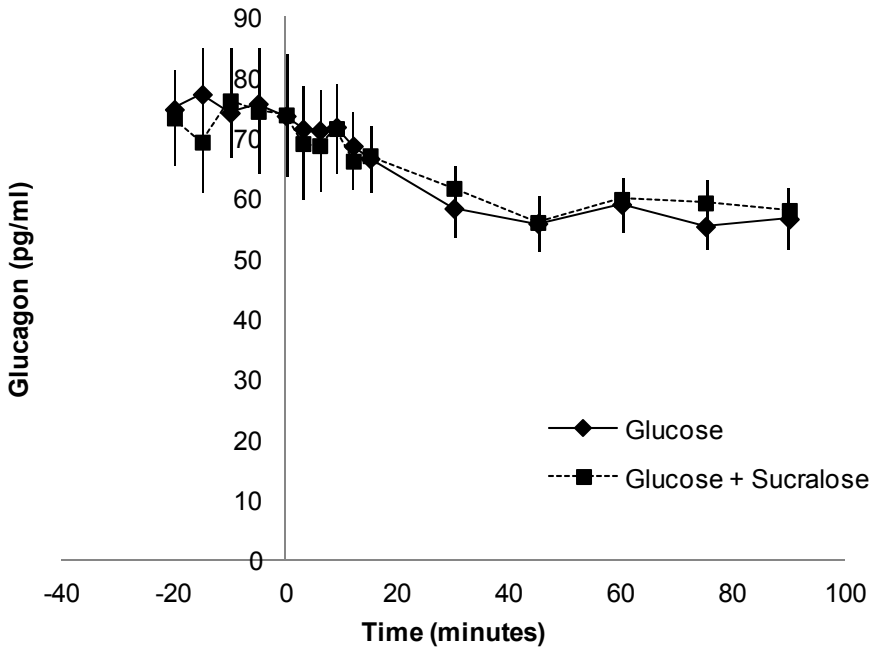

Figure 5-3. Effect of sucralose on plasma glucagon.

Sucralose did not affect plasma glucagon response to a glucose load. There were no main effects or interactions effects of stimulus. There were no differences in glucagon nadir, peak, or AUC between stimuli. Data are means +/- standard error.

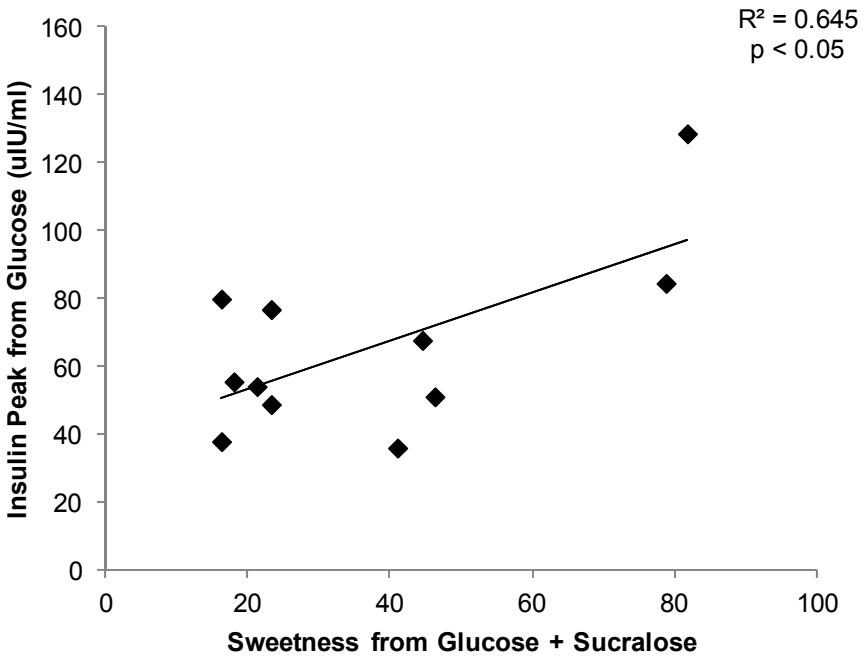

Figure 5-4. Correlation between perceptual and physiological responses.

Scatterplot showing the relationship between peak insulin responses to glucose neat and perceived sweetness from glucose + sucralose. There was a significant correlation between insulin peak from glucose neat and perceived sweetness from glucose + sucralose ($R=0.65$, $p < 0.05$). Individuals who reported greater sweetness intensity from glucose + sucralose showed greater insulin responses to an oral glucose load.

Chapter 6.

Sweet Receptor Antagonism Alters Postprandial Glycemia

ABSTRACT

The sweet taste receptor, T1R2-T1R3, is expressed in endocrine cells in the intestine and pancreas. In animal models, T1R2-T1R3 has been shown to facilitate glucose absorption and assimilation. Its role in humans, however, is unclear. Several studies have shown that high potency sweeteners alter glycemic and hormonal responses to 75 gram oral glucose tolerance tests, but results vary across studies. Furthermore, the effect of sweet taste antagonists on glucose tolerance has received little attention. The purpose of this study was to determine whether the consumption of lactisole, an inverse agonist of the sweet receptor, acutely alters glucose tolerance. We hypothesized that if luminal sweet receptors promote glucose absorption, then lactisole would impair glucose absorption and delay the increase in postprandial glycemia. We conducted oral glucose tolerance tests in 10 human participants with and without lactisole. We also conducted psychophysical studies to investigate the relationship between sweetener detection in the oral cavity and gut. We found that lactisole delayed the rise in postprandial glycemia from baseline as well as its return to baseline. Lactisole impaired glucose absorption in participants who reported the strongest sweetness inhibition. Our findings indicate that the sweet receptor is metabolically active.

INTRODUCTION

Sugars and high potency sweeteners (HPS) activate T1R2-T1R3, a g-protein coupled receptor expressed in taste tissue [20]. In the oral cavity, T1R2-T1R3 activation transduces the perception of sweet taste [4, 5]. T1R2-T1R3 is also expressed in non-taste cells including intestinal L-cells, pancreatic beta cells, and adipocytes [90, 103, 114].

T1R2-T1R3 activation with HPS has been shown to influence glucose homeostasis and adipocyte metabolism. In vitro, HPS stimulate GLP-1 secretion from intestinal L-cells [88, 90] as well as insulin secretion from pancreatic B cells [103, 148]. HPS also stimulate differentiation and inhibit lipolysis in adipocytes [114, 149]. Animal studies have shown that dietary supplementation with HPS upregulates expression of glucose transporters and enhances glucose absorption [90, 91, 147]. In human studies, consumption of HPS acutely elevates plasma glucose, incretin, and insulin responses to an oral glucose load [110-112].

The physiological effects of T1R2-T1R3 inhibition have not been thoroughly examined in human studies. T1R2-T1R3 is inhibited by sodium lactisole, a sweet taste blocker [36]. Sodium lactisole is an inverse agonist of T1R2-T1R3 which binds the transmembrane domain of human T1R3 [145]. When mixed with sugars and sweeteners, lactisole inhibits the perception of sweetness [36]. When lactisole is subsequently released by rinsing with water, the receptor rebounds into the active state, resulting in the perception of sweet water taste [32].

Several lines of reasoning suggest that T1R inhibition may influence human physiology. First, sodium lactisole inhibits GLP-1 and insulin responses to HPS in vitro [88, 104]. Second, T1R2 and T1R3 ablation protects against fat mass gain in mice fed obesogenic diets [115, 116, 150]. Third, clofibric acid, a structural analogue of lactisole which inhibits T1R2-T1R3, reduces plasma triglycerides and cholesterol [119, 153]. Fourth, intragastric infusion of lactisole elevates plasma glucose responses to IG infused glucose [151].

The effects of oral lactisole consumption on glucose metabolism have not been examined. It is also unclear whether oral lactisole consumption influences anticipatory responses to an oral

glucose load. Additionally, previous studies have not controlled for T1R2-T1R3 rebound activity from lactisole.

The purpose of this study was to determine whether sodium lactisole suppresses glycemic responses to an oral glucose load. We conducted oral glucose tolerance tests with and without sodium lactisole in human participants. We also conducted psychophysical studies to determine whether oral whether individual differences in sweet taste perception is associated with differences in glucose metabolism.

METHODS

Subjects

10 non-obese, non-diabetic participants participated in the study. BMI ranged from 19.1 to 25.8 kg/m2 and (mean 23.3 kg/m2). Age ranged from 21-31 years (mean 26 years). 5 males and 5 females participated. Of the participants, 6 were Caucasian, 1 was Asian, 2 were Latin, and 1 was of mixed ethnicity. Fasting glucose was < 100 mg/dl and 2 hour glucose was < 200 mg/dl for all participants. Participants were screened with a medical and dietary questionnaire used in previous studies (25). Participants who reported consuming more than one serving of artificially sweetened beverages or snacks per day were excluded. Participants with diseases and medications affecting digestion and absorption were also excluded. Based on these criteria, 3 participants were excluded. Seven of the ten OGTT participants participated in the taste psychophysical tests. Subjects were paid to participate and provided informed consent on an IRB approved form. This protocol complies with the Declaration of Helsinki for Medical Research involving Human Subjects

Glucose tolerance tests

Participants were tested in a randomized, single blind, cross over design. Participants were tested on two occasions, spaced one week apart. Participants were instructed to fast after midnight before coming to the laboratory. Participants were also instructed to abstain from structured exercise for the 48 hours preceding each test.

In the experimental condition, the stimulus was 1.39 M glucose (Sigma) + 2 mM sodium lactisole (R.C. Treatt) dissolved in water (Millipore). In the control condition, the stimulus was 1.39 M glucose.

Each test session was designed as follows: 1) baseline measurements (-20 to -15 min), 2) oral rinsing with stimulus (-10 to 0 min), 3) consumption of 300 ml stimulus (0 to 3 min), then 4) continuous sipping (3 to 120 min).

During the oral rinse period, participants rinsed and gargled with 30 ml of the stimulus for 20 seconds. After 20 seconds, participants expectorated the sample and began rinsing with another 25 ml of stimulus. This was repeated for 10 minutes.

During the sipping period, participants consumed either 2 mM lactisole dissolved in water (experimental) or plain water (control) at a rate of 10 ml/min. This was done in order to prevent sweet receptor rebound activity in the experimental condition.

Blood samples were collected using an indwelling catheter inserted into an antecubital vein. Samples were drawn at the following time points: -20, -15, 10, -5, 0, 3, 6, 9, 12, 15, 30, 45, 60, 75, 90, 105, and 120 minutes.

Blood samples were collected in chilled EDTA plasma tubes. Samples were centrifuged (4 degrees, 15 minutes, 1300 RCF) and plasma was separated and stored at -80 degrees for later analysis. Plasma insulin and glucagon were measured using a double-antibody radioimmunoassay (EMD Millipore, Billerica MA). Glucose was measured spectrophotometrically using an auto-analyzer (Roche). These measurements were made by a core facility of the University of Pennsylvania Institute for Diabetes, Obesity, and Metabolism.

Calculations

Area under the curve

Area under the curve was determined for plasma glucose, insulin, and glucagon using the trapezoidal method.

Psychophysical studies

Psychophysical studies were conducted one week after completion of the oral glucose tolerance studies. Concentration-intensity functions were determined to determine the inhibitory potency of sodium lactisole.

Three concentration-intensity functions were determined for each participant. 1.39 M glucose was presented with increasing concentrations of sodium lactisole (0-16 mM lactisole). 0.46 M sucrose was presented with increasing concentrations of sodium lactisole (0-16 mM lactisole). Sodium chloride was presented neat in concentrations increasing in half log steps from 4 to 1270 mM. Sodium chloride was used as a negative control to determine whether correlations with psychophysical outcomes were specific to sweet taste perception.

In each psychophysical test session, samples were randomized and presented in 25 ml aliquots. Participants held each solution in the mouth for 5 seconds and rated the taste quality and intensity using the general labeled magnitude scale (gLMS). Participants then expectorated the sample and rinsed with water. To assess rebound effects, participants rated the taste quality and intensity elicited by the water rinse. The interstimulus interval was 2 minutes. Each solution was tested by each participant in triplicate.

Statistical analysis

Differences in OGTT outcomes were determined using repeated measures ANOVA with stimulus and time as within participant factors. Post-hoc Tukey's honestly significant difference (HSD) tests were used to assess differences between glucose, insulin, and glucagon concentrations at each time point. Differences in maximum concentration and area under the curve (AUC) were compared across stimuli using paired t-tests. Analyses were conducted using Statistica (Statsoft, Tulsa, OK). Statistical significance for alpha (Type 1 error) was set at $p < 0.05$.

The relationship between physiological and psychophysical responses to sucralose was assessed using Pearson's correlations.

RESULTS

Plasma glucose

Repeated measures ANOVA revealed an interaction effect of stimulus x time ($p=0.08$), indicating that changes in plasma glucose over time differed across stimuli (**Figure 1A**). Lactisole delayed and extended glycemic responses to oral glucose. Plasma glucose increased from baseline more slowly with lactisole. Plasma glucose increased significantly from baseline within 15 minutes in the glucose condition and within 30 minutes in the glucose + lactisole condition. Similarly, plasma glucose returned to baseline more slowly with lactisole. Plasma glucose returned to baseline within 60 minutes in the glucose condition and within 90 minutes in the glucose + lactisole condition. Plasma glucose peak and AUC tended to be greater with lactisole, but the differences were not significant.

There was a significant correlation between fasting plasma glucose and the effect of lactisole on glucose peak (**Figure 1B**) and glucose AUC (**Figure 1C**). There was a positive association between fasting glucose and the difference in plasma glucose responses between glucose neat

and glucose + lactisole (G-GL). Lactisole lowered plasma glucose in participants with higher fasting glucose. Lactisole elevated plasma glucose in participants with lower fasting glucose.

Plasma insulin

Plasma insulin peak and AUC tended to be greater with lactisole, but the differences were not significant (**Figure 2A**). In parallel with blood glucose outcomes, plasma insulin was elevated in the lactisole condition from 30 – 120 minutes.

There was a significant correlation between fasting glucose and the effect of lactisole on insulin AUC ($R=0.7$, $p=0.05$) (**Figure 2B**). Lactisole elevated insulin AUC in participants with lower fasting glucose.

Plasma glucagon

There were no differences in glucagon outcomes between stimuli (**Figure 3**).

Relationship between lactisole taste perception and physiological effects

We examined relationship between perceptual sensitivity to lactisole and physiological outcomes. To assess lactisole sensitivity for each participant, we identified the concentration of lactisole that reduced sweetness intensity from 1.39 M glucose to a barely detectable level. We then compared metabolic outcomes between individuals showing high and low inhibitory potency from lactisole.

Lactisole significantly inhibited perceived sweetness intensity from 1.39 M glucose (**Figure 4A**). There was a significant correlation between sweetness inhibition and glucose responses from

lactisole (**Figure 4B**). Greater inhibitory potency from lactisole was association with reduced plasma glucose AUC (R^2 = 0.92, $p < 0.01$). We then grouped participants by perceptual sensitivity to lactisole. The difference in the concentration of lactisole needed to inhibit sweetness to barely detectable intensity between groups was significant ($p < 0.05$) (**Figure 4C**). The difference in the plasma glucose change between groups was significant ($p < 0.05$) (**Figure 4D**). Lactisole elevated blood glucose AUC in participants showing weaker perceptual sensitivity to lactisole. Lactisole reduced blood glucose in participants showing stronger perceptual sensitivity to lactisole.

DISCUSSION

In the present study, consumption of 2 mM sodium lactisole delayed and extended glycemic responses to 75 g glucose. Lactisole delayed the elevation of plasma glucose from baseline as well as its return to baseline (**Figure 1A**). This observation supports the hypothesis that T1R2-T1R3 regulates blood glucose homeostasis.

Our findings demonstrate that the effect of T1R2-T1R3 inhibition is not simply the converse of T1R2-T1R3 stimulation with HPS. HPS have been shown to elevate the magnitude of glycemic and/or hormonal responses to an oral glucose load [110-112]. In the present study, lactisole did not reduce the magnitude of glycemic or hormonal responses. Lactisole instead produced a temporal effect, delaying the appearance and clearance of glucose.

Perhaps because glucose absorption was slowed, plasma glucose was elevated for a greater period of time in the lactisole condition (30-75 minutes) versus the glucose only condition (15-45 minutes). As a result, lactisole did not reduce plasma glucose but in fact modestly elevated plasma glucose. Our findings are supported by previous work. T1R3 knockout mice display elevated plasma glucose responses to IP glucose relative to wildtypes [115]. In humans, intragastric infusion of lactisole elevates glucose responses to infused glucose [151].

Lactisole modestly elevated plasma insulin (**Figure 2A**). This was likely a response to elevated plasma glucose, which stimulates insulin secretion. Lactisole did not alter glucagon response (**Figure 3**).

We found relationships between fasting glucose, perceptual sensitivity to lactisole, and the effects of lactisole on glycemia. Participants with higher fasting glucose showed greater sweetness inhibition (not shown) and lower plasma glucose and insulin AUC from lactisole (**Figs 1B, 1C, 2B**). Lactisole inhibited sweet taste intensity from 1.39 M glucose in all participants (**Figure 4A**), but there were significant differences in inhibitory potency from lactisole (**Figure 4C**). Lactisole reduced glucose AUC in participants reporting more potent sweetness inhibition from lactisole (**Figure 4B, 4D**).

Our results may be explained by previous work showing that sweet receptor activity is dysregulated in type II diabetes (T2DM). In T2DM, intestinal sweet receptor activity is elevated [180] and oral sweet taste sensitivity is reduced [181, 182]. Although our participants were not diabetic, it is possible that those with high fasting glucose showed elevated intestinal sweet receptor activity which limited the inhibitory effects of lactisole. Similarly, it is possible that participants with high fasting glucose reported lower sweetness intensity because of poor sweet taste sensitivity. Because each of these variables were significantly correlated, it is difficult to identify causal directions between fasting glucose, sweet taste, and glucose absorption. However, our findings suggest that sweet receptor activity in the oral cavity and intestine may influence fasting and postprandial glucose homeostasis, or vice versa.

This study is the first to demonstrate that feeding sodium lactisole, a sweet taste inhibitor, delays and extends glycemic responses to glucose. These data provide further evidence that T1R2-T1R3 contributes not only to sweet taste perception, but also to nutrient detection and assimilation in the alimentary tract.

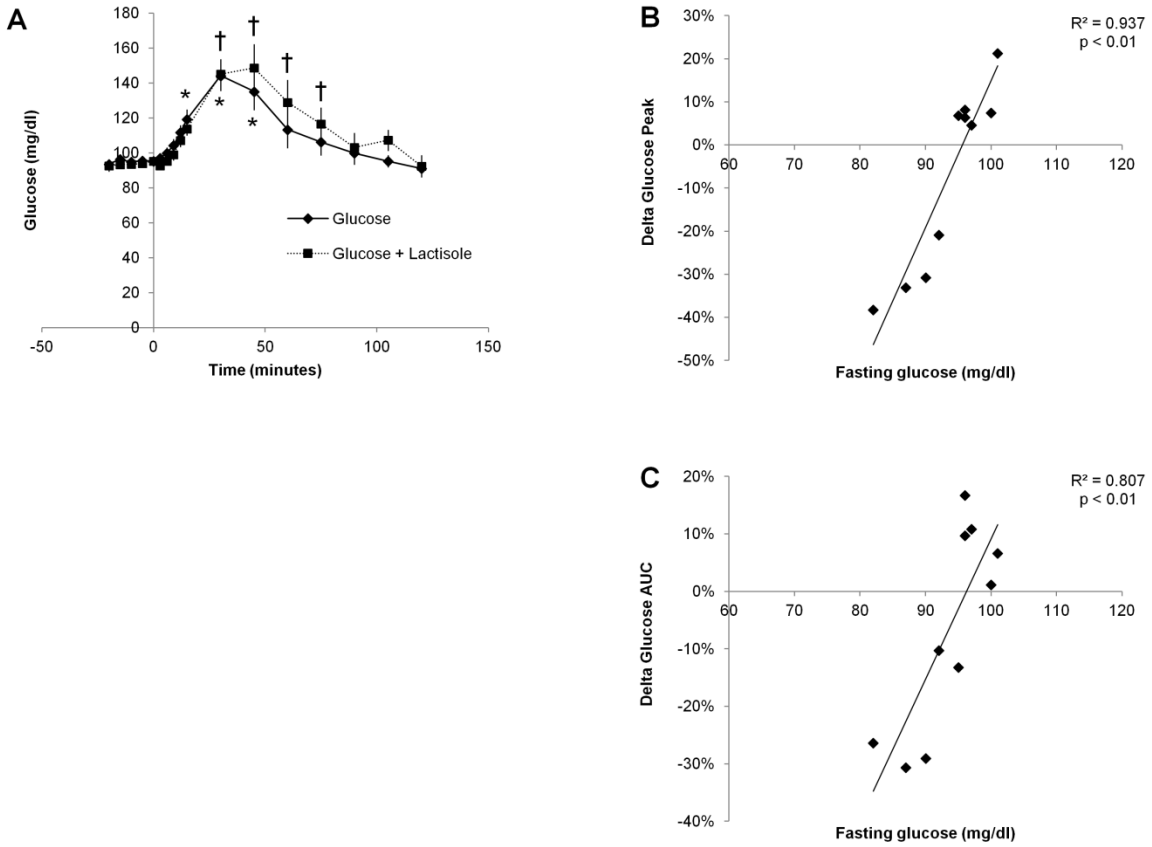

Figure 6-1. Effect of lactisole on plasma glucose.

(A) Plasma glucose responses to glucose and glucose + lactisole. Data are means +/- standard error. There was an interaction of time x stimulus (p=0.08). n=10. * Indicates significant difference from baseline in the glucose condition. † indicates significant difference in the glucose + lactisole condition. **(B)** Scatterplot showing relationship between fasting glucose (x axis) and change in glucose peak between stimuli (y axis). There was a significant, positive correlation between fasting glucose and glucose suppression from lactisole (R=0.94, p<0.01). **(C)** Scatterplot showing relationship between fasting glucose (x axis) and change in glucose AUC between stimuli (y axis). There was a significant, positive correlation between fasting glucose and glucose suppression from lactisole (R=0.81, p<0.01).

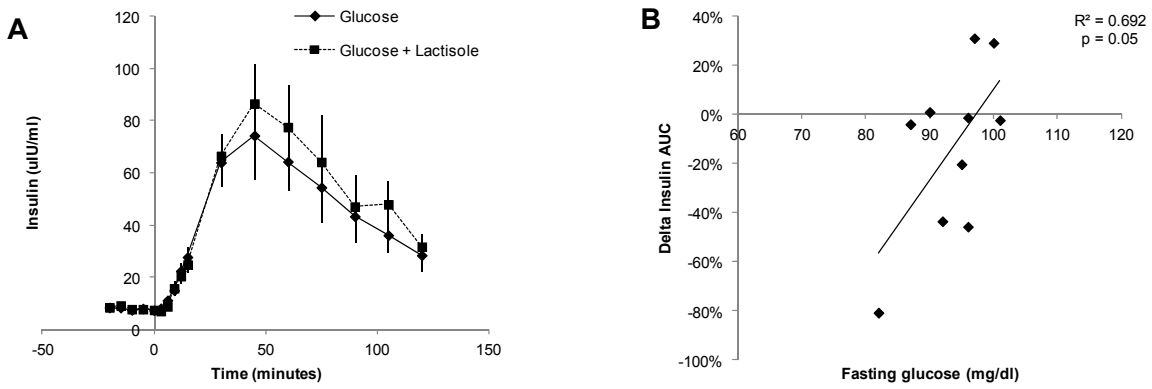

Figure 6-2. Effect of lactisole on plasma insulin.

(A) Plasma insulin responses to glucose (red) and glucose + lactisole (blue). Lactisole tended to elevate plasma insulin, but the effect was not significant. Data are means +/- standard error. **(B)** Scatterplot showing relationship between fasting glucose (x axis) and change in insulin AUC between stimuli (y axis). There was a positive correlation between fasting glucose and insulin suppression from lactisole (R=0.7, p = 0.05). n=10.

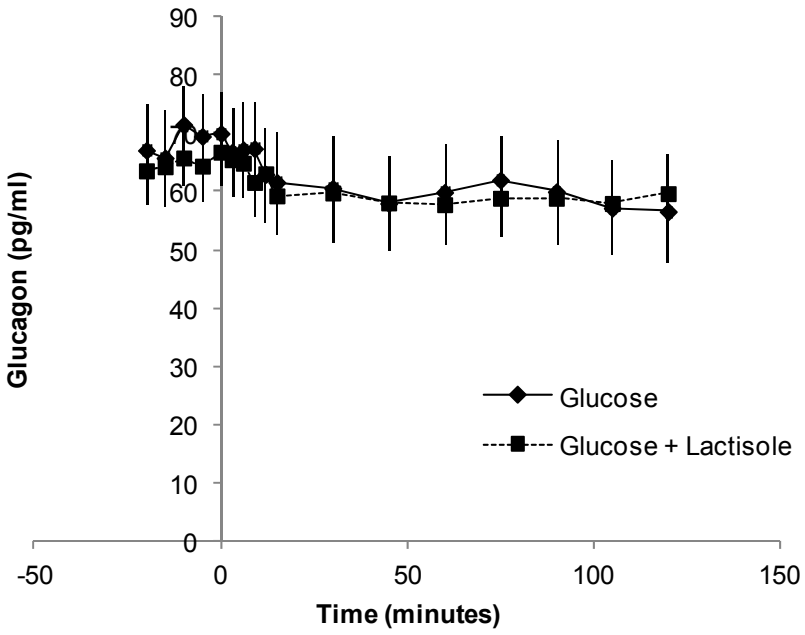

Figure 6-3. Effect of lactisole on plasma glucagon.

Plasma glucagon responses to glucose and glucose + lactisole. There were no differences in glucagon between stimuli. Data are means +/- standard error. n=10.

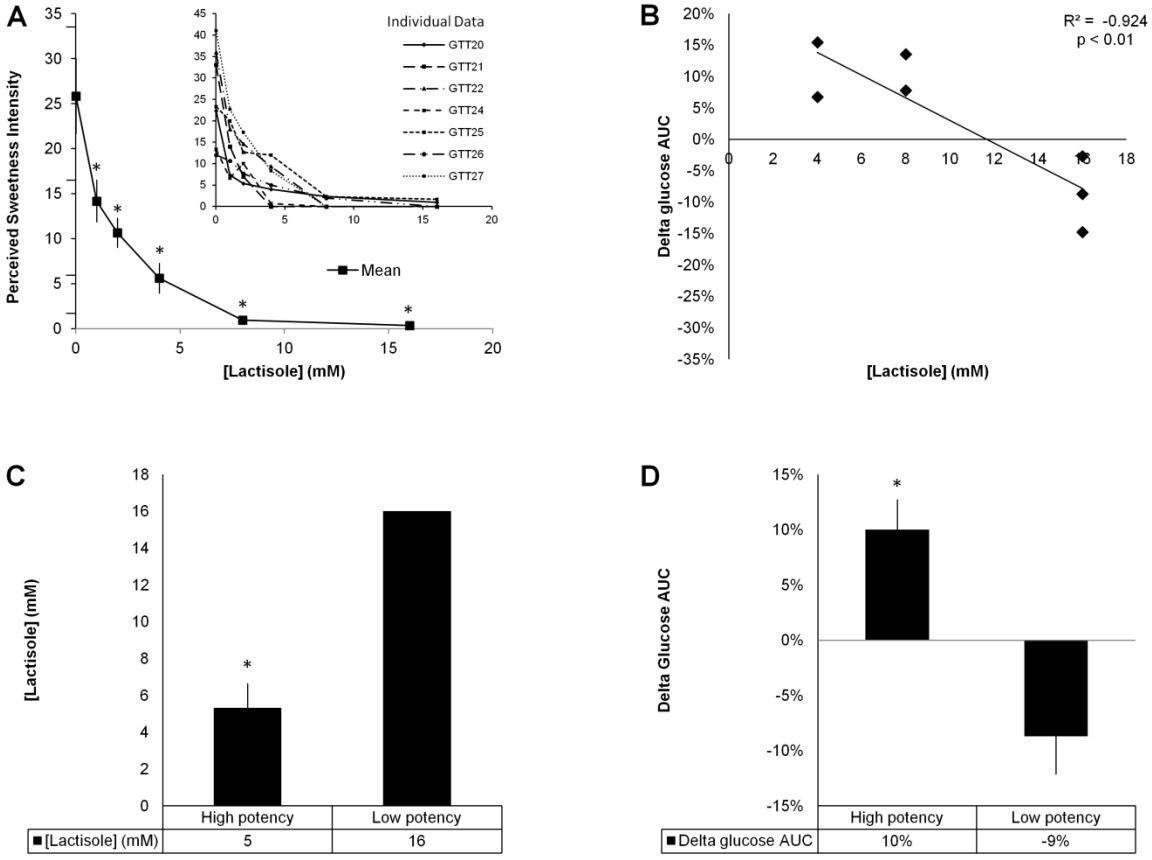

Figure 6-4. Relationships between lactisole perceptual Inhibition and metabolism.

(A) Effect of increasing [lactisole] on perceived sweetness intensity from 1.39 M glucose. * indicates significant difference from 0 mM lactisole (1.39 M glucose neat). **(B)** Scatterplot showing relationship between the [lactisole] needed to reduce sweetness from 1.39 to barely detectable intensity (x axis) and change in glucose AUC between stimuli (y axis). There was a significant, positive correlation between fasting glucose and glucose suppression from lactisole (R^2 = -0.92, $p < 0.01$). **(C)** We grouped participants by perceptual sensitivity to lactisole. The difference in [lactisole] needed to reduce sweetness intensity between groups was significant ($p < 0.05$). **(D)** The difference in delta glucose AUC between stimuli was significantly different between groups ($p < 0.05$).

Chapter 7.

Discussion and Future Directions

Acute effects of sweet receptor activity

There is increasing evidence that the sweet receptor influences glucose metabolism. The sweet receptor is expressed not only in oral taste tissue, but in the intestine, liver, and pancreas. *In vitro*, HPS stimulate GLP-1 secretion and glucose transporter expression in enteroendocrine L-cells. They also stimulate insulin secretion in pancreatic beta cells. As would be expected, GLP-1 and insulin secretion are reduced and glucose tolerance is impaired in T1R knockout animals. Trials in human subjects, however, have lacked the clarity of the *in vitro* experiments and, thus, the role of T1Rs in glucose metabolism has needed further empirical study.

If T1R activity enhances insulin secretion, GLP-1 secretion, and glucose tolerance, then one would logically suspect that HPS consumption similarly enhances insulin secretion, GLP-1 and glucose tolerance. But, this combination of outcomes has not been observed in previous studies. In fact, no combination of outcomes has been consistently observed. This is likely due to differences in sampling and experimental stimuli (both the type of HPS and the dose). In order to better characterize the effect of sweet taste receptor stimulation, I examined the effects of receptor activation and inhibition.

We first examined the effect of T1R2-T1R3 activation with sucralose on oral glucose tolerance test outcomes. I found that sucralose enhances the insulin response to an oral glucose tolerance test. However, sucralose did not reduce postprandial glucose. In fact, postprandial glucose tended to increase with sucralose. This result is supported by previous work [110], but it is surprising nonetheless because insulin promotes glucose clearance. There were no differences in glucagon between stimuli, so glucose was not held stable by increased hepatic glucose output. I suspect that plasma glucose was higher than expected because of increased intestinal absorption. HPS stimulate intestinal glucose transport activity in animal models. In our participants, insulin increases were strongly correlated with glucose increases. Intrahepatic catecholamines are also associated with gluconeogenesis and glycogenolysis, typically occurring with exercise or hypoglycemia. Neither of these conditions were met in our subjects during

testing. Thus, the presence of HPS in mixture with glucose seems to increase plasma glucose without traditional hormonal cues for plasma glucose increases.

To test the hypothesis that intestinal sweet receptors enhance glucose absorption, I then examined the effect of T1R2-T1R3 inhibition on glucose tolerance using lactisole. Lactisole, a GRAS food ingredient used to mask cloying sweetness in high-sugar foods, is an inverse agonist of the sweet receptor. I found that lactisole slows the rise of glucose in the blood during an OGTT. When participants consumed lactisole, plasma glucose remained at baseline 15 minutes longer than it did without lactisole. And, plasma glucose remained elevated from baseline 30 minutes longer than it did without lactisole. In essence, lactisole right-shifted the 'Plasma Glucose x Time' curve. Lactisole did not reduce postprandial insulin. In fact, lactisole tended to increase insulin. This is likely due to the fact that lactisole elevated glucose from baseline for a longer period of time, thereby increasing the total glucose AUC. Our results with lactisole support the hypothesis that intestinal T1R sweetener receptors help regulate glucose absorption.

It is important to note that the effects of sucralose and lactisole were not opposites of each other. Sucralose significantly elevated insulin and tended to elevate plasma glucose. Lactisole delayed the plasma glucose time course and also tended to elevate insulin, but, I believe, for different reasons. Although I conclude that sucralose elevated insulin because it enhanced glucose absorption, I cannot conclude that sucralose significantly hastened the appearance of plasma glucose in all of our participants.

Our results suggest that the effects of T1R2-T1R3 on carbohydrate metabolism varies with individuals' metabolic states. Although all of our participants were non-obese and non-diabetic, there were significant correlations between the effects of sweet receptor manipulation and characteristics, such as fasting glucose. It was shown previously that intestinal T1R activity is dysregulated (overactive) in participants with T2DM relative to non-diabetic controls [92]. In the present work, lactisole reduced postprandial glycemia in participants with higher fasting glucose. Our findings suggest that further investigation into sweet receptor inhibition in the alimentary tract could yield useful insights into disease management or prevention.

Chronic effects of sweet receptor activity

The narrative surrounding the effects of HPS on chronic disease risk has been intriguing, but at times unclear. Before the revelation that sweet receptors are metabolically active, diet sodas and HPS had already been condemned in the court of public opinion. HPS are inappropriately purported by some to cause diabetes, obesity, metabolic syndrome, and other conditions [183, 184]. Some observational studies have shown associations between diet soda consumption and diabetes risk, but such approaches cannot determine causality [185, 186]. The discoveries that sweet receptors appear to stimulate glucose transport and insulin secretion gave credence to the notion that sweet receptor activation (drinking diet soda) might promote chronic disease.

And yet, T1R3 knockout animals show signs of glucose intolerance when maintained on standard chow diets [106, 187]. T1R3 is expressed not only in the oral cavity and intestine, but the pancreas, liver, adipose, and elsewhere. The glucose intolerance observed in T1R3 knockout animals has been ascribed to impaired insulin secretion due to the absence of beta cell sweet receptors. Some researchers posit that intestinal sweet receptors contribute little to glucose control [187]. They extend this logic to argue that HPS have no effect on glucose tolerance because they are not absorbed. But, oral glucose tolerance studies in human participants, such as the present studies, show that HPS alter glucose tolerance. Furthermore, I used sucralose, which is not well absorbed.

The results from the present human oral glucose tolerance studies suggest that HPS consumption may elevate postprandial glycemia and insulin when ingested with food. But I cannot extrapolate the results from an acute exposure to conclude that HPS promote metabolic disease. Yet, there is evidence that T1R activity influences metabolism in the long term. T1R2 and T1R3 ablation attenuates diet-induced obesity, hepatic lipid deposition, and hyperinsulinemia [115, 116, 150]. The mechanisms remain unclear, but it is speculated that the effects may stem from altered substrate metabolism [116].

We took a different approach to exploring the relationship between T1R activity and chronic disease by examining the effects of clofibric acid on taste perception. Clofibric acid is a lipid lowering prescription drug thought to act through PPAR-alpha. PPAR-alpha is a transcription factor which upregulates a host of genes involved in lipid metabolism. Like T1R ablation, clofibric acid ameliorates ectopic lipid deposition and hyperinsulinemia. Clofibric acid, as well as other fibrate drugs and phenoxy herbicides, bind and inhibit T1R3 *in vitro* [153]. I, therefore, sought to determine whether clofibric acid inhibits T1R3-mediated taste perception in humans.

We found that clofibric acid inhibits sweet perception in human participants. Similar to lactisole, clofibric acid appears to be an inverse agonist of the sweet receptor as indicated by its ability to elicit sweet water taste when expectorated and rinsed with water. Importantly, the plasma concentration of clofibric acid achieved from taking a course of clofibrate is adequate to inhibit sweet taste receptors *in vivo*. I conclude that long term consumption of a T1R3 inhibitor could elicit metabolic effects similar to those seen when ingesting a PPAR-α agonist in humans and knocking out *T1r3* in mice.

Clofibric acid is undoubtedly a PPAR-alpha agonist, but it might also exert physiological effects through extra-oral taste receptor inhibition. I demonstrated that acute exposure to lactisole alters glucose tolerance test outcomes. This raises the question of whether the effects of fibrate drugs are mediated in part by T1R3. This question could be answered by investigating the effects of clofibric acid (or other T1R3 inhibitors) on glucose tolerance in either acute or long-term settings.

Saccharide sweetness

The metabolic functions of sweet receptors in extra oral tissues remain to be determined. In the oral cavity, sweet receptors very clearly affect metabolism. Sweet taste guides the perception and consumption of foods and triggers CPIR. Our avidity for sweet foods and beverages is arguably a major driver of the prevalence of obesity and type 2 diabetes. Fructose is a preferred component of sweetener systems because it is roughly twice as sweet as glucose. Unfortunately,

there is reason to believe that it is also more deleterious to health than glucose [53, 188, 189]. Substitutes for fructose sweetened beverages exist in the form of diet soft drinks, but they are easily discriminated from regular soft drinks. I, therefore, sought to clarify the differences in glucose and fructose perception.

Using a self-mixture approach, I found that glucose behaves as a partial agonist of the sweet receptor. Relative to a fructose/fructose self mixture, a fructose/glucose binary mixture elicited lower perceived sweetness. Therefore, glucose suppressed the sweetness of fructose. This suggests that in sweetener systems, fructose and glucose do not interact in a simple additive manner. Glucose may compete with fructose for the same binding site on T1R2-T1R3 and due to its lower intrinsic activity, it reduces the overall activity of the receptor-agonist system. This finding could guide the improvement of saccharide sweetener systems used in beverages.

In light of glucose's weaker intrinsic activity, I asked whether the sweetness intensity of a glucose solution could be matched to that of an equicaloric sucrose solution. Using a duo-trio test trial approach in human participants, I found that a mixture of glucose and stevioside closely matched the sweetness of sucrose, and in many participants was indistinguishable. I provided proof-of-principal that it is possible to formulate fructose free soft drinks that are indiscriminable from sucrose (and presumably HFCS) sweetened soft drinks.

Reducing fructose from the diet would slow or prevent the pathogenesis of metabolic dysfunctions, especially non-alcoholic fatty liver disease, which predisposed patients to metabolic syndrome and T2DM. The replacement of glucose with fructose has been shown to improve significantly several markers of chronic disease [51, 52]. Admittedly, non-caloric options already exist such as water and diet soft drinks. Yet, the consumption of regular sucrose- and HFCS-sweetened soft drinks persists around the world. I further understand that the addition of HPS to a glucose load significantly increases postprandial insulin. But, a key variable in our glucose tolerance study was sweetness. The glucose + sucralose stimulus was substantially more sweet than the glucose stimulus. Our glucose + stevioside beverage is matched for sweetness with equicaloric sucrose. Therefore, I would not expect major metabolic events arising from hyper-

stimulation of the sweet receptor activity to arise. The most important nutritional difference between the experimental beverage and the sucrose beverage is the presence or absence of fructose.

Consistent with competitive antagonism, the relative inhibition from glucose was suppressed when the concentration of fructose was increased, re-attaining its high level of sweetness. The concentrations of added glucose and fructose (200 mM and 100 mM) were weakly sweet relative to the levels of each sweetener used in the latter half of the concentration-intensity functions. It is possible that high levels of glucose and fructose (562 and 1000 mM) increased sweetness intensity to an extent that masked the difference between the +G and +F conditions.

Future Directions

Sweet taste perception

In Aim 1, I demonstrated that glucose and fructose sweetness were non-additive at specific concentrations. It is unclear whether glucose and fructose in HFCS behave additively. Popular soft drinks are sweetened with 39 g HFCS55, which equates to approximately 344 mM fructose + 266 mM glucose. Our approach from Aim 2 could be used to compare the binary mixture of 344 mM fructose + 266 mM glucose with self mixtures of glucose and fructose.

In Aim 3, I demonstrated that a glucose/stevia mixture was indistinguishable from sucrose for 30-40% of participants. This study should be repeated with a larger sample size in order to more thoroughly examine whether glucose/stevia can match the taste of sucrose. The study should control for BMI, chronic disease, HPS consumption, and soda consumption. Future studies should also include other glucose/HPS mixtures, flavorings, carbonation levels, and packaging. If a glucose/HPS mixture performs well in duo-trio testing with large panels, the mixture could be subjected to further sensory and consumer science research. Such studies could examine whether consumers were likely to purchase, consume, and recommend the product. Consumers

could also be given long-term access to the glucose/HPS beverage and the traditional sugar sweetened beverage in order to compare consumption in free living conditions. Ultimately, the engineering of a fructose-free soda for distribution will be pursued with a goal of improving public health by providing a healthier alternative to existing sodas.

Regulatory physiology

In Aims 4 and 5, I demonstrated that sucralose and lactisole acutely influence blood glucose regulation. But, it remains unclear whether sweet receptors in non-taste tissue contribute to disease prevention or pathogenesis. Future studies should examine the physiological effects of different T1R ligands, other outcomes such as plasma free fatty acids and triglycerides, and the long term effects of T1R activity.

In Aim 4, I showed that sucralose significantly elevates plasma insulin and glucose responses to oral glucose. The magnitude of the insulin effect was particularly large, which suggests that sucralose may stimulate sweet receptors in beta cells. Roughly 10-15% of ingested sucralose enters the bloodstream and we used a relatively large dose of sucralose (600 mg), so it is possible that that >60 mg sucralose entered the circulation and activated sweet receptors in pancreatic beta cells. The contribution of pancreatic sweet receptors could be clarified by similar studies using acesulfame K as a stimulus. Acesulfame K is entirely absorbed by the intestine. If beta cells contributed to the increased insulin I observed, then I expect that acesulfame K (equisweet to 5 mM sucralose) would elicit a greater insulin response.

The effect of sucralose on plasma insulin may have consequences for plasma lipids. Insulin stimulates lipid synthesis and suppresses lipolysis. Together, these processes decrease plasma non-esterified fatty acid (NEFA) and increase plasma triglyceride. Therefore, I predict that sucralose lowers plasma NEFA and increases plasma triglycerides. Elevated postprandial triglyceride is a marker for metabolic dysfunction. Previous studies of HPS ingestion have

focused largely on plasma glucose, insulin, and GLP-1. The effects of HPS on plasma lipids are unknown.

Clofibric acid presents another excellent means of examining the intersection of sweet receptors, glucose tolerance, and lipid metabolism. Given that the effects of clofibrate treatment overlap with the effects of T1R ablation, it is possible that clofibrate exerts physiological effects through sweet receptor inhibition in non-taste tissue.

In Aim 3, I found that clofibric acid inhibits sweet taste *in vivo* and therefore inhibits the function of the sweet taste receptor in the oral cavity. Clofibric acid and lactisole exerted similar effects. In Aim 5, I demonstrated that lactisole slows glycemic responses to oral glucose. Therefore, I predict that clofibric acid would slow glycemic response to oral glucose. This prediction could be tested using the approach employed in Aim 5. If clofibric acid slowed glucose appearance, it is possible that habitual clofibric acid consumption chronically inhibits intestinal sweet receptor activity and slows glucose absorption.

In a similar vein, the properties of lactisole should be investigated further. Clofibric acid and lactisole are structurally similar and both compounds bind T1R3. Clofibric acid also binds PPAR-α. It is unknown, however, whether lactisole binds PPAR-α. This could be determined *in vitro* by treating PPAR-α expressing cells (liver, kidney, muscle, heart) with lactisole or clofibric acid (as a positive control) and measuring PPAR-α activity with commercially available PPARα transcription factor assay kits. Alternatively, mRNA levels of PPAR-α target genes could be measured. If lactisole is not a PPAR-α agonist, it could be used in a crossover study to further tease apart the T1R-mediated and PPAR-mediated effects of clofibric acid on plasma glucose and lipids. I would target hyperlipidemic subjects in order to avoid a floor effect and maximize the potential lipid reduction. If IRB approval for a human trial proved problematic, the study could be done in DIO animals. Outcomes of interest would be glucose tolerance and lipid tolerance. If animal models were used, other parameters could include fat weight, liver fat weight, skeletal muscle fat weight, and insulin sensitivity.

Further studies are needed clarify the effects of HPS and sweetness blockers on glucose absorption. In our non-obese, non-diabetic participants, sucralose significantly elevated plasma insulin but not plasma glucose. It is possible that our effects were due entirely to sweet receptor activation in the pancreas. But, we found a significant, positive correlation between insulin delta and plasma glucose delta. When sucralose elevated insulin, it tended to increase plasma glucose. Since our subjects were not diabetic, our findings suggest that sucralose elevated plasma glucose, which elevated insulin. Plasma glucose concentration is influenced by glucose absorption, hepatic glucose output, and glucose disposal. It remains unclear whether HPS enhance glucose absorption in humans. In order to examine glucose absorption more closely, our approach from Aim 4 could be repeated with the inclusion of 3-O-methylglucose (3-OMG) to the bolus. 3-OMG is a nonmetabolized analogue of glucose. It is absorbed in the lumen via SGLT1 and GLUT2, which are presumably responsible for the effects of T1R activation on glucose absorption.

It is unclear whether sweetener and sweetness blockers alter cephalic phase responses. Although HPS alone do not appear to stimulate CPIR, I found that plasma insulin increased from baseline more rapidly from glucose + sucralose relative to glucose alone. I did not detect significant changes in plasma insulin during the 10 minute period of oral stimulation, but my approach could be improved upon with more frequent sampling and a longer baseline period. It is also not known whether HPS/glucose mixtures elicit cephalic phase GLP-1 secretion. HPS consumption has been shown to increase postprandial GLP-1 responses to oral glucose, but previous studies have not incorporated modified sham feeding approaches to closely examine cephalic phase effects. In mice, taste bud cells secrete GLP-1 when exposed to sugars and high potency sweeteners [190]. It is unclear whether oral stimulation with sugars and sweeteners increases GLP-1 in plasma. Since incretin therapies are effective treatments for type 2 diabetes, the effect of sweeteners on GLP-1 secretion merits further investigation.

Conclusion

I have demonstrated that sweeteners and sweet taste blockers modulate metabolism. Sweeteners promote the consumption and assimilation of foodstuffs. Whereas sweet signals are helpful in times of scarcity, they may be harmful in times of plenty. I show that clofibric acid is a sweet taste blocker and that sweet blockers acutely slow glucose appearance. I also show that sweetness enhancement acutely elevates postprandial insulin, but HPS may have significant utility as a practical means of replacing fructose in the diet. Our results can aid in the direction of future projects aimed at moderating the consumption and assimilation of dietary sugars and sweeteners.

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Literature Cited

1. Steiner, J.E., et al., *Comparative expression of hedonic impact: affective reactions to taste by human infants and other primates.* Neurosci Biobehav Rev, 2001. **25**(1): p. 53-74.

2. Max, M., et al., *Tas1r3, encoding a new candidate taste receptor, is allelic to the sweet responsiveness locus Sac.* Nat Genet, 2001. **28**(1): p. 58-63.

3. Montmayeur, J.-P., et al., *A candidate taste receptor gene near a sweet taste locus.* Nature neuroscience, 2001. **4**(5): p. 492-498.

4. Nelson, G., et al., *Mammalian sweet taste receptors.* Cell, 2001. **106**(3): p. 381-90.

5. Li, X., et al., *Human receptors for sweet and umami taste.* Proc Natl Acad Sci U S A, 2002. **99**(7): p. 4692-6.

6. Damak, S., et al., *Detection of sweet and umami taste in the absence of taste receptor T1r3.* Science, 2003. **301**(5634): p. 850-3.

7. Yee, K.K., et al., *Glucose transporters and ATP-gated K+ (KATP) metabolic sensors are present in type 1 taste receptor 3 (T1r3)-expressing taste cells.* Proc Natl Acad Sci U S A, 2011. **108**(13): p. 5431-6.

8. Breslin, P.A. and A.C. Spector, *Mammalian taste perception.* Curr Biol, 2008. **18**(4): p. R148-55.

9. Smith, D.V. and R.F. Margolskee, *Making sense of taste.* Scientific American, 2006. **16**: p. 84-92.

10. Margolskee, R.F., *Molecular mechanisms of bitter and sweet taste transduction.* Journal of Biological Chemistry, 2002. **277**(1): p. 1-4.

11. Merigo, F., et al., *Glucose transporters are expressed in taste receptor cells.* J Anat, 2011. **219**(2): p. 243-52.

12. Glendinning, J.I., et al., *Sugar-induced cephalic-phase insulin release is mediated by a T1r2+T1r3-independent taste transduction pathway in mice.* Am J Physiol Regul Integr Comp Physiol. **309**(5): p. R552-60.

13. Ernest M. Wright, M.S.-R., Donald D.F. Loo, Bruce A. Hirayama, *Sugar Absorption*, in *Physiology of the gastrointestinal tract*, L. Johnson, Editor. 2012, Academic: Boston. p. 1583-1593.

14. Sclafani, A. and D.L. Williams, *Galactose consumption induces conditioned flavor avoidance in rats.* The Journal of nutrition, 1999. **129**(9): p. 1737-1741.

15. Sclafani, A., *Starch and sugar tastes in rodents: an update.* Brain Res Bull, 1991. **27**(3-4): p. 383-6.

16. Chambers, E.S., M.W. Bridge, and D.A. Jones, *Carbohydrate sensing in the human mouth: effects on exercise performance and brain activity.* J Physiol, 2009. **587**(Pt 8): p. 1779-94.

17. Breslin, P.A., G.K. Beauchamp, and E.N. Pugh, Jr., *Monogeusia for fructose, glucose, sucrose, and maltose.* Percept Psychophys, 1996. **58**(3): p. 327-41.

18. Pin, J.P., T. Galvez, and L. Prezeau, *Evolution, structure, and activation mechanism of family 3/C G-protein-coupled receptors.* Pharmacol Ther, 2003. **98**(3): p. 325-54.

19. McLaughlin, S.K., P.J. McKinnon, and R.F. Margolskee, *Gustducin is a taste-cell-specific G protein closely related to the transducins.* Nature, 1992. **357**(6379): p. 563-9.

20. Cui, M., et al., *The heterodimeric sweet taste receptor has multiple potential ligand binding sites.* Curr Pharm Des, 2006. **12**(35): p. 4591-600.

21. Xu, H., et al., *Different functional roles of T1R subunits in the heteromeric taste receptors.* Proc Natl Acad Sci U S A, 2004. **101**(39): p. 14258-63.

22. Jiang, P., et al., *The cysteine-rich region of T1R3 determines responses to intensely sweet proteins.* J Biol Chem, 2004. **279**(43): p. 45068-75.

23. Masuda, T., et al., *Five amino acid residues in cysteine-rich domain of human T1R3 were involved in the response for sweet-tasting protein, thaumatin.* Biochimie. **95**(7): p. 1502-5.

24. Winnig, M., et al., *The binding site for neohesperidin dihydrochalcone at the human sweet taste receptor.* BMC Struct Biol, 2007. **7**: p. 66.

25. Zhang, F., et al., *Molecular mechanism of the sweet taste enhancers.* Proc Natl Acad Sci U S A. **107**(10): p. 4752-7.

26. Masuda, K., et al., *Characterization of the modes of binding between human sweet taste receptor and low-molecular-weight sweet compounds.* PLoS One. **7**(4): p. e35380.

27. Nie, Y., et al., *Distinct contributions of T1R2 and T1R3 taste receptor subunits to the detection of sweet stimuli.* Curr Biol, 2005. **15**(21): p. 1948-52.

28. Atack, J. and H. Lavreysen, *Potency*, in *Encyclopedia of Psychopharmacology*, I.P. Stolerman, Editor, Springer Berlin Heidelberg: Berlin, Heidelberg. p. 1053-1053.

29. Kennedy, S.H. and S.J. Rizvi, *Efficacy*, in *Encyclopedia of Psychopharmacology*, I.P. Stolerman, Editor, Springer Berlin Heidelberg: Berlin, Heidelberg. p. 457-457.

30. DuBois, G.E., et al., *Concentrationâ€"Response Relationships of Sweeteners*, in *Sweeteners*. 1991, American Chemical Society. p. 261-276.

31. Antenucci, R.G. and J.E. Hayes, *Nonnutritive sweeteners are not supernormal stimuli.* Int J Obes (Lond), 2014. **39**(2): p. 254-9.

32. Galindo-Cuspinera, V., et al., *A TAS1R receptor-based explanation of sweet 'water-taste'.* Nature, 2006. **441**(7091): p. 354-7.

33. Sanematsu, K., et al., *Molecular mechanisms for sweet-suppressing effect of gymnemic acids.* J Biol Chem. **289**(37): p. 25711-20.

34. Jiang, P., et al., *Lactisole interacts with the transmembrane domains of human T1R3 to inhibit sweet taste.* J Biol Chem, 2005. **280**(15): p. 15238-46.

35. Diamant, H., et al., *A Comparison of Neural and Psychophysical Responses to Taste Stimuli in Man.* Acta Physiol Scand, 1965. **64**: p. 67-74.

36. Schiffman, S.S., et al., *Selective inhibition of sweetness by the sodium salt of +/-2-(4-methoxyphenoxy)propanoic acid.* Chem Senses, 1999. **24**(4): p. 439-47.

37. Breslin, P.A., *An evolutionary perspective on food and human taste.* Curr Biol. **23**(9): p. R409-18.

38. Peyrot des Gachons, C., et al., *Bitter taste induces nausea.* Curr Biol. **21**(7): p. R247-8.

39. Spielman, A.I., *Chemosensory function and dysfunction.* Crit Rev Oral Biol Med, 1998. **9**(3): p. 267-91.

40. Bolze, M.S., et al., *Taste acuity, plasma zinc levels, and weight loss during radiotherapy: a study of relationships.* Radiology, 1982. **144**(1): p. 163-9.

41. Baharvand, M., et al., *Taste alteration and impact on quality of life after head and neck radiotherapy.* J Oral Pathol Med. **42**(1): p. 106-12.

42. Schiffman, S.S. and B.G. Graham, *Taste and smell perception affect appetite and immunity in the elderly.* Eur J Clin Nutr, 2000. **54 Suppl 3**: p. S54-63.

43. Davis, C. and E. Saltos, *Dietary recommendations and how they have changed over time.* America's eating habits: Changes and consequences, 1999: p. 33-50.

44. Seidell, J.C., *Obesity, insulin resistance and diabetes a worldwide epidemic.* British Journal of Nutrition, 2000. **83**(S1): p. S5-S8.

45. Popkin, B.M., *Patterns of beverage use across the lifecycle.* Physiology & behavior. **100**(1): p. 4-9.

46. Kit, B.K., et al., *Trends in sugar-sweetened beverage consumption among youth and adults in the United States: 1999-2010.* Am J Clin Nutr. **98**(1): p. 180-8.

47. Ogden, C.L., et al., *Consumption of sugar drinks in the United States, 2005-2008.* NCHS Data Brief, (71): p. 1-8.

48. Pollock, N.K., et al., *Greater fructose consumption is associated with cardiometabolic risk markers and visceral adiposity in adolescents.* J Nutr. **142**(2): p. 251-7.

49. Aeberli, I., et al., *Fructose intake is a predictor of LDL particle size in overweight schoolchildren.* Am J Clin Nutr, 2007. **86**(4): p. 1174-8.

50. Hosseini-Esfahani, F., Z. Bahadoran, and F. Azizi, *Dietary Fructose and Risk of Metabolic Syndrome in Adults: Tehran Lipid and Glucose Study.*

51. Lustig, R.H., et al., *Isocaloric fructose restriction and metabolic improvement in children with obesity and metabolic syndrome.* Obesity (Silver Spring). **24**(2): p. 453-60.

52. Gugliucci, A., et al., *Short-term isocaloric fructose restriction lowers apoC-III levels and yields less atherogenic lipoprotein profiles in children with obesity and metabolic syndrome.* Atherosclerosis.

53. Stanhope, K.L., et al., *Consuming fructose-sweetened, not glucose-sweetened, beverages increases visceral adiposity and lipids and decreases insulin sensitivity in overweight/obese humans.* J Clin Invest, 2009. **119**(5): p. 1322-34.

54. Cox, C.L., et al., *Consumption of fructose-sweetened beverages for 10 weeks reduces net fat oxidation and energy expenditure in overweight/obese men and women.* Eur J Clin Nutr. **66**(2): p. 201-8.

55. Brown, C.M., et al., *Fructose ingestion acutely elevates blood pressure in healthy young humans.* American Journal of Physiology-Regulatory, Integrative and Comparative Physiology, 2008. **294**(3): p. R730-R737.

56. Siegel, B., *Sweet nothing: The triumph of diet soda (Hyman Kirsch).* AMERICAN HERITAGE, 2006. **57**(3): p. 40-49.

57. Storey, M.L., R.A. Forshee, and P.A. Anderson, *Beverage consumption in the US population.* Journal of the American Dietetic Association, 2006. **106**(12): p. 1992-2000.

58. DuBois, G.E. and I. Prakash, *Non-caloric sweeteners, sweetness modulators, and sweetener enhancers.* Annu Rev Food Sci Technol. **3**: p. 353-80.

59. Bucher, T. and M. Siegrist, *Children's and parents' health perception of different soft drinks.* British Journal of Nutrition. **113**(03): p. 526-535.

60. Allen, A.L., J.E. McGeary, and J.E. Hayes, *Rebaudioside A and Rebaudioside D Bitterness do not Covary with Acesulfame-K Bitterness or Polymorphisms in TAS2R9 and TAS2R31.* Chemosensory perception. **6**(3): p. 109-117.

61. Woods, S.C., *The eating paradox: how we tolerate food.* Psychological review, 1991. **98**(4): p. 488.

62. Cooper, S.J., *From Claude Bernard to Walter Cannon. Emergence of the concept of homeostasis.* Appetite, 2008. **51**(3): p. 419-427.

63. Pavlov, I.P. and W.H. Thompson, *The work of the digestive glands*. 1902: Charles Griffin.

64. Keefe, J.H. and D.S. Bell, *Postprandial hyperglycemia/hyperlipidemia (postprandial dysmetabolism) is a cardiovascular risk factor.* The American journal of cardiology, 2007. **100**(5): p. 899-904.

65. Leiter, L.A., et al., *Postprandial glucose regulation: New data andnew implications.* Clinical therapeutics, 2005. **27**: p. S42-S56.

66. Rendell, M.S. and L. Jovanovic, *Targeting postprandial hyperglycemia.* Metabolism, 2006. **55**(9): p. 1263-1281.

67. Teff, K.L., B.E. Levin, and K. Engelman, *Oral sensory stimulation in men: effects on insulin, C-peptide, and catecholamines.* American Journal of Physiology-Regulatory, Integrative and Comparative Physiology, 1993. **265**(6): p. R1223-R1230.

68. Teff, K.L., *How neural mediation of anticipatory and compensatory insulin release helps us tolerate food.* Physiology & behavior. **103**(1): p. 44-50.

69. Teff, K., *Nutritional implications of the cephalic-phase reflexes: endocrine responses.* Appetite, 2000. **34**(2): p. 206-213.

70. Schwartz, T., et al., *Vagal, cholinergic regulation of pancreatic polypeptide secretion.* Journal of Clinical Investigation, 1978. **61**(3): p. 781.

71. Teff, K.L. and K. Engelman, *Oral sensory stimulation improves glucose tolerance in humans: effects on insulin, C-peptide, and glucagon.* Am J Physiol, 1996. **270**(6 Pt 2): p. R1371-9.

72. Lorentzen, M., et al., *Effect of sham-feeding on glucose tolerance and insulin secretion.* Acta endocrinologica, 1987. **115**(1): p. 84-86.

73. Grill, H., K. Berridge, and D. Ganster, *Oral glucose is the prime elicitor of preabsorptive insulin secretion.* American Journal of Physiology-Regulatory, Integrative and Comparative Physiology, 1984. **246**(1): p. R88-R95.

74. Just, T., et al., *Cephalic phase insulin release in healthy humans after taste stimulation?* Appetite, 2008. **51**(3): p. 622-7.

75. Teff, K.L., J. Devine, and K. Engelman, *Sweet taste: effect on cephalic phase insulin release in men.* Physiol Behav, 1995. **57**(6): p. 1089-95.

76. Kun, E. and I. Horvath, *The influence of oral saccharin on blood sugar.* Experimental Biology and Medicine, 1947. **66**(1): p. 175-177.

77. Bruce, D., et al., *Cephalic phase metabolic responses in normal weight adults.* Metabolism, 1987. **36**(8): p. 721-725.

78. Abdallah, L., M. Chabert, and J. Louis-Sylvestre, *Cephalic phase responses to sweet taste.* The American journal of clinical nutrition, 1997. **65**(3): p. 737-743.

79. Carter, J.M., A.E. Jeukendrup, and D.A. Jones, *The effect of carbohydrate mouth rinse on 1-h cycle time trial performance.* Med Sci Sports Exerc, 2004. **36**(12): p. 2107-11.

80. Carter, J.M., et al., *The effect of glucose infusion on glucose kinetics during a 1-h time trial.* Medicine and science in sports and exercise, 2004. **36**(9): p. 1543-1550.

81. Pottier, A., et al., *Mouth rinse but not ingestion of a carbohydrate solution improves 1â€h cycle time trial performance.* Scandinavian journal of medicine & science in sports. **20**(1): p. 105-111.

82. Rollo, I., et al., *The influence of carbohydrate mouth rinse on self-selected speeds during a 30-min treadmill run.* International journal of sport nutrition, 2008. **18**(6): p. 585.

83. Sinclair, J., et al., *The effect of different durations of carbohydrate mouth rinse on cycling performance.* European journal of sport science. **14**(3): p. 259-264.

84. Leturque, A., E. Brot-Laroche, and M. Le Gall, *GLUT2 mutations, translocation, and receptor function in diet sugar managing.* Am J Physiol Endocrinol Metab, 2009. **296**(5): p. E985-92.

85. Shirazi-Beechey, S., et al., *Ontogenic development of lamb intestinal sodium-glucose co-transporter is regulated by diet.* The Journal of Physiology, 1991. **437**: p. 699.

86. Cheeseman, C., *Upregulation of SGLT-1 transport activity in rat jejunum induced by GLP-2 infusion in vivo.* American Journal of Physiology-Regulatory, Integrative and Comparative Physiology, 1997. **273**(6): p. R1965-R1971.

87. Kokrashvili, Z., B. Mosinger, and R.F. Margolskee, *T1r3 and alpha-gustducin in gut regulate secretion of glucagon-like peptide-1.* Ann N Y Acad Sci, 2009. **1170**: p. 91-4.

88. Jang, H.J., et al., *Gut-expressed gustducin and taste receptors regulate secretion of glucagon-like peptide-1.* Proc Natl Acad Sci U S A, 2007. **104**(38): p. 15069-74.

89. Daly, K., et al., *Expression of sweet receptor components in equine small intestine: relevance to intestinal glucose transport.* American Journal of Physiology-Regulatory, Integrative and Comparative Physiology. **303**(2): p. R199-R208.

90. Margolskee, R.F., et al., *T1R3 and gustducin in gut sense sugars to regulate expression of Na+-glucose cotransporter 1.* Proc Natl Acad Sci U S A, 2007. **104**(38): p. 15075-80.

91. Mace, O.J., et al., *Sweet taste receptors in rat small intestine stimulate glucose absorption through apical GLUT2.* J Physiol, 2007. **582**(Pt 1): p. 379-92.

92. Young, R.L., et al., *Disordered control of intestinal sweet taste receptor expression and glucose absorption in type 2 diabetes.* Diabetes. **62**(10): p. 3532-3541.

93. Fu, Z., E. R Gilbert, and D. Liu, *Regulation of insulin synthesis and secretion and pancreatic Beta-cell dysfunction in diabetes.* Current diabetes reviews. **9**(1): p. 25-53.

94. Ajjan, R.A. and K.R. Owen, *Glucokinase MODY and implications for treatment goals of common forms of diabetes.* Current diabetes reports. **14**(12): p. 1-7.

95. MacDonald, P.E., et al., *The multiple actions of GLP-1 on the process of glucose-stimulated insulin secretion.* Diabetes, 2002. **51**(suppl 3): p. S434-S442.

96. NAUCK, M.A., et al., *Incretin Effects of Increasing Glucose Loads in Man Calculated from Venous Insulin and C-Peptide Responses*.* The Journal of Clinical Endocrinology & Metabolism, 1986. **63**(2): p. 492-498.

97. Holst, J.J. and C. Ã˜rskov, *The incretin approach for diabetes treatment modulation of islet hormone release by GLP-1 agonism.* Diabetes, 2004. **53**(suppl 3): p. S197-S204.

98. Knop, F.K., et al., *Reduced Incretin Effect in Type 2 Diabetes Cause or Consequence of the Diabetic State?* Diabetes, 2007. **56**(8): p. 1951-1959.

99. Dotson, C.D., et al., *T1R and T2R receptors: the modulation of incretin hormones and potential targets for the treatment of type 2 diabetes mellitus.* Current opinion in investigational drugs (London, England: 2000). **11**(4): p. 447.

100. Buse, J.B., et al., *Exenatide once weekly versus liraglutide once daily in patients with type 2 diabetes (DURATION-6): a randomised, open-label study.* The Lancet. **381**(9861): p. 117-124.

101. Sharma, S.K., et al., *Teneligliptin in management of type 2 diabetes mellitus.* Diabetes, Metabolic Syndrome and Obesity: Targets and Therapy. **9**: p. 251.

102. Taniguchi, K., *Expression of the sweet receptor protein, T1R3, in the human liver and pancreas.* J Vet Med Sci, 2004. **66**(11): p. 1311-4.

103. Nakagawa, Y., et al., *Sweet taste receptor expressed in pancreatic beta-cells activates the calcium and cyclic AMP signaling systems and stimulates insulin secretion.* PLoS One, 2009. **4**(4): p. e5106.

104. Hamano, K., et al., *Lactisole inhibits the glucose-sensing receptor T1R3 expressed in mouse pancreatic beta-cells.* J Endocrinol, 2015. **226**(1): p. 57-66.

105. Kokrashvili, Z., B. Mosinger, and R.F. Margolskee, *Taste signaling elements expressed in gut enteroendocrine cells regulate nutrient-responsive secretion of gut hormones.* Am J Clin Nutr, 2009. **90**(3): p. 822s-825s.

106. Murovets, V.O., A.A. Bachmanov, and V.A. Zolotarev, *Impaired glucose metabolism in mice lacking the Tas1r3 taste receptor gene.* PLoS One. **10**(6): p. e0130997.

107. Ma, J., et al., *Effect of the artificial sweetener, sucralose, on gastric emptying and incretin hormone release in healthy subjects.* American Journal of Physiology-Gastrointestinal and Liver Physiology, 2009. **296**(4): p. G735-G739.

108. Ford, H., et al., *Effects of oral ingestion of sucralose on gut hormone response and appetite in healthy normal-weight subjects.* European Journal of Clinical Nutrition. **65**(4): p. 508-513.

109. Steinert, R.E., et al., *Effects of carbohydrate sugars and artificial sweeteners on appetite and the secretion of gastrointestinal satiety peptides.* British Journal of Nutrition. **105**(09): p. 1320-1328.

110. Pepino, M.Y., et al., *Sucralose affects glycemic and hormonal responses to an oral glucose load.* Diabetes Care, 2013. **36**(9): p. 2530-5.

111. Brown, R.J., M. Walter, and K.I. Rother, *Ingestion of diet soda before a glucose load augments glucagon-like peptide-1 secretion.* Diabetes Care, 2009. **32**(12): p. 2184-6.

112. Brown, R.J., M. Walter, and K.I. Rother, *Effects of diet soda on gut hormones in youths with diabetes.* Diabetes Care, 2012. **35**(5): p. 959-64.

113. Temizkan, S., et al., *Sucralose enhances GLP-1 release and lowers blood glucose in the presence of carbohydrate in healthy subjects but not in patients with type 2 diabetes.* Eur J Clin Nutr, 2015. **69**(2): p. 162-6.

114. Masubuchi, Y., et al., *A novel regulatory function of sweet taste-sensing receptor in adipogenic differentiation of 3T3-L1 cells.* PLoS One, 2013. **8**(1): p. e54500.

115. Simon, B.R., et al., *Sweet taste receptor deficient mice have decreased adiposity and increased bone mass.* PLoS One, 2014. **9**(1): p. e86454.

116. Smith, K.R., et al., *Disruption of the sugar sensing receptor T1R2 attenuates metabolic derangements associated with diet-induced obesity.* Am J Physiol Endocrinol Metab, 2016: p. ajpendo 00484 2015.

117. Ye, J.M., et al., *Peroxisome proliferator-activated receptor (PPAR)-alpha activation lowers muscle lipids and improves insulin sensitivity in high fat-fed rats: comparison with PPAR-gamma activation.* Diabetes, 2001. **50**(2): p. 411-7.

118. Ratzmann, M.L., et al., *Effects of clofibrate therapy on glucose tolerance, insulin secretion and serum lipids in subjects with hyperlipoproteinemia and impaired glucose tolerance. A follow-up study over a five-year period.* Exp Clin Endocrinol, 1983. **82**(2): p. 216-21.

119. Staels, B., et al., *Mechanism of action of fibrates on lipid and lipoprotein metabolism.* Circulation, 1998. **98**(19): p. 2088-93.

120. Bray, G.A., S.J. Nielsen, and B.M. Popkin, *Consumption of high-fructose corn syrup in beverages may play a role in the epidemic of obesity.* Am J Clin Nutr, 2004. **79**(4): p. 537-43.

121. Johnson, R.J., et al., *Potential role of sugar (fructose) in the epidemic of hypertension, obesity and the metabolic syndrome, diabetes, kidney disease, and cardiovascular disease.* Am J Clin Nutr, 2007. **86**(4): p. 899-906.

122. Zhao, G.Q., et al., *The receptors for mammalian sweet and umami taste.* Cell, 2003. **115**(3): p. 255-66.

123. DuBois, G.E., *Molecular mechanism of sweetness sensation.* Physiol Behav.

124. Jackson, A., *Partial Agonist*, in *Encyclopedia of Psychopharmacology*, I.P. Stolerman, Editor, Springer Berlin Heidelberg: Berlin, Heidelberg. p. 959-960.

125. Frank, R.A., S.J.S. Mize, and R. Carter, *An assessment of binary mixture interactions for nine sweeteners.* Chemical Senses, 1989. **14**(5): p. 621-632.

126. Bartoshuk, L.M., et al., *Valid across-group comparisons with labeled scales: the gLMS versus magnitude matching.* Physiol Behav, 2004. **82**(1): p. 109-14.

127. Green, B.G., G.S. Shaffer, and M.M. Gilmore, *Derivation and evaluation of a semantic scale of oral sensation magnitude with apparent ratio properties.* Chemical Senses, 1993. **18**(6): p. 683-702.

128. Hosseini-Esfahani, F., et al., *Dietary fructose and risk of metabolic syndrome in adults: Tehran Lipid and Glucose study.* Nutrition & Metabolism, 2011. **8**.

129. Brown, C.M., et al., *Fructose ingestion acutely raises blood pressure in healthy young adults.* International Journal of Obesity, 2008. **32**: p. S77-S77.

130. Macdonald, I., *Influence of fructose and glucose on serum lipid levels in men and pre- and postmenopausal women.* Am J Clin Nutr, 1966. **18**(5): p. 369-72.

131. Stanhope, K.L., et al., *Consuming fructose-sweetened, not glucose-sweetened, beverages increases visceral adiposity and lipids and decreases insulin sensitivity in overweight/obese humans.* Journal of Clinical Investigation, 2009. **119**(5): p. 1322-1334.

132. Beck-Nielsen, H., O. Pedersen, and H.O. Lindskov, *Impaired cellular insulin binding and insulin sensitivity induced by high-fructose feeding in normal subjects.* Am J Clin Nutr, 1980. **33**(2): p. 273-8.

133. Havel, P.J., *Dietary fructose: implications for dysregulation of energy homeostasis and lipid/carbohydrate metabolism.* Nutr Rev, 2005. **63**(5): p. 133-57.

134. Samuel, V.T., *Fructose induced lipogenesis: from sugar to fat to insulin resistance.* Trends Endocrinol Metab. **22**(2): p. 60-5.

135. Tappy, L. and K.A. Le, *Metabolic effects of fructose and the worldwide increase in obesity.* Physiol Rev. **90**(1): p. 23-46.

136. Schwarz, J.M., et al., *Effect of a High-Fructose Weight-Maintaining Diet on Lipogenesis and Liver Fat.* J Clin Endocrinol Metab. **100**(6): p. 2434-42.

137. United States. Department of Agriculture. Economic Research Service., *Food consumption, prices, and expenditures*, in *Statistical bulletin*, Economic Research Service: [Washington, D.C.]. p. volumes.

138. Perez, C., F. Lucas, and A. Sclafani, *Increased flavor acceptance and preference conditioned by the postingestive actions of glucose.* Physiol Behav, 1998. **64**(4): p. 483-92.

139. DuBois, G.E. and I. Prakash, *Non-Caloric Sweeteners, Sweetness Modulators, and Sweetener Enhancers.* Annual Review of Food Science and Technology, Vol 3, 2012. **3**: p. 353-380.

140. Majchrzak, D., A. Ipsen, and J. Koenig, *Sucrose-replacement by rebaudioside a in a model beverage.* J Food Sci Technol. **52**(9): p. 6031-6.

141. Sclafani, A., *Gut-brain nutrient signaling. Appetition vs. satiation.* Appetite, 2012.

142. Tordoff, M.G. and A.M. Alleva, *Oral stimulation with aspartame increases hunger.* Physiol Behav, 1990. **47**(3): p. 555-9.

143. Black, R.M., L.A. Leiter, and G.H. Anderson, *Consuming aspartame with and without taste: differential effects on appetite and food intake of young adult males.* Physiol Behav, 1993. **53**(3): p. 459-66.

144. Peters, J.C., et al., *The effects of water and non-nutritive sweetened beverages on weight loss during a 12-week weight loss treatment program.* Obesity (Silver Spring). **22**(6): p. 1415-21.

145. Galindo-Cuspinera, V. and P.A. Breslin, *The liaison of sweet and savory.* Chem Senses, 2006. **31**(3): p. 221-5.

146. Mosinger, B., et al., *Genetic loss or pharmacological blockade of testes-expressed taste genes causes male sterility.* Proc Natl Acad Sci U S A, 2013.

147. Moran, A.W., et al., *Expression of Na+/glucose co-transporter 1 (SGLT1) is enhanced by supplementation of the diet of weaning piglets with artificial sweeteners.* Br J Nutr, 2010. **104**(5): p. 637-46.

148. Nakagawa, Y., et al., *Multimodal function of the sweet taste receptor expressed in pancreatic beta-cells: generation of diverse patterns of intracellular signals by sweet agonists.* Endocr J, 2013. **60**(10): p. 1191-206.

149. Simon, B.R., et al., *Artificial sweeteners stimulate adipogenesis and suppress lipolysis independently of sweet taste receptors.* J Biol Chem, 2013. **288**(45): p. 32475-89.

150. Glendinning, J.I., et al., *The role of T1r3 and Trpm5 in carbohydrate-induced obesity in mice.* Physiol Behav, 2012. **107**(1): p. 50-8.

151. Gerspach, A.C., et al., *The role of the gut sweet taste receptor in regulating GLP-1, PYY, and CCK release in humans.* Am J Physiol Endocrinol Metab, 2011. **301**(2): p. E317-25.

152. Lalloyer, F. and B. Staels, *Fibrates, glitazones, and peroxisome proliferator-activated receptors.* Arterioscler Thromb Vasc Biol, 2010. **30**(5): p. 894-9.

153. Maillet, E.L., R.F. Margolskee, and B. Mosinger, *Phenoxy herbicides and fibrates potently inhibit the human chemosensory receptor subunit T1R3.* J Med Chem, 2009. **52**(21): p. 6931-5.

154. Lawless, H.T., *Evidence for neural inhibition in bittersweet taste mixtures.* J Comp Physiol Psychol, 1979. **93**(3): p. 538-47.

155. Ferrari, C., et al., *Effects of short-term clofibrate administration on glucose tolerance and insulin secretion in patients with chemical diabetes or hypertriglyceridemia.* Metabolism, 1977. **26**(2): p. 129-39.

156. Enger, S.C., et al., *The effect of clofibrate on glucose tolerance, insulin secretion, triglycerides and fibrinogen in patients with coronary heart disease.* Acta Med Scand, 1977. **201**(6): p. 563-6.

157. Ren, X., et al., *Sweet taste signaling functions as a hypothalamic glucose sensor.* Front Integr Neurosci, 2009. **3**: p. 12.

158. Dyer, J., et al., *Intestinal glucose sensing and regulation of intestinal glucose absorption.* Biochem Soc Trans, 2007. **35**(Pt 5): p. 1191-4.

159. Nakagawa, Y., *[Function of sweet taste receptor in pancreatic beta-cells].* Seikagaku, 2011. **83**(7): p. 647-51.

160. Roberts, A., et al., *Sucralose metabolism and pharmacokinetics in man.* Food and chemical toxicology, 2000. **38**: p. 31-41.

161. Fujita, Y., et al., *Incretin release from gut is acutely enhanced by sugar but not by sweeteners in vivo.* American Journal of Physiology-Endocrinology and Metabolism, 2009. **296**(3): p. E473-E479.

162. Kim, U.-k., et al., *Variation in the human TAS1R taste receptor genes.* Chemical Senses, 2006. **31**(7): p. 599-611.

163. Fushan, A.A., et al., *Association between common variation in genes encoding sweet taste signaling components and human sucrose perception.* Chemical Senses: p. bjq063.

164. Pepino, M.Y., *Metabolic effects of non-nutritive sweeteners.* Physiology & behavior. **152**: p. 450-455.

165. Stearns, A.T., et al., *Rapid upregulation of sodium-glucose transporter SGLT1 in response to intestinal sweet taste stimulation.* Annals of surgery. **251**(5): p. 865.

166. Trahair, L.G., et al., *Impact of gastric emptying to the glycemic and insulinemic responses to a 75â€g oral glucose load in older subjects with normal and impaired glucose tolerance.* Physiological reports. **2**(11): p. e12204.

167. Wu, T., et al., *Artificial sweeteners have no effect on gastric emptying, glucagon-like peptide-1, or glycemia after oral glucose in healthy humans.* Diabetes Care. **36**(12): p. e202-e203.

168. Wasserman, D.H., *Four grams of glucose.* American Journal of Physiology-Endocrinology and Metabolism, 2009. **296**(1): p. E11-E21.

169. Exton, J.H. and C.R. Park, *Control of gluconeogenesis in liver. II. Effects of glucagon, catecholamines, and adenosine 3',5'-monophosphate on gluconeogenesis in the perfused rat liver.* J Biol Chem, 1968. **243**(16): p. 4189-96.

170. Hwang, L.-D., et al., *Sweet Taste Perception is Associated with Body Mass Index at the Phenotypic and Genotypic Level.* Age (years). **15**(2.6): p. 25.2-4.

171. Suez, J., et al., *Artificial sweeteners induce glucose intolerance by altering the gut microbiota.* Nature. **514**(7521): p. 181-186.

172. Turnbaugh, P.J., et al., *The effect of diet on the human gut microbiome: a metagenomic analysis in humanized gnotobiotic mice.* Science translational medicine, 2009. **1**(6): p. 6ra14-6ra14.

173. Farhadi, A., et al., *Gas chromatographic method for detection of urinary sucralose: application to the assessment of intestinal permeability.* Journal of Chromatography B, 2003. **784**(1): p. 145-154.

174. Yu, J.H., et al., *Decreased sucrose preference in patients with type 2 diabetes mellitus.* Diabetes research and clinical practice. **104**(2): p. 214-219.

175. Wasalathanthri, S., P. Hettiarachchi, and S. Prathapan, *Sweet taste sensitivity in pre-diabetics, diabetics and normoglycemic controls: a comparative cross sectional study.* BMC endocrine disorders. **14**(1): p. 1.

176. Gondivkar, S.M., et al., *Evaluation of gustatory function in patients with diabetes mellitus type 2.* Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology, 2009. **108**(6): p. 876-880.

177. Lawson, W.B., A. Zeidler, and A. Rubenstein, *Taste detection and preferences in diabetics and their relatives.* Psychosomatic medicine, 1979. **41**(3): p. 219-227.

178. Dotson, C.D., et al., *Bitter taste receptors influence glucose homeostasis.* PloS one, 2008. **3**(12): p. e3974.

179. Young, R.L., et al., *Expression of taste molecules in the upper gastrointestinal tract in humans with and without type 2 diabetes.* Gut, 2009. **58**(3): p. 337-346.

180. Young, R.L., et al., *Disordered control of intestinal sweet taste receptor expression and glucose absorption in type 2 diabetes.* Diabetes. **62**(10): p. 3532-41.

181. Wasalathanthri, S., P. Hettiarachchi, and S. Prathapan, *Sweet taste sensitivity in pre-diabetics, diabetics and normoglycemic controls: a comparative cross sectional study.* BMC Endocr Disord. **14**: p. 67.

182. Yu, J.H., et al., *Decreased sucrose preference in patients with type 2 diabetes mellitus.* Diabetes Res Clin Pract. **104**(2): p. 214-9.

183. Mercola, J., *Sweet Deception: Why Splenda, Nutrasweet, and the FDA may be hazardous to your health*. 2006: Harper Collins.

184. Live, S.O., *A Letter From the Calorie Control Council.*

185. Nettleton, J.A., et al., *Diet soda intake and risk of incident metabolic syndrome and type 2 diabetes in the Multi-Ethnic Study of Atherosclerosis (MESA).* Diabetes Care, 2009. **32**(4): p. 688-694.

186. Fowler, S.P., et al., *Fueling the obesity epidemic? Artificially sweetened beverage use and longâ€term weight gain.* Obesity, 2008. **16**(8): p. 1894-1900.

187. Murovets, V., et al., *The involvement of the T1R3 receptor protein in the control of glucose metabolism in mice at different levels of glycemia.* Journal of evolutionary biochemistry and physiology. **50**(4): p. 334-344.

188. Cox, C.L., et al., *Consumption of fructose-but not glucose-sweetened beverages for 10 weeks increases circulating concentrations of uric acid, retinol binding protein-4, and gamma-glutamyl transferase activity in overweight/obese humans.* Nutrition & metabolism. **9**(1): p. 1.

189. Cox, C.L., et al., *Circulating concentrations of monocyte chemoattractant protein-1, plasminogen activator inhibitor-1, and soluble leukocyte adhesion molecule-1 in overweight/obese men and women consuming fructose-or glucose-sweetened beverages for 10 weeks.* The Journal of Clinical Endocrinology & Metabolism. **96**(12): p. E2034-E2038.

190. Takai, S., et al., *Glucagon-like peptide-1 is specifically involved in sweet taste transmission.* The FASEB Journal. **29**(6): p. 2268-2280.