The Role of Intestinal Sweet Taste Receptors (STRS) in the Regulation of Glucose Absorption: Effects of Short Term High Sucrose Diet (HSD)

Tania Hussain
University of Central Florida, thussain@sgu.edu

Find similar works at: https://stars.library.ucf.edu/honortheses1990-2015

Recommended Citation
https://stars.library.ucf.edu/honortheses1990-2015/1839
THE ROLE OF INTESTINAL SWEET TASTE RECEPTORS (STRS) IN
THE REGULATION OF GLUCOSE ABSORPTION: EFFECTS OF SHORT
TERM HIGH SUCROSE DIET (HSD)

by

TANIA HUSSAIN

A thesis submitted in partial fulfillment of the requirements
for the Honors in the Major Program in Biomedical Sciences
in the College of Medicine
and in The Burnett Honors College
at the University of Central Florida
Orlando, Florida

Fall Term 2014

Thesis Chair: Dr. Robert Borgon
ABSTRACT

Sweet taste receptors are primarily found in the oral cavity of the mammalian species. However, recent studies have shown that sweet taste receptors can be found in extraoral tissues such as the pancreas, intestines, and adipose tissue. Our lab has previously found that sweet taste receptors are down-regulated on the pancreas in the presence of high plasma glucose levels. In order to assess the possibility that sweet taste receptors respond to high levels of glucose by suppressing its expression, we wanted to see if they reacted similarly on the intestines. We found that intestinal sweet taste receptors are down regulated in the presence of a 24 hour high sucrose diet (60% sucrose), and a 7 day high sucrose diet in both wild type (WT) mice on the high sucrose diet, and T1R2-KO (lacking sweet taste receptors) mice. We also examined their glucose excursion levels, and found that these mice are lacking a normal response to dietary glucose via an oral glucose tolerance test (OGTT). This led us to conclude that the mice lacking sweet taste receptor expression exhibit abnormal glucose absorption, possibly indicating that sweet taste receptors regulate glucose absorption in the intestines.
ACKNOWLEDGEMENTS

I would like to express my gratitude to those who have helped make this thesis possible. Thank you to Dr. George Kyriazis for being my mentor throughout this process and helping me tremendously with all aspects of my thesis. From editing my thesis to helping me interpret data, I appreciate all the help he has given me. I would also like to thank Kathleen Smith for helping me perform some of the experiments and for teaching me a great deal of laboratory techniques during my time at the Sanford Burnham Medical Research Institute at Lake Nona. I would also like to thank Dr. Richard Pratley for allowing me to work on this project in his lab, and for taking me in as an intern two and a half years ago. The experience I’ve gained is worth more than I hoped for.

I would also like to thank Dr. Robert Borgon for supporting me during this process, and from the time I sat in his QBM course. I appreciate what he did for me as a student by giving me the opportunity to grow by continuing this thesis, and recognizing that I had what it takes to be a part of his PILOT program. Thank you to those that were also a part of my committee, without Dr. William Self, Dr. Dinender Singla, and Dr. Laurence Vonkalm this thesis would not be possible.

I appreciate all the time they’ve taken out of their busy schedules to review it.

I would like to thank my friend, Anna Cruz, for making the illustrations for this thesis. I appreciate the time she took to try to understand a topic that was foreign to her, and being one of my biggest supporters throughout this process. Thank you to my friend, David Abergel, for supporting me throughout this process. Also, allowing me to repeat and explain this information countless times leading up to my defense. Lastly, I would like to thank all my friends and family members who have helped me get through these past years at the University of Central Florida.
# TABLE OF CONTENTS

## CHAPTER 1: INTRODUCTION

- Taste Receptors on the Tongue .......................................................................................... 1
- STR Ligand Binding and Signaling .................................................................................... 2
- STRs on Extra-oral Tissues ............................................................................................... 4
- STRs in the Intestine .......................................................................................................... 6
  - Intestinal glucose absorption and its regulation ............................................................ 6
  - Digestion of Glucose ....................................................................................................... 6
  - Glucose Transport ........................................................................................................... 7
  - Regulation of intestinal glucose absorption .................................................................. 10
- Overview of Study .............................................................................................................. 14

## CHAPTER 2: RESULTS

- Intestinal Sweet Taste Receptors Are Down-Regulated in Response to Short Term High Sucrose Diet (HSD) .......................................................... 15
- T1R3 Receptor is Down-Regulated in T1R2-KO Mice in Response to Short Term High Sucrose Diet (HSD) ................................................................................. 17
- A Short Term High Sucrose Diet (HSD) in T1R2-KO Mice Does Not Alter Plasma Glucose Excursions during an Oral Glucose Tolerance Test (OGTT) ................................................................................................. 18
- Glucose Transporters are Down-Regulated in T1R2-KO Mice Compared to WT Mice ............. 20
- STR Expression is Suppressed in 7 Day HSD-WT and HSD-T1R2 Mice ..................................... 21
- Long Term (7-Day) HSD Causes Glucose Intolerance in WT and T1R2-KO Mice .................. 23
- Glucose Oxidation May Account for the Reduced Glucose Absorption in WT and T1R2-KO mice on 24 hour HSD ........................................................................................................... 25

## CHAPTER 3: MATERIALS AND METHODS

- Animals and Mouse Intestine Isolation .............................................................................. 27
- Quantitative real-time PCR .............................................................................................. 27
- Oral Glucose Tolerance Tests ......................................................................................... 28
- Surgical Catheterization of Mice ..................................................................................... 28
- Plasma Analysis .............................................................................................................. 29
LIST OF FIGURES

Figure 1 - 1: A representation of the sweet taste receptor. ............................................................. 1
Figure 1 - 2: An Illustration depicting the signaling cascade of a lingual sweet taste receptor.... 4
Figure 1 - 3: Depiction of GLUT2 translocation during fasted (left) and fed (right) states. ....... 8
Figure 1 - 4: The effects that GLP-1 has on various tissues. ....................................................... 12

Figure 2 - 1: mRNA expression of 24 hour HSD fed WT mice.. .................................................. 16
Figure 2 - 2: mRNA expression of 24 hour HSD fed T1R2-KO mice. ........................................ 17
Figure 2 - 3: OGTT performed on T1R2-KO mice on CON and 24 hour HSD. ......................... 18
Figure 2 - 4: Absolute mRNA expression of 24 CON fed WT and T1R2-KO.. ......................... 20
Figure 2 - 5: mRNA expression of 7 day HSD and CON fed WT and T1R2-KO. ...................... 22
Figure 2 - 6: OGTT of 7 day HSD and CON fed WT and T1R2-KO. ................................. 24
Figure 2 - 7: OGTT of 24 hour CON or HSD fed WT and T1R2-KO mice using metabolizable (13C-6 glucose) and nonmetabolizable (3-O-methyl-glucose) glucose analogs.. .................. 26
CHAPTER 1: INTRODUCTION

Taste Receptors on the Tongue

Humans use their taste buds to help them distinguish between the types of food they are consuming, whether it is delicious, toxic, or unpleasant. There are five taste sensations: sour, umami (savory), bitter, salty, and sweet compounds, etc [1, 2] (Figure 1-1). The T1R family is a class-C GPCR and it is responsible for sensing both sweet and umami (i.e. savory tastes of amino acids). The T1R gene family encodes for T1R1 (or TAS1R1), T1R2 (or TAS1R2), and T1R3 (or TAS1R3), which hetero-dimerize to form specific receptors for sweet (T1R2 + T1R3) or umami (T1R1 + T1R3) taste [3]. Sweet taste receptors (STRs), the heterodimer of T1R2 and T1R3, is found on the apical surface of the cell, and allows binding of ligands such as sugar, artificial sweeteners, a subset of D-amino acids, and sweet tasting proteins [4].

Figure 1 - 1: A representation of the sweet taste receptor.
STR Ligand Binding and Signaling

STRs bind a variety of ligands that vary across mammalian species. For example, in rodents, aspartame is not sucrose-like, and they avoid it. Cats don’t express T1R2 because it is deemed a pseudo gene, and in humans the sweet inhibitor, lactisole, is ineffective in the rat. Therefore, when conducting these studies, sugar preferences were taken into account [4].

STRs contain four different ligand binding domains on both the T1R2 and T1R3 subunits. Three noteworthy domains include the large extracellular venus flytrap domain (VFTD), which is linked to the heptahelical transmembrane domain (TMD) via the short cysteine rich domain (CRD)[5, 6]. It has been discovered that the CRD contains nine highly conserved cysteines, and the VFTM contains a conserved cysteine that is important for signal transduction once a ligand is bound [6]. Each domain has been shown to bind to different ligands, such as glucose, fructose, sweet proteins such as brazzein, cyclamate, and some artificial sweeteners. Agonists can bind to the VFD of hT1R2 (human) and stabilize the closed conformation, or the agonist can bind to either the TMD of either T1R2 or T1R3 [5]. Residues located on the CRD of hT1r3 have been found to bind to sweet proteins. There are also positive allosteric modulators (PAMs) that are tasteless for human STRs, but are able to enhance the activity of an orthosteric agonist [5]. These PAMs bind to the TMD of the receptors and can enhance the activity of the receptor binding, and/or affinity of the ligand [5].

Signal transduction begins with the ingestion of chemicals/nutrients (tastants), and a signal cascade is mediated via taste receptor cells responsible for umami, sweet, and bitter taste when bound to its tastant. When taste receptors are stimulated, the cells becomes depolarized.
which leads to increased calcium levels within the cell, which in turn leads to exocytosis of a neurotransmitter and the activation of afferent taste neurons (9).

Ligand binding to STRs activates phosphatidylinositol-4,5-bisphosphate phosphodiesterase beta-2 (PLCβ2) causing the hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP2) to inositol triphosphate (IP3) and diacylglycerol (DAG). IP3 binding to IP3-receptors (IP3-Rs) on the endoplasmic reticulum (ER) stimulates calcium release. Calcium then activates the transient receptor potential cation channel subfamily M member 5 (TRPM5) channels on the cell-surface, allowing sodium influx and cell membrane depolarization. The resulting voltage-dependent calcium channel (VDCC) activation leads to calcium influx, and elevated intracellular calcium concentrations ([Ca^{2+}]_i) triggers exocytosis of ATP. In taste cells, the release of ATP acts as a paracrine signal to gustatory afferent axons (Figure 1-2). Loss-of-function studies targeting TRPM5 or PLCβ2 eliminate all sweet and umami taste responses in mice [7, 8].
Figure 1 - 2: An Illustration depicting the signaling cascade of a lingual sweet taste receptor.

**STRs on Extra-oral Tissues**

STRs have been found in various tissues other than the lingual epithelial cells, such as the nasal epithelium, the enteroendocrine cells of the intestine, the pancreas, adipose tissue, and the
Unlike their known function in the mouth, the functions of STRs in nongustatory tissues are still elusive.

**STRs of pancreatic beta-cells** were found to be activated by fructose and exhibit synergistic effects with glucose to amplify insulin secretion in both mice and human islets [10]. By using T1R2-KO mice, Kyriazis et al. was able to determine that circulating fructose and saccharin (an artificial sweetener) potentiate insulin secretion in mice and in vitro mediated by STRs on beta-cells. A STR inhibitor, lactisole, was used in isolated human islets, showing diminished fructose induced insulin secretion by the human islets [10].

STRs on white adipocytes are expressed during adipogenesis of mouse and human precursors upon treatment of artificial sweeteners such as saccharin and acesulfame potassium (AceK) [11].

STRs on the enteroendocrine cells of the intestines have been found to play a role in both glucose absorption and the incretin effect. STR signaling promotes insertion of GLUT2 to the apical surface of the enterocytes, and up regulation of SGTL-1, which are both glucose transporters [7]. They also promote secretion of GLP-1 and GIP, the two incretin hormones. STRs has been purposed to play a role as a gut carbohydrate sensor [8]. Studies in mice lacking STR signaling show defective GLP-1 secretion in response to luminal glucose, compared to the wild type mice [12]. Collectively, these data suggest that STRs function to coordinate adaptive responses of the intestine, beta cells and adipocytes to changes in nutrient availability, optimizing energy absorption, metabolism and storage.
STRs in the Intestine

Intestinal glucose absorption and its regulation

The gastrointestinal (GI) tract detects many nutrients, non-nutrients, mechanical factors, microorganisms, drugs, and toxic chemicals when ingested. Detection of such factors allows the organ to react appropriately through stimulation of sensory nerves, or hormonal response. These responses help to regulate some of the most important pathways in our system, from nutrient digestion and storage, to the many important metabolic pathways needed to keep our body in homeostasis.

Digestion of Glucose

Glucose is an energy source for all tissues in the body. Therefore, it is important to be properly transported where it is needed. Before their absorption carbohydrates must be digested by salivary and pancreatic amylases, and by disaccharidases on the brush border of the enterocytes of the small intestine. These complex sugars are digested to monosaccharides and now in their proper state to be absorbed by mature enterocytes of the small intestine [13]. Sucrose is a common dietary source of carbohydrates, and is digested to both glucose and fructose by the enzyme sucrase. These monosaccharides are absorbed into the bloodstream via specialized receptors on the brush border membrane facing the lumen. Glucose is a polar molecule that requires specialized receptors in order to cross the lipid bilayer of the enterocytes.
Two receptors that transport glucose across the cell membrane are the sodium-glucose cotransporter-1 (SGLT-1) and the glucose transporter 2 (GLUT2).

**Glucose Transport**

SGLT-1 is the primary glucose transporter across the brush border membrane and into the enterocytes. However, it has been seen that not all glucose transport into the cytoplasm of the cell is halted when SGLT-1 is not present. It has been shown that GLUT2 (a glucose transporter) is also present and brought to the apical membrane of the cell when glucose is sensed inside the cell [14]. SGLT-1 is a low capacity, high affinity transporter, and the only transporter capable of moving glucose against its concentration gradient [15]. SGLT-1 is a symport transporter that is mediated by the energy produced from a Na⁺ electrochemical potential gradient across the brush border membrane. This sodium gradient is produced by a sodium/potassium ATPase at the basolateral membrane of the enterocyte, which is responsible for pumping three sodium ions out of the cells as they are being transported into the cell via SGLT-1. As sodium is being pumped out, two potassium ions are being pumped in to maintain the appropriate potential within the cell.

Two sodium ions are attached to SGLT-1 and allow one glucose molecule to adhere to the transporter. Sodium binding allows conformational change of the protein and subsequent binding of glucose. The molecules are then transported into the cell, and dissociation is promoted by low intracellular sodium concentrations and low affinity sodium and glucose binding within the cell[16]. To keep iso-osmolarity within the cell, as SGLT-1 is importing sodium and glucose,
water is also taken into the cell leading to the conclusion that water absorption is glucose dependent within the upper and mid-intestine [16].

GLUT2 is a facilitative glucose transporter that is mainly found in the intestines, liver, kidney, and pancreatic beta cells and helps mediate glucose homeostasis [17]. GLUT2 is a low affinity, and high capacity glucose transporter, and is responsible for the transportation of glucose between the enterocyte and the plasma. GLUT2 is also apically translocated via glucose-induced membrane depolarization triggered by SGLT-1 [17].

Figure 1 - 3: Depiction of GLUT2 translocation during fasted (left) and fed (right) states.
The large absorptive capacity of glucose into the enterocyte suggests the presence of an additional transporter, since SGLT-1 has low absorptive rates [18]. A study was done to confirm GLUT2’s presence in the apical membrane using phloretin, (GLUT2 inhibitor), and observing a 40% decrease in glucose absorption in rat jejunal brush border membrane [18]. It was also shown that 30% of fructose absorption is also reduced when phloretin was infused in the intestine, indicating that GLUT5 was not the only fructose transporter in the lumen [18]. Prior to a meal, there is a low glucose concentration in the lumen, and SGLT-1 is the only active transporter bringing glucose into the cell. Postprandial glucose absorption varies in the mechanism of which GLUT2 exhibits. Due to SGLT-1’s low capacity binding, saturation of SGLT-1 can take place after a meal. To help transport glucose into the cell, GLUT2 is able to translocate to the apical membrane of the enterocyte when activated. GLUT2 is a reversible receptor that is able to secrete and absorb glucose in and out of the cell. When there is low intracellular glucose concentration, GLUT2 is able to transport glucose back into the enterocyte in the absence of glucose in the gut [16].

GLUT2 is also known to transport galactose and fructose out of the enterocyte and into the blood plasma. However, fructose is mainly transported by GLUT5, another GLUT sugar transporter. GLUT5 is a uniporter that mediates fructose secretion from the enterocyte. Fructose is absorbed passively along the brush border membrane of the intestine. This occurs independently of glucose or galactose absorption [15, 16].
**Regulation of intestinal glucose absorption**

In the gastrointestinal (GI) tract, there are 14 or more different types of enteroendocrine cells [8]. Luminal nutrients such as carbohydrates and bitter tastants are able to stimulate GPCRs on enteroendocrine cells of the GI tract. STRs are expressed on the enteroendocrine K-cells and L-cells [19]. K-cells, located in the upper small intestine, secrete glucose-dependent insulinotropic peptide (GIP). L-cells, located in the lower intestine and colon, are enteroendocrine cells that secrete the hormones glucagon-like peptide-1 (GLP-1), GLP-2, and peptide YY (PYY). These hormones play a role in the gut regulatory system contributing to functions such as regulation of appetite, the incretin effect, and gut motility.

**Hormonal Regulation**

GIP, a 42-amino acid hormone, was formerly known as gastric inhibitory polypeptide due to its inhibitory effect on gastric acid secretion [20]. After secretion from the K cells, it enters the bloodstream and is rapidly degraded by dipeptidyl peptidase-4 (DPP-4). Upon cleavage by DPP-4, GIP loses its insulinotropic effect on the pancreas, which affects the incretin response [20]. It is secreted when glucose is present, but it is also responsive when protein and fats are ingested. It was shown that GIP secretion is more rapid and robust when fats are ingested rather than proteins [21].
GLP-1, a 31 amino acid hormone, is encoded by the proglucagon gene, and shares 50% homology to the glucagon hormone [22]. GLP-1 is released from the L cells of the distal ileum and colon, and is stimulated upon nutrient ingestion. Some functions of this hormone include stimulation of insulin secretion via the beta cells of the pancreas, deceleration of gastric emptying, inhibition of glucagon, stimulation of somatostatin via direct contact with delta cells of the pancreas, stimulation of beta cell proliferation, and inhibition of beta cell apoptosis [22, 23]. GLP-1 stimulates insulin release via the pancreatic beta cells, thus contributing to the incretin effect along with GIP. GLP-1 has a biphasic response, one being an early, rapid (10-15 min) phase which is followed by a longer (15-30 min) phase [22]. The longer phase is believed to be attributed to how long it takes for the nutrients to reach the lower intestine, allowing GLP-1 to be released upon stimulation of its enteroendocrine L cells. Similar to GIP, GLP-1 is also inactivated by DPP-4 in less than two minutes of bioactive GLP-1 circulation [22].
Figure 1 - 4: The effects that GLP-1 has on various tissues.

Glucagon-like peptide 2 (GLP-2) is a 33 amino acid peptide, and is derived from the proglucagon gene [24]. Both GLP-1 and GLP-2 are co-secreted from L cells, and are both degraded by DPP-4 having short circulating half-lives. GLP-2 is released in response to ingestion of carbohydrates and fat, but is not stimulated by protein ingestion [24]. It has been seen that GLP-2 is responsible for causing the increased expression of SGLT-1 by enterocytes via activation of the intestinal sweet taste receptors [25]. The GLP-2 receptors are found on enteric neurons, and have not been found on any epithelial cells [25]. GLP-2 has also been shown to increase the mucosal growth of the intestine [26].
The Incretin Effect

GLP-1 and GIP are incretin hormones that help increase insulin release from the pancreas by acting on the pancreatic islets. Incretin hormones are known to help reduce blood glucose levels by augmenting insulin release. The incretin effect is known as the observation that orally ingested glucose is more effective in stimulating glucose secretion from the pancreas as opposed to intravenously injected glucose [19]. GIP and GLP-1 bind to their receptors found on the pancreatic beta cells, GIPR and GLP-1R, respectively [20]. These G-protein coupled receptors activate adneylate cyclase and subsequently increasing levels of cyclic adenosine monophosphate (cAMP) and stimulating glucose dependent insulin secretion.

Regulation of glucose absorption by STRs

STRs expressed on enteroendocrine cells are believed to be the sugar sensors of the intestine. Margolskee et al showed that the STRs sense luminal glucose concentrations. This causes a signaling cascade involving the STRs, alpha gustducin, and other taste signaling elements to be activated in enteroendocrine cells, leading to the secretion of hormones such as GLP-1 and GIP. He proposes that these hormones are responsible for the up regulation of SGLT-1 expression on the enterocytes via a paracrine effect [7]. Mice that were knocked out for either gustducin or T1R3 failed to increase SGLT-1 expression when fed a high carbohydrate diet [7, 27]. It has also been implicated that GLUT 2 trafficking into the apical surface of the enterocyte is also regulated by STRs in the intestine [27]. The signaling cascade for enteroendocrine cell
hormone release via activation of STRs is thought to be similar to the STRs in the tongue. Sweet tastants bind to the STR causing the G protein βγ subunit stimulation of PLCβ2 mediated synthesis of IP3 leading to Ca\(^+\) release from intracellular stores [28]. Increased intracellular Ca\(^+\) concentrations trigger TRPM5 and subsequent hormone secretion from enteroendocrine K or L cells. These hormones are hypothesized to act on the enteroendocrine cells and promotes up regulation of SGLT-1 expression [29].

### Overview of Study

In a previous study focused on human and mouse islet secretion of insulin, our lab found that islets from mice with hyperglycemia or islets cultured in elevated fasting glucose conditions in vitro exhibit a decrease in STR expression, suggesting a link between alterations in plasma glucose and STR function [30]. The regulatory effects of ambient glucose on islet STRs led us to consider that short term exposure to dietary sugars (i.e. high sucrose) may also alter intestinal STRs expression and function.

It was seen that a short term high sucrose diet (HSD) down-regulates intestinal STRs and sugar transporters (SGLT-1, GLUT2, and GLUT5). mRNA expression data indicated similar phenotypes between the wild type mice fed a high sucrose diet and T1R2 knock-out (T1R2-KO) mice, which showed reduced expression of intestinal STRs and sugar transporters. Glucose absorption was compromised amongst the mice lacking normal STR expression and normal glucose transporter expressions. This indicates that STRs regulate intestinal glucose absorption in response to an oral glucose load.
CHAPTER 2: RESULTS

Intestinal Sweet Taste Receptors Are Down-Regulated in Response to Short Term High Sucrose Diet (HSD)

To test whether or not a short term exposure to dietary sugars alters intestinal STR expression we subjected mice to a diet high in sucrose (60%) for 24 hours. We observed that intestinal STRs (T1R2 + T1R3) are significantly down regulated in mice on a HSD (Figure 2-1). This is apparent throughout the entire small intestine (all three sections). There have been studies done indicating that SGLT-1 and GLUT2 expression is regulated by STRs [14]. Therefore, we checked to see how these receptors are affected by a high sucrose diet as well. SGLT-1 did not change in expression (Figure 2-1). On the other hand, GLUT2, a secondary glucose transporter, expression was suppressed (Figure 2-1), while GLUT5 expression is up-regulated significantly.

Our lab has independently shown that WT mice fed a 24 hour HSD had significant reductions in glucose excursions following an oral glucose tolerance test (OGTT). Plasma insulin levels were undisturbed, eliminating any insulin affects to the lowered glucose excursions.
Figure 2-1: mRNA expression of 24 hour HSD fed WT mice. Gene expression of STRs (T1R2 and T1R3), glucose transporters (SGLT-1 and GLUT2), and fructose transporter (GLUT5) in isolated duodenum (D), jejunum (J), and ileum (I) from WT mice in either control diet (CON; black bars) or high sucrose diet (HSD; gray bars). This data was measured using quantitative real-time RT-PCR. Data is expressed as fold change from control diet (set at value 1) normalized to GAPDH (n=10 mice). Student’s t-test. *p<0.05, **p<0.01, ***p<0.001.
T1R3 Receptor is Down-Regulated in T1R2-KO Mice in Response to Short Term High Sucrose Diet (HSD)

To strengthen the correlation between altered intestinal STR expression and glucose absorption, we tested whether mice lacking taste receptor signaling also have altered glucose absorption if fed a short term CON or HSD. In order to investigate this we collected intestine from mice lacking STRs (T1R2-KO), and assessed their gene expression from genes involved in glucose absorption. We noticed that T1R3 is significantly down-regulated in response to HSD (Figure 2-2). This finding supports the STRs heterodimeric structure [6]. There were no significant changes in glucose transporter SGLT-1, and only minor down regulation of GLUT2 expression in the duodenum (Figure 2-2). Similar to WT mice (Figure 2-1), GLUT5 expression is significantly up regulated (Figure 2-2).

![Figure 2-2: mRNA expression of 24 hour HSD fed T1R2-KO mice.](image)

*Figure 2-2: mRNA expression of 24 hour HSD fed T1R2-KO mice.* Gene expression of STRs (T1R3), glucose transporters (SGLT-1 and GLUT2), and fructose transporter (GLUT5) in isolated duodenum (D), jejunum (J), and ileum (I) from T1R2-KO mice in either control diet (CON; black bars) or high sucrose diet (HSD; gray bars). This data was measured using quantitative real-time RT-PCR. Data are expressed as fold change from control diet (set at value 1) normalized to GAPDH (n=10 mice). Student’s t-test. *p<0.05, **p<0.01, ***p<0.001
A Short Term High Sucrose Diet (HSD) in T1R2-KO Mice Does Not Alter Plasma Glucose Excursions during an Oral Glucose Tolerance Test (OGTT)

We performed an OGTT to assess plasma glucose excursions by T1R2-KO mice fed control diet (CON) or HSD. There are no significant changes in glucose excursions between T1R2-CON and T1R2-HSD (Figure 2-3A). This suggests that the effects of HSD in WT mice on glucose excursions are likely mediated via mechanisms involving STR signaling. These findings are further confirmed using OGTT area under the curve (AUC) calculations comparing WT and T1R2-KO mice on CON and HSD. T1R2-KO mice on either diet have similar AUC during the first 30 minutes of an OGTT to WT mice on HSD (Figure 2-3B). This suggests a HSD regulates STR expression leading to alterations in glucose absorption. Thus, WT mice on HSD mimic the T1R2-KO phenotypic response to glucose consumption, strengthening our hypothesis that STRs regulate glucose absorption.

Figure 2-3: OGTT performed on T1R2-KO mice on CON and 24 hour HSD. 2-3 A: Plasma glucose excursions during an OGTT (1.0 g/kg) in 5-hour fasted T1R2-KO mice fed control diet (T1R2-KO CON; black trace) and high sucrose diet (T1R2-KO HSD; gray trace) (n=8 mice/group). 2-3 B: Plasma glucose area under the curve (AUC) calculated between 0-30 minutes of the OGTT WT and T1R2-KO mice fed either control diet or high sucrose diet.
Glucose Transporters are Down-Regulated in T1R2-KO Mice Compared to WT Mice

Because T1R2-KO mice have altered glucose absorption irrespective of the diet, we compared basal mRNA expression of relevant genes in WT and T1R2-KO mice fed control diet. T1R2-KO mice have significantly lower expression of sugar transporters (i.e. glucose and fructose) compared to WT mice (Figure 2-4). This further exemplifies our conclusions that STRs alter glucose absorption via glucose transporter regulation.

![Figure 2-4: Absolute mRNA expression of 24 CON fed WT and T1R2-KO. Gene expression of STRs (T1R3), glucose transporters (SGLT-1 and GLUT2), and fructose transporter (GLUT5) in isolated duodenum (D), jejunum (J), and ileum (I) from T1R2-KO mice in either control diet (CON; black bars) or high sucrose diet (HSD; gray bars). This data was measured using quantitative real-time RT-PCR. Data are expressed as absolute values normalized to GAPDH (n=10 mice). Student’s t-test. *p<0.05, **p<0.01, ***p<0.001.](image-url)
STR Expression is Suppressed in 7 Day HSD-WT and HSD- T1R2 Mice

Next, we examined whether STR changes persist during longer term dietary interventions. Similar to the 24 hour HSD mice, intestinal gene expression of STRs in mice on a 7-day HSD are significantly lower than CON (Figure 2-5). This suggests that the transcriptional regulation of STR expression in mice fed a HSD persists. We also checked glucose transporter expression to see if long term HSD would affect glucose transporter expression as well. SGLT-1 expression seems to be reduced during a long term HSD feeding (Figure 2-5). This suggests that a long term exposure to glucose is able to down regulate SGLT-1 expression, which was not present during short term diet (Figure 2-1 and 2-2). GLUT2 expression was also marginally down regulated in the ileum of WT mice on the HSD and the jejunum of T1R2-KO mice on HSD (Figure 2-5). Consistent with previous findings (Figure 2-4) T1R2-KO mice express lower amounts of each receptor.
Figure 2-5: mRNA expression of 7 day HSD and CON fed WT and T1R2-KO. Gene expression of STRs and glucose transporters in isolated duodenum (D), jejunum (J), and ileum (I) from WT and T1R2-KO mice on either control diet (CON; black bars) or high sucrose diet (HSD; gray bars) for 7 days. This data was measured using quantitative real-time RT-PCR. Data is expressed as fold change from control diet (set at value 1) normalized to GAPDH (n=10 mice). Student’s t-test. *p<0.05, **p<0.01, ***p<0.001.
Long Term (7-Day) HSD Causes Glucose Intolerance in WT and T1R2-KO Mice

To further investigate the long term effects of a HSD we performed an OGTT on both wild type (WT) mice, and mice lacking STRs (T1R2-KO). WT and T1R2-KO mice on HSD have increased plasma glucose excursions indicating that a week of HSD is adequate to induce glucose intolerance (Figure 2-6A). Plasma glucose area under curve (AUC) calculations confirmed these findings (Figure 2-6B). No significant changes in body weight between the WT and T1R2-KO mice on the HSD were observed (Figure 2-6C). Consistent with the OGTT data, fed plasma glucose was elevated in WT mice on HSD, but these effects were absent in T1R2-KO mice. (Figure 2-6D).
Figure 2-6: OGTT of 7 day HSD and CON fed WT and T1R2-KO. Figure 2-6A: Plasma glucose responses during an OGTT (1.0g/kg) in 5-hour fasted WT and T1R2-KO mice fed control diet (WT-CON and T1R2-CON; black and gray filled trace, respectively) and HSD (WT-HSD and T1R2-HSD); black and gray discontinuous trace, respectively (n=5-6 mice/group). Figure 2-6B: Plasma glucose area under the curve (AUC) calculated for a duration of the OGTT (0-120 min) in WT mice compared to T1R2-KO mice fed either CON or HSD. Student’s t-test, *p<0.05, **p<0.01. Figure 2-6C: Body weight (in grams) comparison between WT and T1R2-KO mice on the high sucrose diet (HSD). Figure 2-6D: Fed plasma glucose of WT mice on CON and HSD, and of T1R2-KO mice on CON and HSD. Student’s t-test, ***p<0.001.
Glucose Oxidation May Account for the Reduced Glucose Absorption in WT and T1R2-KO mice on 24 hour HSD

There is a possibility that glucose is being metabolized within the enterocytes at a higher rate. This would lead to reduced levels of plasma glucose excursions. An oral gavage was performed administering 0.50 g/ kg $^{13}$C-6-glucose (metabolizable) and 0.50 g/kg 3-O-methylglucose (nonmetabolizable) glucose analogs. $^{13}$C-6-glucose in WT mice on HSD exhibit lower rates of excursion, as opposed to the T1R2-KO mice which exhibit no changes between CON and HSD (Figure 2-7A), consistent with previous data (Figure 2-3). Plasma 3-OMG appearance did not change between both genotypes and diets (Figure 2-7B). However, at 0 and 5 minutes, there are significantly lowered levels of 3-OMG being absorbed into the blood, as compared to $^{13}$C-6-glucose excursions (Figure 2-7A-B).
Figure 2-7: OGTT of 24 hour CON or HSD fed WT and T1R2-KO mice using metabolizable (13C-6 glucose) and nonmetabolizable (3-O-methyl-glucose) glucose analogs. Figure 2-7 A: Plasma 13C-6-glucose appearance during an OGTT (0.50 g/kg per analogue) in 5-hour fasted WT and T1R2-KO mice fed control (CON-WT; black trace) or high sucrose diet (HST-WT; gray trace) (n=7-8 mice/group). AUC from 0-30 minutes is represented for each via a bar graph.
CHAPTER 3: MATERIALS AND METHODS

Animals and Mouse Intestine Isolation

All animal experiments were approved by the Institutional Animal Care and Use Committee (IACUC). Mice with a homozygous deletion for the T1R2 gene (kindly provided by Dr. Zuker and back-crossed on the C57BI/6J strain for 9 generations) were used for our T1R2-KO cohort. We used nonlittermate WT mice (C57BI/6J) as controls. Cohorts of 6-12 mice were placed on a 24 hour or 7 day high sucrose diet (60% sucrose D12329, Research diets) or control diet (60% Corn starch D12328, Research diets). Male mice (C57BI/6J or T1R2-KO) between 8-10 weeks of age were acclimatized on the control diet for a week prior to diet switch to HSD or continuation of control diet.

mRNA was extracted from intestinal tissue via TRIzol (Ambion, 15596018) and tested to assess the regulation of genes involved in STR signaling and glucose absorption. Intestinal tissue was extracted under fed conditions. Mice were euthanized via a CO₂ chamber followed by cervical dislocation. The transcription of genes involved in sugar absorption (i.e. SGLT-1, GLUT2, GLUT5) and STR signaling (T1R2, T1R3) were assessed via quantitative real-time PCR.

Quantitative real-time PCR
Total RNA from sections of the duodenum, jejunum, and ileum were isolated and was reverse transcribed to cDNA (1.0µg) using New England BioLabs M-Mulv Reverse Transcriptase Kit (M0253L). Quantitative PCRs were performed on an Eppendorf MasterCycler using iQ SYBR Green from BioRad Laboratories using the protocol and primer sequences.

**Oral Glucose Tolerance Tests**

Oral glucose tolerance tests (OGTTs) were performed in 5-hour fasted mice to measure plasma glucose excursions after the 24 hour or 7 day diet intervention. Administration of glucose (1 g of glucose per 1 kg of body weight) was performed through an oral gavage and blood glucose levels were taken at time points 0, 5, 15, 30, 60, 90, and 120 minutes. Mice gavaged with glucose analogs were administered 1.0 g/kg of $^{13}$C-6-glucose or, for glucose absorption analysis, 0.50 g/kg $^{13}$C-6-glucose and 0.50 g/kg 3-O-methyl-glucose (3-OMG).

**Surgical Catheterization of Mice**

The Cardiometabolic Phenotyping core implanted catheters into the left common carotid artery. They are inserted under isoflurane anesthesia using sterile surgical techniques. The free ends of the catheters were externalized behind the head of the rodent and affixed to a silicone cap.
Plasma Analysis

Blood was collected at time points 0, 5, 15, and 30 minutes during the OGTT for tracer analysis, and 0 and 5 minutes for insulin analysis. The blood was spun down in a centrifuge at 1.5 g for 20 minutes at 4°C. Plasma (upper phase) was collected and sent to the Pharmacology core for plasma analysis of tracers ($^{13}$C-6-glucose and 3-OMG). Insulin levels were also quantified from plasma samples at 0 and 5 minutes by using the Mercodia, Ultra-sensitive mouse insulin Elisa (10-1249-01) kit.
CHAPTER 4: DISCUSSION

Based on our data, this study can help further investigation to elucidate how intestinal STRs regulate glucose absorption. Since homeostatic glucose absorption rates are important to maintain proper metabolic function, it is important to note how dysregulation can play a role in metabolic disease such as type 2 diabetes and obesity. Previous studies have shown that high-fructose consumption has been linked to obesity and the development of adverse metabolic effects [31]. Therefore, as the data presented here has proposed, changes in dietary carbohydrate composition or glycaemia, frequently seen in obesity and diabetes, modulate glucose availability and homeostasis via intestinal and pancreatic regulation of TRs and their signaling [30]. Elucidation of this pathway can help formulate treatments for those with metabolic diseases pertaining to homeostatic glucose concentrations in the future.

In this study we tested the effects of acute (24h) and long-term (7-days) high sucrose diet (HSD) on glucose absorption and homeostasis. Our lab has independently demonstrated that WT mice on a 24 hour HSD exhibit reduced plasma glucose excursions during an oral glucose tolerance test (OGTT). Our mRNA expression data performed on WT mice shows that sweet taste receptors (T1R2 and T1R3) are significantly down regulated after being placed on the HSD. Based on the mRNA expression data (Figure 2-1), we have hypothesized that sweet taste receptors may be linked to reduced glucose absorption responses. HSD in T1R2-KO did not change plasma glucose excursions during an OGTT (Figure 2-2A), further supporting the link between glucose absorption and STR regulation. A previous study suggested that intestinal T1R2
signals proper regulation of glucose absorption via SGLT-1 up regulation [32]. However, our 24 hour HSD-WT expression data suggests that there is no change in SGLT-1 expression (Figure 2-1C). We observed that the secondary glucose transporter, GLUT2, was also down-regulated (Figure 2-1D). This has led us to believe that there may be a mechanism in which basal SGLT-1 expression is regulated by another pathway besides intestinal STRs signaling [33].

Although up regulation of SGLT-1 and GLUT2 has been reported previously with intestinal STR signaling [14, 25], we see no significant up regulation of SGLT-1 expression in our data. There is significant up regulation of GLUT5 in the WT mice fed HSD and the T1R2-KO fed HSD (Figure 2-1 and 2-2), which is likely due to increase dietary fructose (sucrose is a disaccharide of glucose and fructose monomers).

T1R2-KO mice express lower levels of the STR subunit T1R3 and sugar transporters (SGLT-1, GLUT2 and GLUT5) suggesting that STRs regulate these receptors (Figure 2-4). There have been studies done demonstrating STR regulation of glucose transporter SGLT-1, concluding that STRs signal an increase in SGLT-1 expression [7, 25]. STRs stimulate enteroendocrine hormone secretion of GLP-1, GLP-2, and GIP, which in turn helps stimulate SGLT-1 and GLUT2 expression [18, 25]. In order to mimic these stimulatory effects, we fed mice a diet high in carbohydrates to stimulate STRs with luminal sugars. However, we observe no significant mRNA changes in SGLT-1 between diets. Nevertheless, T1R2-KO mice suggest that STRs may regulate GLUT2 and GLUT5 expression, suggesting its role in glucose absorption and homeostasis.
After 7 day HSD both genotypes exhibited glucose intolerance (Figure 2-6A). These findings are similar to a study done showing that chronic HSD leads to glucose intolerance [34]. Unfortunately, glucose intolerance leads to many other pathway alterations, making it difficult to study glucose absorption directly. However, most of the mRNA changes in each receptor persist (Figure 2-5). Interestingly, the T1R2-KO mice exhibited a down-regulation in GLUT5 expression on a CON diet, indicating its basal expression (Figure 2-5). It has been found that fructose binds to T1R3, possibly have a similar signaling cascade for GLUT5 [35]. This led us to think that fructose transport may also be mediated by STRs in the intestine. T1R2-KO mice, under fed conditions, exhibit less glucose as opposed to its WT counterparts (Figure 2-6D). This may be due to reduced fructose transport since GLUT5 is down regulated, preventing hyperglycemia for mice on a HSD. This led to the hypothesis that STRs may mediate GLUT5, protecting the blood from glucose concentration imbalances. This must be investigated further.

An alternative pathway that may occur in mice lacking STR regulation is higher rates of glucose oxidation within the enterocytes, which may account for lower glucose excursions. Using an oral mix of equal amounts of the non-metabolizable 3-O-methylglucose and the metabolizable $^{13}$C-6-glucose we measured their rate of appearance in the circulation for direct comparisons. This way we can indirectly assess the relative contributions of glucose transport and glucose metabolism by the enterocytes. Plasma $^{13}$C-6-glucose appearance was reduced in WT-HSD mice and in T1R2 mice compared to WT-CON, confirming our previous OGTT data. However, no diet or genotype differences were observed in 3-OMG excursion into the blood, supporting the possibility that enterocytes of mice lacking STR signaling metabolize glucose at higher rates than WT mice.


Limitations

There are various limitations to this study that could be addressed in the future. (i) We did not quantify protein expression from both our WT and T1R2-KO mice on HSD. Although mRNA expression is a good indicator of the amount of potential protein that is being made, there may be an absence in the correlation between the mRNA expression levels compared to protein expression levels. One reason for this is the possibility of varied post-transcriptional mechanisms that are involved in turning mRNA into protein, therefore limiting our guarantee in protein quantification via mRNA concentrations [36]. (ii) We were unable to look for plasma GLP-1 and GLP-2 from collected blood samples during an OGTT and on fed mice, which may or may not have strengthened the correlation between STR regulations on glucose absorption via hormone stimulatory effects on glucose transporters [25]. (iii) There may also be variability in the diet response due to the amount of food intake in CON vs HSD mice. This variation can lead to differences in mRNA expression due to glucose load. (iv) In order to assess this hypothesis in a more controlled environment, studies of in vitro glucose absorption would have been ideal. Finally, (v) checking to see if the glucose not being absorbed was excreted from the body as waste via fecal glucose content analysis.

Summary

This study examined the effects of STR dysregulation on glucose absorption from the enterocytes to the blood. It also examined the response that a diet high in carbohydrates had on intestinal STR regulation. From our findings we can conclude that intestinal STRs are down regulated in response to a high sucrose diet. Along with diet induced suppression of STRs and
genetically ablated STR (T1R2-KO) mice, we noticed that STRs play a role in glucose regulation into the blood. Along with data indicating lower glucose excursions, we showed lower mRNA expression of sugar transporters responsible for glucose absorption.
APPENDIX A:
IACUC PROTOCOL APPROVAL FORM
I, Tanja Hussain, have received the following training and assume responsibility for animal well-being as part of the animal program at Sanford-Burnham and as approved personnel listed on the protocol(s) below.

Principal Investigator Name: Richard Pratley

IACUC Protocol(s) #: 2012-0081

* Personnel must be listed on an IACUC approved protocol before handling animals or receiving access to the Vivarium. Personnel cannot be added to an approved protocol prior to completing required online AALAS training courses.

Animal Facility Orientation Part 1

- [ ] New
- [ ] Refresher

Safety Training
- [ ] General Safety
- [ ] Chemical Safety
- [ ] Biological Safety
- [ ] Radiation Safety (as needed)

(Signature of EH&S Trainer) [Signature]
(Date) [9/6/12]

Animal Facility Orientation Part 2

- [ ] New
- [ ] Refresher

- [ ] Power Point Animal Facility Part 2
- [ ] Facility Tour
- [ ] Microisolator Training

(Signature of Project Manager/Designee) [Signature]
(Date) [9/29/12]

Return completed form to IACUC/Compliance Coordinator

(Signature of IACUC/Compliance Coordinator) [Signature]
(Date) [10/9/12]
APPENDIX B:
AALAS ANIMAL CARE AND USE COURSES TRANSCRIPT
<table>
<thead>
<tr>
<th>Course Title</th>
<th>Course Initiation</th>
<th>Course Completion</th>
<th>Lessons Remaining</th>
<th>Score (%)</th>
<th>Exam Passed</th>
<th>CEUs Earned</th>
</tr>
</thead>
<tbody>
<tr>
<td>Laboratory Animal Allergy</td>
<td>8/30/2012</td>
<td>8/30/2012</td>
<td></td>
<td>100</td>
<td>8/30/2012</td>
<td>0.75</td>
</tr>
<tr>
<td>exam certificate</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>completion certificate</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Post-Procedural Care of Mice and Rats in Research: Minimizing Pain and Distress</td>
<td>8/30/2012</td>
<td></td>
<td>1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15</td>
<td>93</td>
<td>8/30/2012</td>
<td></td>
</tr>
<tr>
<td>exam certificate</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>completion certificate</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Working with the IACUC: non-VA version</td>
<td>8/27/2012</td>
<td>8/27/2012</td>
<td></td>
<td>100</td>
<td>8/27/2012</td>
<td></td>
</tr>
<tr>
<td>exam certificate</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>completion certificate</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Working with the Laboratory Mouse</td>
<td>8/27/2012</td>
<td>8/28/2012</td>
<td></td>
<td>88</td>
<td>8/28/2012</td>
<td></td>
</tr>
<tr>
<td>exam certificate</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>completion certificate</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
APPENDIX C:
LABORATORY ANIMAL ALLERGY COMPLETION CERTIFICATE
This certifies that on
August 30, 2012

Tania Hussain
of
SANFORD-BURNHAM MEDICAL RESEARCH
INSTITUTE at Lake Nona

completed the course and passed the examination for the

Laboratory Animal Allergy
and earned 0.75 CEU on the

AALAS Learning Library

www.aalaslearninglibrary.org

Exam #: 2491571

https://www.aalaslearninglibrary.org/members/personalaccess/certificate.asp?strKeyID=4...

8/30/2012
APPENDIX D:
POST-PROCEDURE CARE OF MICE AND RATS IN RESEARCH COMPLETION CERTIFICATE
This certifies that on
August 30, 2012

___

Tania Hussain
of
SANFORD-BURNHAM MEDICAL RESEARCH
INSTITUTE at Lake Nona

___

passed the examination for the
Post-Procedure Care of Mice and Rats in
Research: Minimizing Pain and Distress

on the
AALAS Learning Library

Exam #: 2491487

www.aalaslearninglibrary.com

https://www.aalaslearninglibrary.org/members/personalaccess/certificateexam.asp?strKey1... 8/30/2012
APPENDIX E:
WORKING WITH THE IACUC COMPLETION CERTIFICATE
This certifies that on
August 27, 2012

Tania Hussain
of
SANFORD-BURNHAM MEDICAL RESEARCH
INSTITUTE at Lake Nona

completed the course and passed the examination for the
Working with the IACUC
on the
AALAS Learning Library

Exam #: 2488282

https://www.aalaslearninglibrary.org/members/personalaccess/certificate.asp?strKeyId=60... 8/27/2012
APPENDIX F:
WORKING WITH THE LABORATORY MOUSE COMPLETION CERTIFICATE
This certifies that on
August 28, 2012

Tania Hussain
of
SANFORD-BURNHAM MEDICAL RESEARCH
INSTITUTE at Lake Nona

completed the course and passed the examination for the
Working with the Laboratory Mouse
on the
AALAS Learning Library

Exam #: 2489213

https://www.aalaslearninglibrary.org/members/personalaccess/certificate.asp?strKeyID=97... 8/28/2012
LIST OF REFERENCES


