The Role of the Fatty Acid Signaling Pathway in Dietary-Induced Obesity

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THE ROLE OF THE FATTY ACID SIGNALING PATHWAY IN
DIETARY-INDUCED OBESITY

by

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ABSTRACT

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Utah State University, 2017

Major Professor: Timothy A. Gilbertson
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In recent years, evidence has accumulated that alludes to dietary fat being capable of activating taste receptor cells and eliciting a unique chemosensory experience, i.e., a taste of fat. Fatty acids, the chemical cue found in dietary fat, have been shown to activate a G-protein coupled receptor pathway, very similar to known transduction pathways for sweet, bitter, and umami taste stimuli. In this pathway, the cation channel TrpM5 is of crucial importance; it is responsible for cellular depolarization, which is a necessary prerequisite to the cellular response. Based on the importance of TrpM5’s role in the fatty acid (FA) signaling pathway, in this current study, mice lacking the TrpM5 gene (TrpM5-/-; KOs) were used to elucidate the role that TrpM5, and the FA signaling pathway in general, have on detection and intake of FAs, as well as weight gain and fat absorption on a high fat diet. These studies show that TrpM5, and by extension, the entire signaling pathway for FAs, is essential for detection of FAs in the oral cavity. Additionally, it’s shown that male TrpM5-/- mice consume significantly less calories and gain less weight than wildtypes (WTs) when placed on a high fat diet.
This current study also shows that the TrpM5 FA pathway is specific to long-chain polyunsaturated fatty acids (PUFA). This was seen by altering the FA profile of the high fat diet, from a 1:1 ratio of unsaturated to saturated fat, to primarily saturated or primarily unsaturated high fat diets. Increased phenotypic responses, (increased divergences between caloric intake and weight gain between WT and KO mice) were seen on the unsaturated high fat diet while decreased phenotypic responses were seen on the saturated high fat diet. However, these differences were only seen in males. In females, KOs do not show decreased intake on any diet, yet still show decreased weight gain, illuminating a sex-specific response at the level of intake. Finally, this study shows that KO mice, male and female, excrete less lipids in their feces than WTs, suggesting that lack of TrpM5 is not causing a deficit in fat absorption.
PUBLIC ABSTRACT

The Role of the Fatty Acid Signaling Pathway in Dietary-Induced Obesity

Melissa N. Nelson

In recent years, dietary fat has been shown to be capable of activating taste receptor cells in the tongue. Fatty acids (FAs), which act as the chemical cue and are found in dietary fat, activate a cellular signaling pathway that results in a unique signal being sent to the brain that is then interpreted as the taste of fat. One important element in this pathway is the ion channel TrpM5. It is responsible for depolarizing the taste cells that are activated by fatty acids; depolarization is an essential step in cellular response, making TrpM5 essential in the functioning of the FA signaling pathway.

To study the potential roles of the FA signaling pathway, a mouse model, in which mice lacked the TrpM5 gene (TrpM5−/−), was used. From this model, I show that TrpM5 is essential for detection of fatty acids in the oral cavity; without TrpM5, mice were not able to detect FAs in the mouth. I also show here that TrpM5−/− mice eat significantly less and gain significantly less weight on a high fat diet than wildtype mice, who have the TrpM5 gene, linking TrpM5 to both fat intake and weight gain. Interestingly, these responses are only seen in male mice. Females lacking TrpM5 show no deficit in calorie intake compared to the wildtype females. Despite taking in the same amount of calories as the wildtype females, TrpM5−/− females still gain significantly less weight than the wildtypes. This posits a sex-specific response in terms of calorie intake on a high fat diet. Additionally, I show that the TrpM5 pathway is specific for a subtype
of fatty acids, primarily the long-chain polyunsaturated fatty acids (PUFAs) and does not contribute to saturated fatty acid taste transduction. Lastly, in this study I show that both male and female mice who do not have TrpM5 excrete significantly less lipids in their feces than the wildtype mice; surprisingly not implicating TrpM5 in fat malabsorption. We are currently looking for other roles of TrpM5 in fat metabolism.
I would like to thank, first and foremost, my advisor Dr. Timothy Gilbertson. I am grateful that he took a chance on me and allowed me to work with him as an undergraduate, an employee, and finally as a graduate student. I also want to thank him for all of his helpful insight and guidance throughout this entire process. It was a great opportunity to work in his lab, learn from him, and practice how to be a productive scientist. Additionally, I want to acknowledge Dr. Frank Messina and Dr. Robert Ward for agreeing to be on my committee and providing their knowledge and feedback to my experiments. I appreciate the input and advice they were always willing to offer.

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<td>Adenosine diphosphate</td>
</tr>
<tr>
<td>ATP</td>
<td>Adenosine triphosphate</td>
</tr>
<tr>
<td>CCK</td>
<td>Cholecystokinin</td>
</tr>
<tr>
<td>CD36</td>
<td>Cluster of Differentiation 36</td>
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<tr>
<td>CS</td>
<td>Conditioned Stimulus</td>
</tr>
<tr>
<td>CTA</td>
<td>Conditioned Taste Aversion</td>
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<td>DAG</td>
<td>Diacylglycerol</td>
</tr>
<tr>
<td>DRK</td>
<td>Delayed-rectifying Potassium</td>
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<tr>
<td>EEC</td>
<td>Enteroendocrine Cells</td>
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<tr>
<td>ENaC</td>
<td>Epithelial sodium channel</td>
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<tr>
<td>g</td>
<td>Grams</td>
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<td>GLAST</td>
<td>Glutamate transporter</td>
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<tr>
<td>GLP-1</td>
<td>Glucagon-like peptide-1</td>
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<tr>
<td>GPCR</td>
<td>G-protein coupled receptor</td>
</tr>
<tr>
<td>IP$_3$</td>
<td>Inositol 1, 4, 5-triphosphate</td>
</tr>
<tr>
<td>IP$_3$R3</td>
<td>Inositol 1, 4, 5-triphosphate receptor 3</td>
</tr>
<tr>
<td>kCal</td>
<td>Kilocalories</td>
</tr>
<tr>
<td>KO</td>
<td>Knock-out</td>
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<tr>
<td>LA</td>
<td>Linoleic acid</td>
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<tr>
<td>LiCl</td>
<td>Lithium Chloride</td>
</tr>
<tr>
<td>mg</td>
<td>Milligrams</td>
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<tr>
<td>mL</td>
<td>Milliliters</td>
</tr>
<tr>
<td>mM</td>
<td>Millimolar</td>
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<tr>
<td>NaCl</td>
<td>Sodium Chloride</td>
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<tr>
<td>NCAM</td>
<td>Neural adhesion molecule</td>
</tr>
<tr>
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<td>Description</td>
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<td>PIP&lt;sub&gt;2&lt;/sub&gt;</td>
<td>Phosphatidylinositol 4, 4- biphosphate</td>
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<td>PKD1L3/PKD2L1</td>
<td>Polycystic kidney disease 1-like protein 3/ 2-like protein 1</td>
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<td>PLCβ2</td>
<td>Phospholipase C β2 isoform</td>
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<td>PUFA</td>
<td>Polyunsaturated fatty acid</td>
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<td>RT-PCR</td>
<td>Real-time Polymerase Chain Reaction</td>
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<td>SCFA</td>
<td>Short-chain fatty acid</td>
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<td>SEM</td>
<td>Standard error of the mean</td>
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<td>Synaptosomal-associated protein 25</td>
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<td>Secretin tumor cell line-1</td>
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<td>TM</td>
<td>Transmembrane</td>
</tr>
<tr>
<td>TRC</td>
<td>Taste receptor cell</td>
</tr>
<tr>
<td>T1R/T2R</td>
<td>Taste receptor type 1/2</td>
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<tr>
<td>TRP</td>
<td>Transient Receptor Potential</td>
</tr>
<tr>
<td>TrpM</td>
<td>Transient Receptor Potential- Melastatin subfamily</td>
</tr>
<tr>
<td>μL</td>
<td>Microliter</td>
</tr>
<tr>
<td>μM</td>
<td>Micromolar</td>
</tr>
<tr>
<td>VCAM</td>
<td>Voltage-modulate, Ca&lt;sup&gt;2+&lt;/sup&gt;-activated monovalent-specific</td>
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<tr>
<td>WT</td>
<td>Wild-type</td>
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CHAPTER I

LITERATURE REVIEW

The mammalian taste system has two specific roles. The first being to recognize beneficial nutrients, from simple ions to complex molecules, such as sweet, salty, or umami stimuli. The second role is to detect and avoid noxious or toxic compounds, usually bitter or sour tastants. Though this detection can be aided by smell and visual cues, the final quality check on food before its ingested is dependent on the chemical interactions that take place in the mouth. These chemoreceptive events can be anything from changes in membrane area, ligands binding to a receptor, or membrane potential changes due to interactions with ion channels. Through whichever of these pathways this nutrient recognition is achieved, the compounds are then recognized as either beneficial or harmful. The taste system must then relay this information to the central nervous system (Herness and Gilbertson, 1999; Lindemann, 2001).

The peripheral taste system is able to recognize nutrients as harmful or nutritive. In addition, it is also able to discriminate different taste qualities; i.e., which of the five basic taste modalities—sweet, salty, umami, bitter, or sour is being presented. There are two different theories on how this takes place: the “across-fibre pattern” and the “labelled line” theories. The “labelled line” view states that taste receptor cells are tuned to respond to only one single taste modality, such as sweet taste. These taste receptor cells (TRCs) are innervated by individually tuned nerve fibers. Therefore, each TRC and its nerve fibers respond to the same tastant, and only that one tastant. The other theory, the
“across-fibre pattern” view is actually comprised of two contrasting models. The first model states that instead of TRCs being tuned to one tastant, they are tuned to multiple tastants and they are innervated by fibers that can carry information for multiple taste modalities. The second model is amenable to the idea that TRCs are tuned for only one taste quality, but the afferent nerve fibers are capable of carrying information for multiple taste qualities. While there is evidence for both theories, the “across-fibre pattern” theory is more prominent (Chandrashekar et al., 2006; Huang et al., 2006).

**Taste Receptor Cells**

On the mammalian tongue, taste receptor cells (TRCs) are found clustered within taste buds, with about 50-150 TRCs found within a single taste bud. These onion-shaped taste buds have a pore at the top where interactions with tastants occur. Taste buds are organized and distributed in groups, known as papillae. There are four groups of papillae on the tongue: the fungiform, foliate, and circumvallate papillae are sensory in nature, while the non-sensory filiform papillae play roles in more mechanical aspects related to food intake. The circumvallate papillae are located at the back of the tongue, the foliate papillae are on the posterior lateral edge, and the fungiform papillae are located near the tip of the tongue. Within each of these chemosensory papillae, there are varying numbers of taste buds, ranging from a few single taste buds to hundreds to thousands, correlating with the papillae group, i.e., fungiform, foliate, or circumvallate respectively (Chandrashekar et al., 2006). In addition to being on the tongue, taste buds are also found
in the soft palate, epiglottis, pharynx, and the larynx (Breslin and Specter, 2008; Roper, 2013).

Research has elucidated that there are three types of taste receptor cells; Type I, Type II, and Type III. Type I taste cells are referred to as the glial-like cells because of their similarities to neural glial cells; they have extended lamellae that surround and support other cells and also they express the glutamate aspartate transporter (GLAST) which is expressed in glial cells in the brain. GLAST is involved in the reuptake of glutamate, which removes it from the extracellular space and takes it into the cell. This implies that Type I cells are capable of regulating the molecular environment found in the taste bud. Type II cells are known as receptor cells and they are the primary sensors for taste stimuli. They express GPCRs, T1R, T2R, alpha-gustducin, PLCβ2, IP3R3, and TrpM5, all elements necessary for sweet, bitter, umami and fat taste transduction. Both Type I and Type II TRCs also express voltage-gated Na+ and K+ channels. This allows for the firing of action potentials and the release of neurotransmitters, one of which is adenosine-triphosphate (ATP). Protein pannexin-1 hemi-channels act as a pathway for ATP to be released from the taste receptor cells and transmit taste information (Murata et al., 2010; Kinnamon and Finger, 2013).

Type III cells are considered presynaptic cells because they make conventional synapses with afferent nerve terminals. Additionally, they express a neural adhesion molecule (NCAM), proteins associated with neurotransmitter release, such as synaptosomal-associated protein 25 (SNAP-25) and synapsin-II, as well as neurotransmitter-synthesizing enzymes for serotonin, ATP, and other neurotransmitters
(Yee et al., 2001; Katoaka et al., 2008; DeFazio et al., 2006). ATP released from Type I
and Type II TRCs can interact with and excite the Type III cells which allows for the
cellular response of neurotransmitter release and signal transduction to higher cellular
locations (Huang et al., 2009; Kinnamon and Cummings, 1992; Ruiz et al., 2001).
Because of these similarities with neuronal cells, it is believed that Type III taste
receptors cells main function is to transduce sensory information received by the taste
receptor cells (cf. Fig. 1).

**Gustatory Synapses**

Once a taste receptor cell recognizes and responds to a taste quality, this sensory
information must be relayed to nerve fibers and eventually to the brain. Taste responses
are initiated when tastants interact with receptors on the apical membrane of TRCs. This
interaction causes a change in membrane potential, depolarization, and a Ca\(^{2+}\) influx
which leads to neurotransmitter release onto gustatory afferent neurons. TRCs can also
express voltage-gated Na\(^+\) and K\(^+\) channels which allows for action potentials to fire and
neurotransmitter release as well. These gustatory afferent neurons make synapses with
the facial, glossopharyngeal, or vagus nerve fibers (Finger et al., 2000; Yarmolinsky et
al., 2009). There are believed to be a variety of neurotransmitters that are involved in
signal transmission between these taste cells and the afferent nerve fibers. While it
remains an open area of research, serotonin, norepinephrine, ATP, glutamate,
acetylcholine, and others have been hypothesized to act as neurotransmitters for gustatory
cells (Chadhauri and Roper, 2010).
Figure 1. The three types of taste receptor cells and ion channels expressed in the oral cavity. Taken from Chadhauri and Roper, 2010.

Basic Taste Qualities

There are many chemical stimuli that TRCs are exposed to and must respond to, and several different transduction mechanisms have been proposed for how this is done. Primarily, it is proposed that all of the basic taste qualities are transduced through either
ion channel, or G-protein coupled receptor (GPCR) mechanisms. Salty and sour tastants are hypothesized to be transduced by ion channels, while sweet, bitter, umami, and fat stimuli utilize GPCRs for taste transduction.

**Salty Taste Transduction**

Salty taste, typically characterized by Na\(^+\) ions, is thought to be mediated by an ion channel in the family of amiloride-sensitive epithelial-type sodium channels, or simply called ENaC (Heck et al., 1984; Lyall et al., 2004). While ENaC plays an important role in salt taste perception, it is responsible for only part of the total sodium transduction pathway in humans, suggesting that there may be another component in the salt transduction mechanism (Gilbertson et al., 2000). Recent research has provided evidence that these ENaCs are present in taste receptor cells, epithelial cells in the kidney, bladder, intestines, and colon and are believed to play a major role in the detection of salt (Loffing et al., 2000). ENaC is crucial in regulating salt reabsorption; it helps control overall salt and water homeostasis in an organism, and it also contributes to blood pressure (Alvarez de la Rosa, 2000). Type I taste receptor cells specifically express ENaC, suggesting that these type I taste cells are directly involved in salt taste transduction (Vandenbeuch et al., 2008).

**Sour Taste Transduction**

Similar to salty taste transduction, sour taste transduction is also believed to be mediated through ion channels. Though much remains uncertain, two possible channels
belonging to the transient receptor potential (TRP) family have been named as possible candidates for sour taste transduction; PKD1L3 and PKD2L1. These two channels, when coexpressed, are activated by various acidic stimuli and are not activated by any other taste stimuli. These two channels are expressed in certain taste cells, primarily type III presynaptic cells (Huang et al., 2006; Ishimaru et al., 2006; Katoaka et al., 2008). A study by Huang et al. further validated this by exposing isolated presynaptic cells to acetic acid, a common sour stimulus. They found that the presynaptic cells responded to the sour stimulus by releasing the neurotransmitter serotonin (Huang et al., 2008). Therefore, it is hypothesized that PKD1L3 and PKD2L1 are plausible receptors for sour taste transduction.

**Sweet, Bitter, and Umami Taste Transduction**

Sweet, bitter, and umami taste stimuli are all mediated by G-protein coupled receptors (GPCRs). Sweet and umami taste is mediated by the T1R family, while bitter taste is mediated by the T2R family of G-protein coupled receptors (GPCRs; Chandrashekhar et al., 2000; Li et al., 2002; Meyerhof 2005; Nelson et al., 2001; Oike et al., 2006; Zhao et al., 2003). Ligands (tastants) bind to their respective GPCR and cause a conformational change to the receptor that activates the alpha portion of the GPCR: in taste that portion is alpha-gustducin. Alpha-gustducin is considered very important in bitter and sweet taste (Caicedo et al., 2003). A study by He et al. showed mice lacking alpha-gustducin had diminished responses to many bitter and sweet tastants (He et al., 2004), thereby supporting a critical role of the protein. Alpha-gustducin activation leads
to a cascade of second messenger events. Initially, alpha-gustducin activates phospholipase C beta 2 (PLCβ2), which acts as a catalyst to phosphorylate phosphatidylinositol-4,5-bisphosphate (PIP2) into inositol triphosphate (IP3) and diacylglycerol (DAG). IP3 goes on to bind to its receptor, IP3R3, which causes an increase in intracellular calcium via endoplasmic reticulum calcium store release, which further leads to the activation of a channel known as TrpM5 that depolarizes the taste cell. This allows for subsequent neurotransmitter release and cellular response (Gilbertson et al., 2010; Sclafani et al., 2007).

**Fat Taste Transduction**

Until fairly recently, fat was considered tasteless. Its detection was believed to be due to its unique texture, olfactory cues, and post-ingestive effects. However, it has recently been theorized that fats do have a detectable taste, possibly leading to the addition of a sixth basic taste quality. In order to address this, researchers have eliminated all of these known cues for fat - texture, smell, and post-ingestive effects - and discovered that rats were still capable of recognizing the presence of fat and even showed a preference for it (Fukuwatari et al., 2003). Therefore, it is established that there must be some palatable and detectable taste for fat. Research has shown that free fatty acids, such as linoleic acid, found in dietary fat are capable of activating taste cells (Gilbertson, 1998; Shah et al., 2012). Free fatty acids are usually found grouped as triglycerides. Triglycerides are composed of three fatty acids bound together by a glycerol backbone. Triglycerides are ingested from our food and quickly degraded in our mouths by lingual
lipase into free fatty acids. Fatty acids can exist in a wide variety of sizes, ranging from 2-22 carbon atoms. These fatty acids then go on to interact with and activate our taste receptor cells (Gilbertson et al., 1997).

Fat taste is believed to be transduced in a manner very similar to sweet, umami, and bitter tastants, utilizing a G-protein coupled receptor (GPCR) pathway (Liu et al., 2011). While much is still uncertain about this pathway, it is hypothesized that fatty acids activate a specific G-protein coupled receptor, and in the case of long-chain polyunsaturated fatty acids, the cognate receptor appears to be GPR120. Fatty acids are guided to GPCR by the binding protein, CD36. Upon binding to their receptor, second messenger pathways are activated, leading to phosphorylation of PLCβ2 into DAG and IP₃. IP₃ then binds to its receptor, IP₃R3. This causes an influx of calcium which depletes intracellular stores of Ca²⁺ found in the endoplasmic reticulum. This depletion then activates a monovalent cation channel, known as TrpM5 (Liu et al., 2011). Upon activation, TrpM5 opens and allows for an influx of sodium into the cell, thus depolarizing the cell. This depolarization activates delayed-rectifying potassium (DRK) channels. A subset of these DRK channels become blocked by free fatty-acids, preventing K⁺ from leaving the cell, thereby prolonging the cellular depolarization and allowing for subsequent cellular responses (Pittman et al., 2008). The proposed model for fat taste transduction is shown below (cf. Fig. 2).

This same pathway is also found in the enteroendocrine cells (EECs) of the gut, again utilizing GPR120. Fatty acids are transduced and activate not only taste cells on our tongues, but EECs in the small intestine as well. The activation of this pathway in the gut
Figure 2. Proposed transduction pathway for polyunsaturated fatty acids. Fatty acids are guided by CD36 to their G-Protein coupled receptor, GPR120. Upon binding, second messenger pathways are activated, leading to phosphorylation of PLCβ2 into DAG and IP₃. IP₃ then binds to its receptor, IP₃R3. This causes an increase in intracellular calcium that binds to and activates TrpM5. Upon activation, TrpM5 opens and allows for an influx of sodium into the cell, thus depolarizing the cell. This depolarization activates delayed-rectifying potassium (DRK) channels. A subset of these DRK channels are inhibited by free fatty-acids and this inhibition prolongs the cellular depolarization and allows for subsequent cellular response.

leads to a release of hormones, such as cholecystokinin (CCK) and glucagon-like-peptide-1 (GLP-1) which signal satiety and aid in digestion of fat (Shah et al., 2012; Rozengurt and Sternini, 2007; Tanaka et al., 2008).
G-Protein Coupled Receptors (GPCRs) in Fat Taste

There a wide range of G-protein coupled receptors found in the mammalian body. Different GPCRs play a role in a variety of physiological functions. One of these physiological functions is presiding as a receptor for taste ligands. GPCRs are utilized in sweet, bitter, and umami taste. Recently, they have also been discovered to assist in fat taste transduction as well, with a few “orphan” GPCRs being named as receptors for free fatty acids (Montmayeur et al., 2011). Specifically, these are GPR40, GPR41 and 43, GPR84, and GPR120 and they all respond to fatty acids of different chain lengths and chemical compositions. Fatty acids are named and grouped based on the number of carbons they possess in the carbon chain as well as the level of hydrogen saturation. Short-chain fatty acids contain 2-5 carbons, medium-chain fatty acids contain 6-12 carbons, and long-chain fatty acids contain 14-22 carbons. Fatty acids can also be saturated or unsaturated, depending on whether each carbon is fully saturated with hydrogen.

In a study by Hong et al., they discovered that GPR41 and GPR43 responded to the ligands acetate and propionate, two prototypical short chain fatty acids (SCFAs). Propionate is thought to induce leptin expression. Leptin is a satiety hormone that signals fullness by inhibiting hunger. While both of these GPCRs are involved in short-chain fatty acid transduction, they are very different in SCFA selection and also in location. GPR41 typically has ligands that are 3-5 carbon SCFAs and is expressed in brain, lung and adipose tissue. GPR43 usually binds ligands that are 2-3 carbon SCFAs and is found
in leukocyte and adipose tissue primarily (Hong et al., 2005; Kimura et al., 2011; Xiong et al., 2004).

GPR40 is thought to be activated by both medium and long-chain fatty acids (carbon chains from 6-22). This receptor is expressed in pancreatic beta cells, the brain, and in Type I taste cells (Cartoni et al., 2010; Itoh et al., 2003; Rayasam et al., 2007). In a study by Cartoni et al., they showed that genetic knockouts of the GPR40 receptor in mice made them indifferent to linoleic acid (tested up to a concentration of 350 mM). This showed that GPR40 is crucial in detecting medium and long-chain fatty acids (Cartoni et al., 2010). Another GPCR believed to aid in medium-chain fatty acid transduction is GPR84. Much is still unknown about this receptor, including uncertainty about the pathway it activates, second messengers involved, and even its function. However, previous studies using RT-PCR show that GPR84 is expressed mainly in bone marrow, the lungs, and peripheral blood leukocytes. More recently, work done in the Gilbertson lab shows that GPR84 is highly expressed in taste cells, strongly eluding to a role of GPR84 in fatty acid taste transduction (Liu et al., 2016). It is also certain that GPR84 is the receptor for medium chain fatty acids, within the range of 9-14 carbons (Wang et al., 2006).

In a study by Cartoni et al., researchers performed preference tests for linoleic acid in mice lacking GPR120. As previously discussed, the G-protein coupled receptor, GPR120 is believed to play a crucial role in polyunsaturated fatty acid (PUFA) taste transduction. When mice lacking this receptor were exposed to differing concentrations of linoleic acid (a PUFA), they were indifferent to it and were unable to detect the fatty
acid. They found that knocking out GPR120 only had an effect on long-chain fatty acid taste transduction; it showed no effects when mice were exposed to bitter, sweet, salty, sour, or umami compounds (Cartoni et al., 2010). GPR120 is highly expressed in the intestine, in enteroendocrine cells, and in Type II taste receptor cells. Activation of GPR120 mediates secretion of cholecystokinin (CCK) and glucagon-like-peptide-1 (GLP-1) in the enteroendocrine cells of the gut. Both CCK and GLP-1 are satiety hormones that increase circulating insulin levels in the body, making GPR120 a very important target in diabetes research (Hirasawa et al., 2005; Tanaka et al., 2008).

It has also been proposed that GPR120 regulates adipogenic processes and plays a major role in adipocyte development and differentiation. Adipocytes are considered “fat cells” and they make up our adipose tissue. They are specialized in storing fat and using it for energy. Recently, obesity has been shown to elicit a chronic, low-grade systemic inflammatory response. This inflammation results from insulin resistance in adipocytes, and increasing the number and size of adipose cells. This adds another reason that GPR120 is of extreme importance in understanding obesity-related diseases, which have become increasingly prevalent in our day (Gotoh et al., 2007; Ichimura et al., 2012; Vachharajani and Granger, 2009).

**TrpM5**

TrpM5 is a member of a very large family of ion channels called transient receptor potential (TRP) channels. Within this family, there are 7 subfamilies: TRPC (classical or canonical), TRPV (vanilloid), TRPM (melastatin), TRPP (polycystin),
TRPML (mucolipin), TRPA (ANKTM1 homologues) and TRPN (NOMP-C homologues; Jemal et al., 2013; Owsianik et al., 2006). Each of these subfamilies contains groups and subgroups, adding up to about 28 members in the Trp family. One example of the variety and range of the Trp family can be found in the TrpV (vanilloid) subfamily, which contains 6 members that provide a wide range of functions. It is proposed that each of the 6 TrpV channels is tuned to respond to a variety of stimuli, from heat sensing to pain sensation to hearing modulation. TRP channels are present in almost all types of cells in both invertebrates and vertebrates, making these ion channels very important in cellular and molecular functioning (Ramsey et al., 2006; Zheng, 2013; Pedersen et al., 2005).

Within the TrpM subfamily, there are 8 members, making it the largest subfamily in the Trp channel family. This subfamily is difficult to categorize and understand because each of the channels expresses unusual means of activation. A large range of sources can activate different TrpM channels, from Ca\(^{2+}\) to ADP to thermal changes and even one that just shows constitutive activation. Additionally, all the channels differ in selectivity. The TrpM family mediates a wide variety of sensations, from noxious cold sensing, Mg\(^{2+}\) and Ca\(^{2+}\) homeostasis, to taste transduction (Clapham, 2003; Montell et al., 2002).

TrpM5, which belongs to the TrpM (melastatin) subgroup, is very important in taste transduction, specifically in transduction of long-chain polyunsaturated fatty acids (PUFAs). Previous studies done in our lab have shown that mice lacking TrpM5, while they still prefer fatty acid containing foods, they eat less of a high fat diet, gain less weight, and gain less body fat mass when compared to animals that have TrpM5.
Additionally, TrpM5<sup>−/−</sup> mice briefly exposed to a long-chain PUFA, linoleic acid, are unable to detect the fatty acid, even at concentrations of up to 100 µM (Liu et al., 2011; Minaya, 2013). This work leads us to believe that TrpM5 plays a crucial role in long-chain PUFA transduction.

TrpM5 is encoded by the TrpM5 gene and is found concentrated in taste cells as well as in olfactory sensory neurons, the stomach and the gut. It is often found co-expressed with α-gustducin (Lin et al., 2006; Minke and Cook, 2002; Perez et al., 2003.) It is activated by a G-protein coupled receptor and a phospholipase C (PLC) signaling pathway, which includes second messengers such as inositol triphosphate (IP<sub>3</sub>) and diacylglycerol (DAG). TrpM5 is a monovalent cation-selective channel which preferentially allows Na<sup>+</sup> ions to cross - it is one of two TrpM channels that is impermeable to calcium (the other being TrpM4). It responds to depletions of intracellular calcium stores and is dependent on membrane potential changes, therefore TrpM5 is characterized as a voltage-modulated and Ca<sup>2+</sup>-activated monovalent-specific cation channel (VCAM; Hofman et al., 2003).

Structurally, like all Trp channels, TrpM5 is a membrane protein that has six putative transmembrane (TM) subunits. These subunits assemble as tetramers and form a cation-permeable pore between the TM5 and TM6 subunits; this is where ionic flow occurs. On each end, in the cytoplasm, is located an amino or a carboxyl terminus. At the amino terminus (N-terminus), there are ankyrin repeats, which are about 33 amino acids long. These repeats mediate protein-protein interactions and are highly conserved.
throughout TRP channel evolution (Clapham et al., 2001; Clapham, 2003; Minke and Cook, 2002; Owsianik et al., 2006; Peeters et al., 2011; Yoo et al., 2014; cf. Fig. 3).

**Sex Differences**

In a preliminary study done in the Gilbertson lab, male and female mice were placed on a high fat diet for 4 weeks to gauge effects of knocking out TrpM5 (discussed above) on mice eating a high fat diet. In this study, we tested both TrpM5<sup>−/−</sup> male and female mice as well as male and female C57Bl/6 mice to act as a control group. All mice were placed on a control diet for one week then switched to a high fat diet for five weeks. We monitored both food intake, fat mass, and weight gain throughout the study. We found that male TrpM5<sup>−/−</sup> (KO) mice ate significantly less than their wild-type (WT) counterparts. Surprisingly, we didn’t see this response in females; both the KO and WT females ate the same amount of calories. While we see significant differences between the KO males and females in caloric intake, we see the same response in both male and female TrpM5 KOs in terms of body composition. At the end of the study, both male and female KOs gained significantly less body weight and fat mass than compared to their WT counterparts. This leads us to believe that TrpM5 might play a differential role in fatty acid transduction and hormone release in each of the sexes.

In humans, it is observed that women usually have more fat mass that contributes to their body weight compared to men. Additionally, women generally store their fat deposits subcutaneously and usually on their hips and thighs. Men, however, tend to deposit fat in their abdominal regions. Storing fat and having excess adipose cells in the
Figure 3. Proposed structure of Trp channels. Trp channels are believed to be composed of 6 transmembrane (TM) domains with a pore, where the ion channel is found, forming in between the 5\textsuperscript{th} and 6\textsuperscript{th} TM. Taken and adapted from Yoo et al., 2014.

abdominal region is more dangerous in terms of health risks than storing fat subcutaneously (Black 2001; Geer and Shen, 2009). In summation, women tend to have more body fat than men, but men tend to be at more risk health wise than women because of where the excess fat is stored. Many other studies highlight the fact that females tend to have more fat mass and gain more weight than males and the reasons are varied. One group of scientists believes that women carrying excess fat on their thighs and hips is due to evolutionary adaptation that increases female reproductive success. Additionally, differences in where fat is stored and how much is stored is attributed to estrogen and
other hormone differences between men and women (Lemieux et al., 1993; Power and Schulkin, 2008).

This same phenomenon is found in mice as well. In a study by Hwang et al., they discovered that male mice placed on a long term high fat diet are much more susceptible to metabolic diseases, such as hyperglycemia, hypercholesterolemia, insulin resistance, and hyperleptinemia, than female mice placed on the same long term high fat diet. This is significant because many of these metabolic diseases expressed in the obese male mice have analogs to human diseases caused by obesity (Hwang et al., 2010). This area of research, obesity and sex differences, is something that needs a much closer look in the coming years. Understanding obesity, hormones, sex and how all these factors interact is a crucial step to eventually overcoming the obesity epidemic.

References


CHAPTER II

INTRODUCTION

In recent years, the problem of obesity has risen to epidemic proportions. Obesity, and its related complications, such as diabetes, stroke, and cancer, rank among the highest causes of death in the United States. While obesity is a highly complicated, multifaceted disease with many interacting causes, research has shown that there is a connection between fat intake and obesity outcome. Environmental, food availability, and lifestyle changes have altered the normal ‘Western diet’, with the result being a sharp increase in dietary fat intake in the population (Hill et al., 2000). The increased fat in the diet can then lead to problematic metabolic profiles, such as raised cholesterol, high blood pressure, and obesity. Recent research has begun to elucidate an important connection; the interaction between tasting fat in the oral cavity, and the effect that it has on these metabolic outcomes.

A number of studies highlight this important connection, and shows the effect that tasting, or not tasting, fat can have on the metabolic response to ingested fat. Research has shown a crucial role in activation of the taste receptor cells in the oral cavity and downstream responses such as lipid processing, peptide hormone release for digestion of fat, and serum triglyceride levels (Mattes, 2001; Mattes, 2011; Robertson et al., 2002). Additionally, in sham feeding studies, where participants are exposed orally to fat but do not ingest the fat, show that there is an increase in peptide hormones released in response. This provides evidence that oral fat exposure, even without ingestion, is sufficient to
cause a digestive response comparable to if the fat was actually ingested (Chavez-Jauregui et al., 2010). This places a large role on the oral cavity and the fatty acid taste transduction pathway in mediating a physiological response to dietary fat. Therefore, understanding the exact mechanisms of how we taste fat, the first step in that signaling pathway, is a crucial stage in understanding obesity more thoroughly.

Until fairly recently, fat was considered to be tasteless, with the only cues being the unique texture and noticeable odor associated with fat. However, seminal work by Gilbertson and colleagues showed that fats, in the form of fatty acids, are capable of activating taste receptor cells in the oral cavity, which is a necessary hallmark of a taste stimulus. In this study, isolated taste cells, taken from mice, were assayed using patch clamp recording techniques. It was shown that the fatty acid linoleic acid, was capable of producing a response in a subset of taste cells. This response was in the form of a cellular depolarization and a rise in intracellular calcium, both of which are indicative of cellular response and signaling. Additionally, Gilbertson found that primarily polyunsaturated fatty acids, or the essential fatty acids, elicited this response (Gilbertson, 1998; Gilbertson et al., 2005). Despite knowing that FAs activate TRCs, it was still unknown exactly what they were activating and what fatty acids were the ligand for. It was hypothesized that a type of delayed rectifying potassium (DRK) channel, Kv1.5, was acting as a receptor for fatty acids (Gilbertson et al., 2005). However, because these DRK channels are activated (opened) by depolarization, it has since been concluded that activity upstream must be responsible for the initial depolarization and be a precursor to the activation of the DRK channels. Therefore, a different element must be the cognate receptor for fatty acids.
During the search for the fatty acid receptor, many ‘orphan’ G-protein coupled receptors were implicated in fat taste transduction. For polyunsaturated fatty acids, such as linoleic acid, GPR120 was identified and was shown to be highly expressed in taste receptor cells in rats (Matsumura et al., 2007). Additionally, in mice, the absence of GPR120 produces diminished responses to polyunsaturated fatty acids. This was shown in behavioral studies, as well as in nerve recording studies where a diminished signal in response to fatty acid stimulation is observed in mice who do not have GPR120 (Cartoni et al., 2010). Another plausible receptor has been named for PUFAs as well, and that is the transporter protein Cluster of Differentiation 36, or shortened as CD36. Laugerette et al., showed that CD36 is also highly expressed in taste receptor cells and that mice lacking CD36 also show much decreased responses to oral linoleic acid exposure (Laugerette et al., 2005). Another study by Liu et al., shows that while CD36 is indeed involved in fatty acid sensing and detection in the oral cavity, CD36 acts merely as a chaperone to guide fatty acids to GPR120, which acts as the prominent receptor for PUFAs (Xu, 2014). Current work is still being focused on confirming the exact roles, restrictions, and interactions of both CD36 and GPR120 in fatty acid detection in the oral cavity.

After naming GPR120 as a plausible receptor for PUFAs, further work was dedicated to the elucidation of other elements involved and eventually to the proposal of a transduction pathway for polyunsaturated fatty acids, which is highly similar to the pathways for sweet, bitter, and umami stimuli. Specifically, the α-subunit of the GPR120 receptor activates 1-phosphatidylinositol-4,5-bisphosphate phosphodiesterase β-2,
(PLCβ2), which results in the phosphorylation of phosphatidylinositol 4,5-bisphosphate, (PIP2) into second messengers diacylglycerol, (DAG) and inositol trisphosphate, (IP3).

The latter goes on to bind to its receptor, IP3R3, which is located on the endoplasmic reticulum. Binding opens this channel and results in an increase in intracellular calcium. The rise in intracellular calcium is sufficient to activate the TrpM5 ion channel which depolarizes the taste cell. Depolarization activates DRK channels, of which a subset become blocked by external free fatty acids. This results in a stronger depolarization and leads to release of neurotransmitter and cellular signaling (Liu et al., 2011).

Experiments involving immunohistochemistry show that the ion channel, TrpM5, is strongly, (~90%), co-expressed with GPR120, in Type II taste receptor cells in the oral cavity, providing further evidence of the importance of GPR120 in fat taste transduction (Cartoni et al., 2010). Additionally, TrpM5 has been shown to be essential in mediating a response to polyunsaturated fatty acids. Liu et al., shows that mice lacking TrpM5 exhibit significantly reduced responses to linoleic acid exposures, both at the behavioral level as well as at the cellular level (Liu et al., 2011). Due to the proposed role of TrpM5 in being responsible for depolarizing the taste cell, TrpM5 becomes a crucial element in the taste transduction pathway; without TrpM5, the taste cell cannot depolarize in response to fatty acid stimulation and therefore, cannot respond and release neurotransmitter to send a signal. This current study, through the use of TrpM5 knock-out mice, investigated how this pathway affects the detection of fatty acids in the oral cavity and any implications the inability to detect fat orally has on metabolic responses like lipid processing and weight gain.
In this report I show that the TrpM5 transduction pathway is essential for detection of PUFAs in the oral cavity since mice without TrpM5 cannot detect linoleic acid when exposed for brief periods. Additionally, I show that TrpM5 and the fatty acid transduction pathway are capable of influencing metabolic processes as is evidenced by decreased weight gain and decreased fecal lipid content in mice who are lacking TrpM5. From my studies, I also provide evidence that the TrpM5 pathway is specific to unsaturated fatty acids through the use of altered high fat diets containing different portions of saturated and unsaturated fats. Finally, it is shown that there are observable sex differences in the TrpM5+/- mice. Males, when compared to their wildtype (WT) counterparts, show decreased food intake as well as a corresponding decreased weight gain. In females, however, there is no observable difference in food intake between TrpM5+/- and WT females, yet there is a significant difference in weight gain despite this isocaloric intake. While the exact mechanisms behind the observed sex differences remain uncertain, I show that knocking out TrpM5 produces phenotypic responses that differ between males and females.

**Materials and Methods**

**Animals**

Details of TrpM5 knock-out mice has been described previously by Damak (Damak et al., 2006). TrpM5+/- mice were bred at the Laboratory Animal Research Center (LARC) at Utah State University (USU) on a 100% C57bl/6 background. Wild-type C57bl/6 mice were purchased from Jackson Laboratories and transported to the LARC.
facility at USU, where they were allowed to acclimate for 5-7 days prior to being used for any experiments. C57bl/6 mice acted as controls in all experiments. All mice used in experiments, both TrpM5 knockouts and wild-type C57bl/6, were 7-14 weeks old at the time of experiments. Mice were maintained on a 12-hour day/night cycle with normal mouse chow (Harlan Laboratories, rodent feed 8604), and water provided ad-libitum. All experiments and procedures were approved by the Institutional Animal Care and Use Committee (IACUC) of Utah State University.

**Diets**

All diets were provided by Research Diets, Inc. A 60% high fat lard diet (D12492), a 60% saturated fat diet (D06062302), a 60% unsaturated fat diet (D06062303), a high sucrose diet (D06062304), and a 10% fat run-in control diet (D07020902) were used. All diet compositions are listed in Table 1.

**Aim 1: The Role of TrpM5 in the Oral Cavity and the Gut:**

**Role of TrpM5 in the oral cavity: Conditioned Taste Aversion**

A conditioned taste aversion (CTA) assay was performed to test for differences between female TrpM5 KO mice and C57bl/6 WT mice. This was complementary to a previous study done in the lab testing male TrpM5 KO and WT mice using the CTA
Table 1: Experimental Diet Composition. Taken and altered from Research Diets, Inc.

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technique. The assay was designed to test for differences in how mice detect and respond to a fatty acid stimulus, linoleic acid, when given only brief exposure to it. This was done in an effort to eliminate post-ingestive effects, since lipid absorption takes around ten minutes and signaling cues take slightly less time (Mattes, 2009). Two groups of female mice, TrpM5+/− and C57bl/6 WT, were further divided into categories to receive either a LiCl injection to induce gastric malaise, or NaCl, used as a control injection. Mice were placed on a 23.5-hour water restriction prior to the first day of conditioning and were maintained on this schedule for the duration of the experiment.

Following this, mice were trained to lick during water stimulus trials using a Davis Rig lickometer (DiLog Instruments, Tallahassee FL) for 4-6 days. Mice were considered fully trained if they tested each stimulus presented and drank for the full 5 second exposure. After each day of training, mice were allowed 30 minutes of ad libitum access to water. After training, 2 consecutive days of conditioning took place. Mice were exposed to the conditioned stimulus (CS), 100 µM linoleic acid, by 1 mL intraoral application, and then immediately injected, based on their group, with either 150 mM LiCl or 150 mM NaCl. All injections were dose dependent based on body weight (20 µL/g body weight). Mice received 30-minute ad libitum access to water immediately following their injections.

Following conditioning, mice were tested in the Davis Rig for two days to assess whether they had formed an aversion to the linoleic acid (CS). Each daily testing session consisted of two blocks of 13 test stimuli, randomly ordered. Each stimulus was presented for five seconds, with a maximum of 150 seconds until the first lick. Between
each of the taste stimuli, a two-second rinse (tap water) was presented to cleanse the mouse’s palette. To reduce olfactory cues, fans were placed by each Davis Rig chamber to blow air perpendicularly across the opening for the spout.

Of the taste stimuli provided, fatty acid concentrations were selected based on previous data showing fatty acid concentrations that elicited a response from taste cells. The taste stimuli were as follows: Water, 0.1 µM linoleic acid (LA), 0.3 µM LA, 1 µM LA, 3 µM LA, 10 µM LA, 30 µM LA, and 100 µM LA. 3 mM denatonium benzoate, 100 mM NaCl, and 100 mM sucrose were used to assess innate aversions against other recognized tastants. 100 µM oleic acid and 200 µM capric acid were also included to test for cross-generalized aversions to other fatty acids. Total lick number for each stimulus in both blocks was measured and normalized using a lick ratio of mean licks per stimulus/mean licks of water. This helps account for differences in thirst motivation and training across the mice. Differences between the C57bl/6 wild-type mice and the TrpM5−/− mice were analyzed with a student’s t-test for statistical significance using an alpha level of 0.05.

Role of TrpM5 in the gut: Fatty acid absorption

One of the phenotypic hallmarks of TrpM5 deficient mice is a lower increase in weight gain and body fat on a high fat diet. This was observed in both male and female TrpM5 knockouts, with the knockout mice gaining significantly less on a high fat diet than their wild type counterparts. Interestingly, in males, the TrpM5−/− mice ate
significantly less than the wild type males. As would be intuitively expected, the knockout mice who ate less also gained less weight. However, this was not seen in the females. Both knockout and wildtype females eat the same amount of a high fat diet. Despite taking in the same amount of calories, TrpM5\(^{-/-}\) females still gain significantly less weight than the wildtypes. Due to this interesting phenomena, of TrpM5 deficient mice gaining less weight than wild-type, it warrants the hypothesis that TrpM5 could affect other downstream processes, such as fat absorption. This experiment tested if there were any differences in fatty acid absorption between TrpM5 KO mice and C57bl/6 WT mice. Additionally, it evaluated differences in weight gain due to differences in fat absorption.

This was tested using a variation of a basic lipid extraction technique designed by Kraus and colleagues, with slight variations from their protocol (Folch et al., 1956; Kraus et al., 2015). Briefly, feces were collected on days 0, 2, and 4 of a 4-day \textit{ad libitum} feeding period where individually housed mice were placed on a 60\% high fat diet (1:1 SFA:UFA; the lard diet). Food intake was measured every other day as well. Within groups, i.e. male wildtypes, female wildtypes, male knockouts, and female knockouts, feces were pooled, in 1000 ± 3 mg aliquots and placed in 15 ml conical tubes. Feces were crushed into powder and suspended in a normal saline solution, (0.15 M) and vortexed to mix. Following this, 7 mL of chloroform in methanol (2:1 by volume) was added to each sample and vortexed. All samples were then centrifuged at 2000 rpm for 10 minutes at room temperature. Glass tubes or cylinders were weighed and labeled for each sample. By poking two holes in the 15 ml conical tube with a small needle and syringe, the
bottom liquid phase containing the extracted lipids in the chloroform and methanol solution is pushed out of the conical tube and into one of the pre-weighed glass vials. After 3-4 days of evaporation, glass tubes were reweighed to get the weight of the lipids extracted. Using this protocol, the lipids in the feces were separated and weighed to determine total lipid content in feces. Average amounts were then compared across strains to assess any significant differences.

Aim 2: The Role of TrpM5 in Weight Gain and Weight Loss:

*TrpM5’s role in weight gain*

Previous studies done in our lab show that male TrpM5 deficient mice eat significantly less of a 60% high fat diet than their WT counterparts. These TrpM5<sup>−/−</sup> mice take in less calories and gain less body weight than the male WT mice. We re-examined this by replicating previous feeding studies. Twenty mice were used in this feeding study. Ten male C57bl/6 mice along with 10 TrpM5<sup>−/−</sup> mice were individually housed in wire-bottom cages and switched from their normal chow diet and placed on a run-in control diet (Table 1), containing 10% fat, for one week. Following this week, all mice were switched to a 60% high fat lard diet for 7 weeks. Mice were allowed *ad libitum* access to both food and water throughout the duration of the study. Weight and food intake of each mouse was taken every other day with fresh food provided every other day as well. Using a body composition analyzer (EchoMRI LLC, Houston TX), body fat composition was
measured at the start of the run-in diet, at the start of the high fat diet, and at the completion of the study to analyze body composition changes in accordance with diet changes.

*TrpM5’s role in weight loss*

For 28 consecutive days, male mice were switched from their normal chow diet and placed on either a 60% high fat lard diet or a run-in control diet (high carbohydrate diet, 10% fat). Mice were individually housed with *ad libitum* access to their respective diets. Mice were divided into 4 groups: 7 male C57bl/6 wildtype and 6 male TrpM5 KO mice placed on the control diet, and 8 male C57bl/6 wildtype and 7 male TrpM5 KO mice placed on the high fat diet. Food intake was not measured during these 28 days with the expectation that mice never lacked available food. Weight was measured every other day during this preliminary 28-day period. Body composition measurements were made weekly throughout the duration of the study using the EchoMRI system.

At the end of the 28-day period, final weight measurements were taken and used as a baseline weight for the deprivation period. Following this, mice previously on the HF diet were switched to the run-in control diet, and mice on the control diet remained on the control diet. All mice were then placed on a food restriction and allowed access to only 60% of their regular caloric intake, which was determined during the last week of the initial 28-day feeding period. Water was provided *ad libitum* throughout the study. The restricted food was pre-measured daily and recorded and was administered every day at 9:00 a.m. Weight was also monitored every day and any remaining food was weighed as
well. Mice were removed from the study as soon as they reached 80% of their baseline weight (Toth and Gardiner, 2000). Body composition measurements were made weekly throughout the food restriction period and immediately following the removal of the mice from the study.

Aim 3: The Specificity of TrpM5

*Saturated vs. unsaturated high fat diets*

TrpM5 is believed to be primarily involved in the transduction pathway of long chain poly-unsaturated fatty acids (PUFAs). We aimed to test this specificity by evaluating TrpM5−/− mice on a variety of different high fat diet compositions. The previous feeding study mentioned above was done using a 60% fat diet with a 1:1 ratio of unsaturated : saturated fatty acids; essentially a lard-based diet. We wanted to test other diets that are composed of primarily unsaturated or primarily saturated fatty acids to compare if a TrpM5 KO would elicit similar phenotypic effects on body composition and intake in response to saturated and unsaturated fatty acid diets. These feeding studies followed a very similar protocol to that used in the 60% high fat, lard diet feeding study.

Ten male C57bl/6 mice along with 6 TrpM5−/− mice were individually housed in wire-bottom cages and switched from their normal chow diet and placed on a run-in control diet, containing 10% fat, for two weeks. Following this, mice were switched to a 0.1:1 (unsaturated: saturated fatty acids; primarily saturated) high fat diet for 6 weeks. Mice were allowed *ad libitum* access to both food and water throughout the duration of
the study. Weight and food intake of each mouse was taken every other day with fresh food provided every other day as well. Using an EchoMRI system, body fat composition was measured at the start of the run-in diet, at the start of the high fat diet, and at the completion of the study to analyze body composition changes in accordance with diet changes. Feces were collected over the last 4 days of the study, to be used for lipid extraction. For the second half of this study, a group of new mice were placed on a 3.3:1 (unsaturated: saturated fatty acids; primarily unsaturated) high fat diet for 6 weeks and followed the same protocol. Feces were collected at the end of the 6-week high fat period and analyzed using the lipid extraction method described above (Kraus et al., 2015). Averages were compared across strains to assess for differences.

**Aim 4: Sex Differences of TrpM5**

*Weight gain, weight loss, and response to differing fatty acid profiles in high fat diets in female mice*

While we’ve seen differences in food intake and weight gain in male mice on a high fat diet, we don’t see these differences in female WT and TrpM5 KO mice on the same diet. Females, regardless of whether or not they have TrpM5, don’t show any differences in caloric intake. Again, we re-evaluated this claim in an effort to tease apart the differences of a TrpM5 KO in a female compared to a male on a variety of high fat diets. Females were tested following identical protocols to those used on their male counterparts on the high fat lard (1:1) diet, primarily saturated fat diet, primarily
unsaturated fat diet, lipid extraction, and the food deprivation feeding study to assess differences between the sexes.

**Aim 5: The Role of TrpM5 in the Transduction Pathway of Other Tastants**

It is believed that TrpM5 is involved in the sweet taste transduction pathway, so we tested if knocking out TrpM5 would have any phenotypic effects on sweet taste detection. TrpM5 KO along with wildtype C57bl/6 mice were used to evaluate if differences were present in preference or body composition of mice on a high sucrose diet. 40 mice total were used in this feeding study. Twenty C57bl/6 mice (10 male and 10 female), along with 20 TrpM5−/− mice (10 male and 10 female) were individually housed in wire-bottom cages and switched from their normal chow diet and placed on a run-in control diet, containing 10% fat, for one week. Following this, all mice were switched to a 70% high sucrose diet for 5 weeks. Mice were allowed *ad libitum* access to food and water throughout the duration of the study. Weight and food intake of each mouse was measured every other day with fresh food provided every other day as well. Using an EchoMRI system, body fat composition was measured at the start of the run-in diet, at the start of the high sucrose diet, and at the completion of the study to analyze body composition changes in accordance with diet changes.
Statistical Analyses

All statistical analyses were performed using GraphPad Prism 7.00. Two-sample t-tests or one-way ANOVA were used to evaluate any significant differences in the data for each parameter measured. A significant result was accepted with an alpha level of 0.05. Level of significance is further notated in all figures as follows: *, p-value<0.05; **, p-value<0.01; ***, p-value<0.001; and ****, p-value<0.0001.

Results

Aim 1: The Role of TrpM5 in the Oral Cavity as Well as in the Gut:

Role of TrpM5 in the oral cavity: Conditioned Taste Aversion

To assess the role of TrpM5 in the oral cavity, a Conditioned Taste Aversion (CTA) test was performed to assess the ability of mice lacking TrpM5 in forming an aversion to a variety of different concentrations of linoleic acid (LA), a long-chain polyunsaturated fatty acid. A CTA removes the possibility of post-ingestive responses, since mice are only allowed a 5-second exposure to the test stimuli. It was shown that female mice lacking TrpM5 are incapable of forming an aversion against the conditioned stimulus, 100 µM LA. There was no difference between TrpM5 knock-out mice who received the control injection of NaCl, versus the test injection of LiCl. In contrast, wildtype C57bl/6 females are capable of forming a strong aversion to LA. The WT
females show an aversion to LA at concentrations as low as 1 µM and show a LA concentration dependent decrease in lick number (Fig. 4). This corresponds with similar experiments done previously on males lacking TrpM5 (Liu et al., 2011, data shown below; Fig. 5).
Figure 4: Role of TrpM5 in the oral cavity in WT and KO female mice. Average preference ratios, shown as lick number of stimulus/lick number of water ± SEM for day 1 of testing after CTA in WT (A) and KO (B) females. WT NaCl group n=9; WT LiCl group n=6; TrpM5^+/^- NaCl group n=6; TrpM5^+/^- LiCl group n=7. Two-sample t-tests were performed on each data point to assess differences between test and control groups as well as WT and KO groups. Significance set at α= 0.05 (p-value<0.0001).
Figure 5: Role of TrpM5 in the oral cavity in WT and KO male mice. Previous data obtained from Liu et al., 2011. Average preference ratios, shown as lick number of stimulus/lick number of water ± SEM for day 1 of testing after the CTA. Two-sample t-tests were performed on each data point to assess differences between injections groups. Significance set at α= 0.05. Asterisks are shown above each point where there is a significant difference between the NaCl groups and the LiCl groups (p-value<0.0001).
Role of TrpM5 in the gut: Fatty acid absorption

Fecal lipid content, extracted from collected feces shows significant differences in total lipid content on days 0, 2 and 4. Both male and female TrpM5<sup>−/−</sup> mice show significantly less lipid content in their feces compared to the WT mice (Figs. 6, 7). Additionally, all groups show dramatic decreases in the amount of feces excreted, in milligrams. Within four days of being on the high fat diet, feces excreted, in milligrams, decreases to about 25% of what was seen on the chow diet, before being placed on the 1:1 high fat diet (Figs. 8, 9).
Figure 6: Lipid content in feces in male mice on a 1:1 high fat diet on days 0, 2, and 4. Average lipids, in percent per mille (‰) ± SEM, excreted in the feces after 0, 2, or 4 days on a 1:1 high fat diet. TrpM5⁻/⁻ males n=7, WT n=8. Two-sample t-tests were performed to assess significant differences between WT and TrpM5⁻/⁻ mice fecal lipid content. Results were significant for all days (p-value = 0.0059; 0.0114; 0.0166 for days 0, 2, and 4 respectively).
Figure 7: Lipid content in feces in female mice on a 1:1 high fat diet on days 0, 2, and 4. Average lipids, in percent per mille (‰) ± SEM, excreted in the feces after 0, 2, or 4 days on a 1:1 high fat diet. TrpM5⁻/⁻ females n=7, WT n=8. Two-sample t-tests were performed to assess significant differences between WT and TrpM5⁻/⁻ mice fecal lipid content. Results were significant for all days (p-values= 0.0193; 0.0041; 0.0019 for days 0, 2, and 4 respectively).
Figure 8: Amount of feces excreted in male mice on a 1:1 high fat diet on days 0, 2, and 4. Average amount of feces, in milligrams ± SEM, excreted after 0, 2, or 4 days on a 1:1 high fat diet. TrpM5⁻/⁻ males n=7, WT n=8. Two-sample t-tests were performed to assess significant differences between WT and TrpM5⁻/⁻ mice fecal lipid content. Results were not significant for all days (p-value = 0.7816; 0.3709; 0.4807 for days 0, 2, and 4 respectively).
Figure 9: Amount of feces excreted in female mice on a 1:1 high fat diet on days 0, 2, and 4. Average amount of feces, in milligrams ± SEM, excreted after 0, 2, or 4 days on a 1:1 high fat diet. TrpM5−/− females n=7, WT n=8. Two-sample t-tests were performed to assess significant differences between WT and TrpM5−/− mice fecal lipid content. Results were not significant for all days (p-value = 0.8080; 0.1688; 0.8080 for days 0, 2, and 4 respectively).
Aim 2: The Role of TrpM5 in Weight Gain and Weight Loss:

TrpM5’s role in weight gain

Male mice lacking TrpM5 exhibit no significant differences in calorie intake in comparison to WT C57bl/6 mice when placed on a 10% fat run-in control diet for 7 days. When placed on a 60% high fat lard diet for 53 days, TrpM5<sup>−/−</sup> males eat significantly less calories than WT males (Fig. 10). Additionally, TrpM5<sup>−/−</sup> males show no difference in weight gain compared to WT males while on the control diet. However, TrpM5<sup>−/−</sup> males gain significantly less body weight than their WT male counterparts while on the high fat diet (Fig. 11).

Body composition data, including lean and fat mass, were assessed using an EchoMRI system. TrpM5<sup>−/−</sup> males show no significant differences in changes in lean or fat mass while on the control diet compared to the WT males. On the high fat feeding period, TrpM5<sup>−/−</sup> male mice gain significantly less fat mass as well as significantly less lean mass when compared to their wildtype counterparts (Figs. 12, 13).
Figure 10: Cumulative calorie intake of males on a 60% high fat lard diet. Average cumulative caloric intake (in Kcal) per day ± SEM, over days of the study. TrpM5−/− males n=10, WT males n=10. Two-sample t-tests were performed for each data point to assess significant differences between WT and TrpM5−/− male’s calorie intake. Results were significant (p-values ≤ 0.037).
Figure 11: Change in body weight of males on a control diet and 60% high fat lard diet. Average change in body weight, ± SEM, on a run-in diet (A) for 7 days, or a 60% high fat lard diet (B) for 53 days. TrpM5−/− males n=10, WT males n=10. Two-sample t-tests were performed to assess significant differences between WT and TrpM5−/− males change in body weight. Results were not significant for the run-in diet period (p-value=0.5844). Results were significant for the high-fat diet period (p-value<0.0001).
Figure 12: Change in lean mass of males on a control diet and 60% high fat lard diet. Average change in lean mass, ± SEM, on a run-in diet (A) for 7 days, or a 60% high fat lard diet (B) for 53 days. TrpM5⁻/⁻ males n=10, WT males n=10. Two-sample t-tests were performed to assess significant differences between WT and TrpM5⁻/⁻ males change in lean mass. Results were not significant for the run-in diet period (p-value= 0.2634). Results were significant for the high-fat diet period (p-value= 0.001).
Figure 13: Change in fat mass of males on a control diet and 60% high fat lard diet.
Average change in fat mass, ± SEM, on a run-in diet (A) for 7 days, or a 60% high fat lard diet (B) for 53 days. TrpM5−/− males, n=10; WT males, n=10. Two-sample t-tests were performed to assess significant differences between WT and TrpM5−/− males change in fat mass. Results were not significant for the run-in diet period (p-value= 0.0733). Results were significant for the high-fat diet period (p-value= 0.0011).
TrpM5’s role in weight loss

TrpM5+/− males show no significant differences in rate of weight loss after being on either a control diet, or a 60% high fat diet for four weeks when compared to their wildtype counterparts. All males lost weight at similar rates (Fig. 14). In terms of body composition, there were no significant differences in changes in lean mass, whether mice had been on the control or the high fat diet prior to the deprivation period. There were no significant differences, between WT and KO mice who had previously been on the control diet in changes in fat mass. However, there were significant differences in the changes of fat mass in males who had previously been on the high fat diet before the deprivation period, with TrpM5+/− males losing significantly less fat mass while on the food deprivation compared to the C57bl/6 wildtype mice (Figs. 15, 16).
Figure 14: Body weight percentages of males over days on food deprivation. Average percent body weight shown ± SEM. TrpM5−/− males on control diet n=7, WT males on control diet n=8 (A). TrpM5−/− males on HF diet n=6, WT males on HF diet n=8 (B). One-way ANOVA was used to assess any overall differences between C57bl/6 males and TrpM5−/− males on either a control diet or a HF diet prior to beginning a food deprivation period. Results were not significant for both the control (p-value= 0.9885) and the HF diet group (p-value= 0.9296) in terms of rate of weight loss.
Figure 15: Change in lean mass of males on a control or high fat diet. Average change in lean mass, ± SEM, on either a run-in diet (A) or a 60% high fat lard diet for 28 days (B) prior to beginning a food deprivation period. TrpM5^+/− males on control diet n=6, WT males on control diet n=7. TrpM5^+/− males on HF diet n=7, WT males on HF diet n=7. Two-sample t-tests were performed to assess significant differences between WT and TrpM5^+/− males change in lean mass. Results were not significant for the control diet group (p-value= 0.2818). Results were also not significant for the high-fat diet period (p-value= 0.0566).
Figure 16: Change in fat mass of males on a control or high fat diet. Average change in fat mass, ± SEM, on either a run-in diet (A) or a 60% high fat lard diet (B) for 28 days prior to beginning a food deprivation period. TrpM5−/− males on control diet n=6, WT males on control diet n=7. TrpM5−/− males on HF diet n=7, WT males on HF diet n=7. Two-sample t-tests were performed to assess significant differences between WT and TrpM5−/− males change in lean mass. Results were not significant for the control diet group (p-value= 0.7570). Results were significant for the high-fat diet period (p-value= 0.0104).
Aim 3: The Specificity of TrpM5

*Saturated high fat diets*

Male TrpM5 deficient mice placed on a control diet for two weeks, then switched to a 60% saturated high fat diet for 6 weeks show no significant differences in caloric intake when compared to their wildtype counterparts (Fig. 17). Despite similar caloric intakes, a significant difference in body weight between KO and WT mice is observed, with TrpM5⁺/⁻ males gaining significantly less weight than WT C57bl/6 males while on the high fat diet. No differences in body weight were observed during the run-in period (Fig. 18). Additionally, there are no significant differences in lean or fat mass changes while on the control diet or the high fat diet between the TrpM5 KO males and the WT males (Figs. 19, 20).
Figure 17: Cumulative calorie intake of males on a 60% saturated high fat diet. Average cumulative caloric intake (in Kcal) per day ± SEM, over days of the study. TrpM5^{-/-} males n=6, C57Bl/6 males n=10. Two-sample t-tests were performed for each data point to assess significant differences between WT and TrpM5^{-/-} male’s calorie intake. Results were not significant (p-values ≥ 0.381).
Figure 18: Change in body weight of males on a control diet and 60% saturated high fat diet. Average change in body weight, ± SEM, on a run-in diet (A) for 14 days, or a 60% saturated high fat diet (B) for 42 days. TrpM5^-/- males n=6, WT males n=10. Two-sample t-tests were performed to assess significant differences between WT and TrpM5^-/- males change in body weight. Results were not significant for the run-in diet period (p-value= 0.0713). Results were significant for the high-fat diet period (p-value= 0.0245).
Figure 19: Change in lean mass of males on a control diet and 60% saturated high fat diet. Average change in lean mass, ± SEM, on a run-in diet (A) for 14 days, or a 60% saturated high fat diet (B) for 42 days. TrpM5−/− males, n=6; WT males, n=10. Two-sample t-tests were performed to assess significant differences between WT and TrpM5−/− males change in lean mass. Results were not significant for the run-in diet period (p-value= 0.6270). Results were not significant for the high-fat diet period (p-value= 0.6086).
Figure 20: Change in fat mass of males on a control diet and 60% saturated high fat diet. Average change in fat mass, ± SEM, on a run-in diet (A) for 14 days, or a 60% saturated high fat diet (B) for 42 days. TrpM5⁻/⁻ males, n=6; WT males, n=10. Two-sample t-tests were performed to assess significant differences between WT and TrpM5⁻/⁻ males change in fat mass. Results were not significant for the run-in diet period (p-value=0.7139). Results were also not significant for the high-fat diet period (p-value=0.1485).
**Unsaturated high fat diets**

Male knockout mice placed on a control diet for two weeks, then switched to a 60% primarily unsaturated high fat diet for 6 weeks show highly significant differences in caloric intake when compared to their wildtype counterparts (Fig. 21). Male TrpM5\textsuperscript{−/−} mice show no differences in body weight on the run-in diet compared to the wildtype mice. However, there are significant differences in weight gain during the high fat feeding period, with TrpM5 KO males gaining significantly less body weight than WT males (Fig. 22). In terms of body composition, there are no differences in changes in lean or fat mass while on the run-in diet compared to the wildtype mice. Additionally, there are significant differences seen in fat and lean mass changes between KO and WT mice when on the unsaturated high fat diet; male KO mice gain significantly less fat mass as well as lean mass during this period (Figs. 23, 24).
Figure 21: Cumulative calorie intake of males on a 60% unsaturated high fat diet.
Average cumulative caloric intake (in Kcal) per day ± SEM, over days of the study.
TrpM5⁻/⁻ males n=7; WT males n=10. Two-sample t-tests were performed for each data point to assess significant differences between WT and TrpM5⁻/⁻ male’s calorie intake. Results were significant (p-values ≤ 0.013).
Figure 22: Change in body weight of males on a control diet and 60% unsaturated high fat diet. Average change in body weight, ± SEM, on a run-in diet (A) for 14 days, or a 60% unsaturated high fat diet (B) for 42 days. TrpM5⁻/⁻ males n=7; WT males n=10. Two-sample t-tests were performed to assess significant differences between WT and TrpM5⁻/⁻ males change in body weight. Results were not significant for the run-in diet period (p-value = 0.7828). Results were significant for the high-fat diet period (p-value ≤ 0.0001).
Figure 23: Change in lean mass of males on a control diet and 60% unsaturated high fat diet. Average change in lean mass, ± SEM, on a run-in diet (A) for 14 days, or a 60% unsaturated high fat diet (B) for 42 days. TrpM5−/− males n=7; WT males n=10. Two-sample t-tests were performed to assess significant differences between WT and TrpM5−/− males change in lean mass. Results were not significant for the run-in diet period (p-value= 0.4716). Results were significant for the high-fat diet period (p-value= 0.003).
Figure 24: Change in fat mass of males on a control diet and 60% unsaturated high fat diet. Average change in fat mass, ± SEM, on a run-in diet (A) for 14 days, or a 60% unsaturated high fat diet (B) for 42 days. TrpM5−/− males n=6; WT males n=10. Two-sample t-tests were performed to assess significant differences between WT and TrpM5−/− males change in fat mass. Results were not significant for the run-in diet period (p-value=0.0007). Results were significant for the high-fat diet period (p-value=0.1485).
Fecal lipid content on a saturated and unsaturated high fat diet

Lipid extraction on a saturated high fat diet: Male KO mice who had been on a control diet for 2 weeks followed by a 60% primarily saturated high fat diet for 6 weeks show no significant differences in the amount, in percentage per mille, (percent per 1000), of excreted lipids when compared to their wildtype counterparts, with averages of 15.17‰ compared to 15.44‰ for the KO and WT males, respectively (Fig. 25).

Lipid extraction on an unsaturated high fat diet: Male TrpM5/- mice who had been on a control diet for 2 weeks then switched to a 60% primarily unsaturated high fat diet for 6 weeks show significant differences in the amount, in percentage per mille, (percentage per 1000), of excreted lipids when compared to their wildtype counterparts, with averages of 14.09‰ compared to 20.40‰ for the KO and WT males, respectively (Fig. 25).
Figure 25: Lipid content in feces in male mice on a saturated high fat diet (SHF) or an unsaturated high fat diet (UHF). Average lipids, in percent per mille ± SEM, excreted in the feces after six weeks of a saturated or unsaturated high fat diet. TrpM5\textsuperscript{-/-} n=6, WT n=10, for the saturated HF diet. TrpM5\textsuperscript{-/-} n=7, WT n=10, for the unsaturated HF diet. Two-sample t-tests were performed to assess significant differences between WT and TrpM5\textsuperscript{-/-} mice fecal lipid content. Results were not significant for the saturated HF diet (p-value = 0.326) but were significant for the unsaturated HF diet (p-value < 0.0001).
Aim 4: Sex Differences of TrpM5

Weight gain

Female mice lacking TrpM5 have displayed differences, in terms of calorie intake, from the males when compared to wildtype females. TrpM5\(^{-/-}\) females show no differences in caloric intake when placed on a 60\% high fat, lard-based diet, compared to wildtype females (Fig. 26). Despite eating the same amount of calories, TrpM5 KO females gain significantly less body weight than WT females, both on the control diet as well as on the high fat diet (Fig. 27). There are no significant differences in the changes in lean mass while on the run-in diet, however, there are significant differences in the change in lean mass while on the high fat diet, with the TrpM5\(^{-/-}\) females gaining significantly less lean mass. Additionally, there are significant differences between WT and KO mice in terms of changes in fat mass. These differences are significant on the both the run-in diet as well as on the high fat diet (Figs. 28, 29).
Figure 26: Cumulative calorie intake of females on a 60% high fat lard diet. Average cumulative caloric intake (in Kcal) per day ± SEM, over days of the study. TrpM5⁻/⁻ females n=10, WT females n=10. Two-sample t-tests were performed for each data point to assess significant differences between WT and TrpM5⁻/⁻ females calorie intake. Results were not significant (p-values ≥ 0.175).
Figure 27: Change in body weight of females on a control diet and 60% high fat lard diet. Average change in body weight, ± SEM, on a run-in diet (A) for 7 days, or a 60% high fat lard diet (B) for 53 days. TrpM5−/− females n=10, WT females n=10. Two-sample t-tests were performed to assess significant differences between WT and TrpM5−/− females change in body weight. Results were significant for the run-in diet period (p-value= 0.002). Results were also significant for the high-fat diet period (p-value= 0.0004).
Figure 28: Change in lean mass of females on a control diet and 60% high fat lard diet. Average change in lean mass, ± SEM, on a run-in diet (A) for 7 days, or a 60% high fat lard diet (B) for 53 days. TrpM5⁻/⁻ females n=10, WT females n=10. Two-sample t-tests were performed to assess significant differences between WT and TrpM5⁻/⁻ females change in lean mass. Results were not significant for the run-in diet period (p-value=0.874). Results were significant for the high-fat diet period (p-value=0.0012).
Figure 29: Change in fat mass of females on a control diet and 60% high fat lard diet. Average change in fat mass, ± SEM, on a run-in diet (A) for 7 days, or a 60% high fat lard diet (B) for 53 days. TrpM5⁻/⁻ females n=10, WT females n=10. Two-sample t-tests were performed to assess significant differences between WT and TrpM5⁻/⁻ females change in fat mass. Results were slightly significant for the run-in diet period (p-value=0.046). Results were significant for the high-fat diet period (p-value=0.0171).
Weight loss

TrpM5⁻/⁻ females show no significant differences in rate of weight loss after being on a control diet for four weeks when compared to their wildtype counterparts. However, a significant difference in rate of weight loss is seen between WT and KO females who had previously been on the 60% high fat diet. The TrpM5⁻/⁻ females lost weight much more slowly than the C57bl/6 WT females (Fig. 30). In terms of body composition, in mice who had been on the control diet, there were no significant differences between WT and KO females in changes in lean mass. There were, however, significant differences in fat mass following the food deprivation period, with WT females losing significantly more fat mass than the TrpM5⁻/⁻ females. There were also significant differences, between WT and KO mice who had previously been on the 60% high fat diet, in changes in both lean and fat mass. TrpM5⁻/⁻ females lost significantly more lean mass, but lost significantly less fat mass when compared to the C57bl/6 WT females (Figs. 31, 32).
Figure 30: Body weight percentages of females over days on food deprivation.
Average percent body weight shown ± SEM. Mice were removed from the study as soon as they reached 80% of their starting body weight prior to beginning the deprivation. TrpM5⁻/⁻ females on control diet n=8, WT females on control diet n=8 (A). TrpM5⁻/⁻ females on HF diet n=8, WT females on HF diet n=8 (B). One-way ANOVA was used to assess any overall differences between WT females and TrpM5⁻/⁻ females on either a control diet or a HF diet prior to beginning a food deprivation period. Results were not significant for the control (p-value= 0.9518) and significant for the HF diet group (p-value= 0.0141).
Figure 31: Change in lean mass of females on a control diet or high fat diet. Average change in lean mass, ± SEM, on either a run-in diet (A) or a 60% high fat lard diet (B) for 28 days prior to beginning a food deprivation period. TrpM5<sup>−/−</sup> females on control diet n=8, WT females on control diet n=8. TrpM5<sup>−/−</sup> females on HF diet n=8, WT females on HF diet n=8. Two-sample t-tests were performed to assess significant differences between WT and TrpM5<sup>−/−</sup> females change in lean mass. Results were not significant for the control diet group (p-value= 0.4001). Results were significant for the high-fat diet period (p-value= 0.0006).
Figure 32: Change in fat mass of females on a control diet or high fat diet. Average change in fat mass, ± SEM, on either a run-in diet (A) or a 60% high fat lard diet (B) for 28 days prior to beginning a food deprivation period. TrpM5⁻⁄⁻ females on control diet n=8, WT females on control diet n=8. TrpM5⁻⁄⁻ females on HF diet n=8, WT females on HF diet n=8. Two-sample t-tests were performed to assess significant differences between WT and TrpM5⁻⁄⁻ females change in fat mass. Results were significant for the control diet group (p-value= 0.0270). Results were significant for the high-fat diet period (p-value= 0.0001).
Response to differing fatty acid profiles in high fat diets in female mice

Saturated High Fat diet: Female TrpM5<sup>−/−</sup> mice placed on a control diet for two weeks, then switched to a 60% saturated high fat diet for 6 weeks show no significant differences in caloric intake when compared to their wildtype counterparts (Fig. 33). Despite similar caloric intakes, a significant difference in body weight between KO and WT mice is observed, with TrpM5<sup>−/−</sup> females gaining significantly less weight than WT C57bl/6 females, just as was seen in the males (Fig. 34). Additionally, there are no significant differences in lean or fat mass changes while on the control diet between the TrpM5 KO females and the WT females. However, on the saturated high fat diet period, significant differences were observed in the change in lean mass, with the TrpM5 deficient females gaining significantly less lean mass than the WT females. There were no significant differences in fat mass changes on the high fat period between the two groups (Figs. 35, 36).
Figure 33: Cumulative calorie intake of females on a 60% saturated high fat diet. Average cumulative caloric intake (in Kcal) per day ± SEM, over days of the study. TrpM5⁻/⁻ females n=7, WT females n=10. Two-sample t-tests were performed for each data point to assess significant differences between WT and TrpM5⁻/⁻ females calorie intake. Results were not significant (p-values ≥ 0.212).
Figure 34: Change in body weight of females on a control diet and 60% saturated high fat diet. Average change in body weight, ± SEM, on a run-in diet (A) for 14 days, or a 60% saturated high fat diet (B) for 42 days. TrpM5^{-/-} females n=7, WT females n=10. Two-sample t-tests were performed to assess significant differences between WT and TrpM5^{-/-} females change in body weight. Results were not significant for the run-in diet period (p-value= 0.9044). Results were significant for the high-fat diet period (p-value= 0.0194).
Figure 35: Change in lean mass of females on a control diet and 60% saturated high fat diet. Average change in lean mass, ± SEM, on a run-in diet (A) for 14 days, or a 60% saturated high fat diet (B) for 42 days. TrpM5⁻/⁻ females n=7, WT females n=10. Two-sample t-tests were performed to assess significant differences between WT and TrpM5⁻/⁻ females change in lean mass. Results were not significant for the run-in diet period (p-value= 0.8166). Results were significant for the high-fat diet period (p-value= 0.0188).
Figure 36: Change in fat mass of females on a control diet and 60% saturated high fat diet. Average change in fat mass, ± SEM, on a run-in diet (A) for 14 days, or a 60% saturated high fat diet (B) for 42 days. TrpM5−/− females n=7, WT females n=10. Two-sample t-tests were performed to assess significant differences between WT and TrpM5−/− females change in fat mass. Results were not significant for the run-in diet period (p-value= 0.9150). Results were also not significant for the high-fat diet period (p-value= 0.2421).
**Unsaturated High Fat diet:** Female mice lacking TrpM5 show no significant differences in caloric intake from the wildtype females when placed on a control diet for two weeks, then switched to a 60% primarily unsaturated fat diet (Fig. 37). Female TrpM5−/− mice show no differences in body weight change, compared to the WT females, when on the control diet. However, during the high fat feeding period, KO females gain significantly less weight than the WT females (Fig. 38). Additionally, while on the run-in diet, there are no significant differences between wildtype and knock-out females in terms of changes in lean and fat mass. However, during the high fat feeding period, there are significant differences in lean mass, with the TrpM5 KO females gaining significantly less lean mass than the WT females. There were no significant differences between the groups in fat mass changes during the high fat feeding period (Figs. 39, 40).
Figure 37: Cumulative calorie intake of females on a 60% unsaturated high fat diet. Average cumulative caloric intake (in Kcal) per day ± SEM, over days of the study. TrpM5⁻/⁻ females n=6, WT females n=10. Two-sample t-tests were performed for each data point to assess significant differences between WT and TrpM5⁻/⁻ females calorie intake. Results were not significant (p-values ≥ 0.877).
Figure 38: Change in body weight of females on a control diet and 60% unsaturated high fat diet. Average change in body weight, ± SEM, on a run-in diet (A) for 14 days, or a 60% saturated high fat diet (B) for 42 days. TrpM5<sup>-/-</sup> females n=6, WT females n=10. Two-sample t-tests were performed to assess significant differences between WT and TrpM5<sup>-/-</sup> females change in body weight. Results were not significant for the run-in diet period (p-value= 0.3475). Results were significant for the high-fat diet period (p-value= 0.0006).
Figure 39: Change in lean mass of females on a control diet and 60% unsaturated high fat diet. Average change in lean mass, ± SEM, on a run-in diet for 14 days, or a 60% unsaturated high fat diet for 42 days. TrpM5\(^{-/-}\) females n=6, WT females n=10. Two-sample t-tests were performed to assess significant differences between WT and TrpM5\(^{-/-}\) females change in lean mass. Results were significant for the run-in diet period (p-value= 0.0151). Results were significant for the high-fat diet period (p-value= 0.0002).
Figure 40: Change in fat mass of females on a control diet and 60% unsaturated high fat diet. Average change in fat mass, ± SEM, on a run-in diet (A) for 14 days, or a 60% unsaturated high fat diet (B) for 42 days. TrpM5−/− females n=6, WT females n=10. Two-sample t-tests were performed to assess significant differences between WT and TrpM5−/− females change in fat mass. Results were not significant for the run-in diet period (p-value= 0.1958). Results were also not significant for the high-fat diet period (p-value= 0.4987).
Fecal lipid content on a saturated and unsaturated high fat diet

**Lipid extraction on a saturated high fat diet:** Female mice lacking TrpM5, who had previously been on a control diet for 2 weeks followed by a 60% primarily saturated high fat diet for 6 weeks show significant differences in the amount, in percentage per mille, (percentage per 1000), of excreted lipids when compared to their wildtype counterparts. KO females excreted, on average, 14.25‰ compared to the WT females who had an average of 17.64‰ (Fig. 41).

**Lipid extraction on an unsaturated high fat diet:** Female TrpM5^{−/−} mice, who had previously been on a control diet for 2 weeks followed by a 60% primarily unsaturated high fat diet for 6 weeks show significant differences in the amount, in percentage per mille, (percentage per 1000), of excreted lipids when compared to their wildtype counterparts. KO females excreted, on average, 13.98‰ compared to the WT females who had an average of 17.12‰ (Fig. 41).
Figure 41: Lipid content in feces in female mice on a saturated high fat diet (SHF) or an unsaturated high fat diet (UHF). Average lipids, in percent per mille ± SEM, excreted in the feces after six weeks of a saturated or unsaturated high fat diet. TrpM5−/− females n=7, WT n=10, for the saturated HF diet. TrpM5−/− n=6, WT n=10, for the unsaturated HF diet. Two-sample t-tests were performed to assess significant differences between WT and TrpM5−/− mice fecal lipid content. Results were significant for the saturated HF diet (p-value = 0.0137) as well as the unsaturated HF diet (p-value= 0.028).
Aim 5: The Role of TrpM5 in the Transduction Pathway of Other Tastants

Male and female TrpM5<sup>−/−</sup> mice show no significant differences in calorie intake when placed on a control diet for 7 days then switched to a 70% high sucrose diet (Fig. 42). Males additionally show no significant differences in weight gain on the control or the high sucrose feeding periods (Fig. 43). Females, however, show significant differences in body weight, both during the control period as well as the high sucrose period, with the KO females gaining significantly less weight than the wildtype females (Fig. 44).
Figure 42: Cumulative calorie intake on a high sucrose diet. Average cumulative caloric intake (in Kcal) per day ± SEM, over days of the study. TrpM5−/− n=10, WT n=10, for both males and females. Two-sample t-tests were performed for each data point to assess significant differences between WT and TrpM5−/− calorie intake. Results were not significant (p-value ≥ 0.3633 for males, and p-value ≥ 0.4273 for females).
Figure 43: Change in body weight of males on a control diet and high sucrose diet. Average change in body weight, ± SEM, on a run-in diet (A) for 8 days, or a high sucrose diet (B) for 34 days. TrpM5⁻/⁻ males n=10, WT males n=10. Two-sample t-tests were performed to assess significant differences between WT and TrpM5⁻/⁻ males change in body weight. Results were not significant for the run-in diet period (p-value = 0.0144). Results were also not significant for the high-fat diet period (p-value = 0.0006).
Figure 44: Change in body weight of females on a control diet and high sucrose diet. Average change in body weight, ± SEM, on a run-in diet (A) for 8 days, or a high sucrose diet (B) for 34 days. TrpM5⁻/⁻ females n=10, WT females n=10. Two-sample t-tests were performed to assess significant differences between WT and TrpM5⁻/⁻ females change in body weight. Results were significant for the run-in diet period (p-value= 0.0144). Results were also significant for the high-fat diet period (p-value= 0.0006).
Discussion

While still controversial, fat is closer to becoming accepted as a basic taste primer. Texture and smell, which used to be considered the sole sensory cues for fat, are not necessary for fat, in the form of fatty acids, to be detected in the oral cavity (Besnard et al., 2015, Mattes, 2009). Many studies involving both rodents and humans have shown that fatty acids elicit a unique chemosensory experience. Previous studies have shown that rodents are capable of detecting the presence of fatty acids, even while smell and texture of the fatty acids are blocked (Fukuwatari et al., 2003). Humans can also distinguish not only if fat is present, but can also discriminate between concentrations and types of fatty acids (Stewart et al., 2010). From this evidence, it can be argued that fatty acids act as a unique taste stimuli and elicit a response in the form of a taste of fat from the oral cavity.

The taste of fatty acids is believed to be transduced by a pathway very similar to that used in sweet, bitter, and umami taste transduction. For these three classes of aforementioned tastants, this pathway employs a variety of different G-protein coupled receptors, (GPCRs), which are unique for each class of tastants. After ligand binding, these GPCRs start a transduction cascade that eventually leads to the release of intracellular calcium, membrane depolarization through TrpM5 cation channels, and eventual release of neurotransmitter (Huang and Roper, 2010). This pathway has been elucidated by a number of different techniques, including functional cellular imaging, in vitro assays such as patch clamp recording, and corresponding molecular expression data.
corroborating the presence of all these channels and elements involved (Liu et al., 2011, Peréz et al., 2002; Shah et al., 2012).

In addition to sweet, bitter, and umami taste transduction, this same pathway has also been shown to be involved in long-chain polyunsaturated fatty acid (PUFA) taste transduction. The same mechanisms, including GPCRs, PLCβ2, IP3R3, and calcium release, lead to eventual TrpM5 activation, which leads to depolarization and cell signaling through the release of neurotransmitter (Gilbertson et al., 2010; Kaske et al., 2007; Zhang et al., 2007). One highly important element in this pathway is TrpM5, the cation channel responsible for depolarizing the taste cell in response to activation by fatty acids. Studies have shown that without TrpM5, diminished responses occur; specifically, decreased membrane depolarization and decreased subsequent cellular response (Liu et al., 2011; Shah et al., 2012).

In addition to the changes seen at the cellular level, removing TrpM5 in a rodent model can also result in changed responses at the whole organism level, in the form of measurable phenotypic differences. Previous studies have shown that TrpM5−/− (KO) mice show decreased or abolished responses, in terms of preference and intake, to sweet, bitter, and umami stimuli (Damak et al., 2006). In my own work, to validate the importance of TrpM5 in fatty acid signal transduction, TrpM5 knockout mice were used to show the role of TrpM5 in fat preference, calorie intake, and subsequent body composition. In my studies, and previous studies done in the Gilbertson lab, it’s been found that TrpM5 KO mice show a decreased taste-mediated response to fatty acids. Through the use of a conditioned taste aversion assay (CTA), I show that TrpM5−/− mice are incapable of
forming an aversion to the long-chain polyunsaturated fatty acid, linoleic acid (LA). This result is seen in both males and females lacking TrpM5. This result is contrasting to what is seen in wildtype mice; both males and females can form an aversion against LA and can detect the fatty acid at concentrations as low as 1 µM. From this, it can be inferred that mice lacking TrpM5 are incapable of detecting the fatty acid when exposed to it for a brief period. This helps exemplify the critical role of TrpM5 in the LCPUFA taste transduction pathway and fat taste recognition in the oral cavity.

The importance of TrpM5 in fatty acid detection is further verified by placing both WT and KO males on a high fat diet. Male mice lacking TrpM5 show significant differences in calorie intake when placed on a 1:1 (unsaturated: saturated) high fat diet compared to the wildtype males. WT males eat significantly more of the high fat diet. Fatty foods are known to activate pleasure centers in the brain. This reward pathway, brought on by tasting fat, encourages further fat intake; acting as a positive feedback mechanism. In mice lacking TrpM5, the diminished activation of taste cells, leading to a decreased signal to the pleasure centers and therefore, a decreased cephalic phase response, could explain the lower caloric intake compared to the wildtype males. Knockout males, because they lack TrpM5, find the high fat diet much less rewarding than the WT males and consume less of the high fat diet (Berridge et al., 2010; Oliveira-Maia, 2011).

This result is not seen in the female mice. TrpM5⁻/⁻ females do not show a decreased calorie intake compared to their wildtype counterparts on the same high fat diet. Despite not being able to ‘taste’ fat, as discovered with the CTA, female KOs show
no differences in how much of a high fat diet they consume compared to wildtypes; the
two groups have almost identical caloric intakes. This leads to the possible conclusion
that TrpM5 might play sex-specific roles in males and females, with differences
occurring at the level of intake. Additionally, this apparent lack of response in females
without TrpM5 could be due to an unrelated or redundant pathway that monitors caloric
intake, only found in females. This pathway would have to be unrelated to fat taste, since
it’s been shown that TrpM5⁻/⁻ females are also incapable of tasting fat, just like males.

In a seminal study by Wade and Schneider (1992), energy intake is shown to be
highly affected by estradiol and ovarian hormones in females. Females require significant
amounts of energy to maintain a regular reproductive cycle, so strong means of energy
regulation must exist. They found that changes in macronutrient availability, like fat, can
result in an adjusted response, mainly from estradiol, in compensating for that lack of
energy. Additionally, they found that estradiol can act in the brain to increase food intake
and other regulatory functions related to nutrient intake (Wade and Schneider, 1992). It is
plausible that this is the case in females lacking TrpM5. If a component of a crucial
nutrient sensing pathway is missing, i.e., TrpM5 in the fat taste pathway, an estradiol
response could adjust calorie intake to ensure adequate energy is available to maintain
regular cycling.

Another possible mechanism for the different phenotypic response in females
(i.e., no apparent decrease in calorie intake), could be due to an increased expression of
TrpM4 in females compared to males. TrpM4 is in the same family of TrpM5, the
melastatin subfamily. TrpM4 is characterized as a non-selective monovalent cation
channel; much like TrpM5, it is activated by calcium and activation leads to cellular depolarization and response (Nilius et al., 2004). TrpM4 is also a plasma membrane protein (Launay et al., 2002). TrpM4 is widely expressed throughout the body, including the immune system as well as the gastrointestinal tract (Guinimard et al., 2011). In a study by Liu et al., they found that TrpM4 was also expressed in the oral cavity (Liu et al., 2011). The exact role of TrpM4 in the oral cavity remains elusive, but it is possible that this channel could act as a protective mechanism for fat intake in females. These plausible pathways could be there simply to monitor calorie intake and ensure that adequate calories were being consumed.

Previous studies have shown that the TrpM5 transduction pathway is primarily involved in the taste transduction of long-chain PUFAs (Hara et al., 2014; Laugerette et al., 2007; Shah et al., 2012). I verify this in my current work through altering the fatty acid profile of a high fat diet. On a high fat diet with an equal amount of saturated and unsaturated fatty acids (1:1), we see a pronounced phenotypic response in males without TrpM5. These males eat significantly less than the WT males. To test the specificity of the TrpM5 fatty acid taste transduction pathway, I then compared WT and TrpM5−/− mice on an altered high fat diet, one with comparatively little unsaturated fatty acids and primarily saturated fatty acids (10:1 SFA: UFA), to see if the phenotypic response would be lessened because there would be a much smaller portion of the diet that we would expect to be transduced using this pathway. The results do indeed show a lessened phenotypic response in calorie intake. I saw no significant differences between the WT and the KO males in calorie intake on this saturated high fat diet.
On an unsaturated high fat diet, (1: 3.3; SFA: UFA), the phenotypic response of calorie intake is increased from the 1:1 high fat. More of the diet is composed of fatty acids that are transduced through the TrpM5 pathway, so there is a stronger phenotypic effect of knocking TrpM5 out. As expected, male KOs eat significantly less calories than WT males when placed on a primarily unsaturated high fat diet. From this, it can be inferred that the fatty acid transduction pathway involving TrpM5 is primarily involved in the detection of long-chain polyunsaturated fatty acids, like linoleic acid or linolenic acid, both of which are considered essential fatty acids.

These phenotypic differences in response to the fatty acid profile of the diet are only seen in males. Again, just as was seen in the females on the 1:1 high fat diet, there were no significant differences in calorie intake in females with or without TrpM5 on either the unsaturated or the saturated high fat diets. Again, this observable sex difference could be due to a sex-specific pathway, possibly involving estrogen, that encourages and monitors calorie intake to ensure adequate calories are being consumed, unrelated to the ability to taste fat (Wade and Schneider, 1992).

Additionally, I show here that TrpM5 plays a role post-ingestively, and can affect body composition. In TrpM5−/− males, we see a decreased caloric intake on the 1:1 and the unsaturated high fat diets. TrpM5−/− males also show a corresponding decreased weight gain, as well as decreased gains in both lean and fat mass. This result is intuitive; taking in fewer calories leads to gaining less weight. However, on the saturated high fat diet, there were no significant differences in calorie intake between KO and WT males, yet despite this similar caloric intake, there is still a significant difference in weight gain.
TrpM5<sup>−/−</sup> males consume the same amount of calories as the WT males, yet gain significantly less weight than the WTs. Additionally, there were no significant differences in changes in fat or lean mass gain on the saturated high fat diet. This difference in weight gain appears to be proportionate to the difference in calorie intake in male mice. A larger difference in weight gain is seen on the unsaturated and 1:1 high fat diets, where there are larger differences in calorie intake, than on the saturated high fat diet, where no differences in calorie intake were observed. On each diet, we see a very different phenotypic response between WT males and TrpM5<sup>−/−</sup> males, with the result of TrpM5<sup>−/−</sup> males gaining less weight. From this, it can be concluded that TrpM5 not only affects oral detection of fatty acids, but may also play an undefined role downstream in body composition and weight gain.

In females, this concept is further verified. Despite similar caloric intakes on all three diets, female TrpM5<sup>−/−</sup> mice gain significantly less body weight than the WT females. Contrary to what would be expected, we observe an incongruence of nutrient intake and resulting weight gain; the amount of calories consumed does not dictate the amount of weight gained in the TrpM5<sup>−/−</sup> females. Even if the same amount and quality of nutrients are being ingested, there is a consistent difference observed in weight gain between WT and KO female mice. Something downstream, after ingestion of fatty acids, is being affected by TrpM5 and causing decreased weight gain in mice that do not have TrpM5. One possible hypothesis is that nutrient (specifically lipid) metabolism is different in mice lacking TrpM5.
To quantify these differences in nutrient metabolism, I hypothesized that TrpM5 knockout mice were metabolizing lipids differently than the WT mice. In the oral cavity, after fatty acids activate taste cells, depolarization through TrpM5 allows for neurotransmitters, like ATP, to be released. ATP then interacts with afferent nerves that innervate the taste cells and a signal is sent to the nucleus of the solitary tract, and then on to the gustatory cortex (Kinnamon and Finger, 2013). It is here that these signals, mediated by taste stimuli, are decoded into a unique chemosensory experience, such as the ‘taste’ of fat. Intestinal epithelial cells are known to also express the TrpM5 FA detection pathway, the same pathway found in taste cells on the tongue (Bezencon et al., 2007; Kaske et al., 2007). The role of this pathway in the oral cavity is to detect fatty acids that are about to be consumed. In the intestines, this pathway is thought to aid in preparing the gut to digest incoming fatty acids by producing cholecystokinin (CCK) and other pancreatic enzymes that are necessary for digesting food (Kokrashvili et al., 2009).

It is hypothesized that, from here, a reflexive efferent signal is then sent to the gut that affects lipid digestion, lipid storage, peptide secretions, and chylomicron synthesis. This response of the gut has been shown to be neurally-mediated, meaning that activation in the oral cavity, leading to activation in the gustatory cortex, is sufficient to produce these changes in the gut. This is evidenced in current research showing that sham feeding of fatty foods, (oral exposure without ingestion), leads to the same gut response: elevated levels of enzymes and hormones needed for digestion of fat. This physiological response from oral detection alone was of the same magnitude as is seen with actual ingestion of fat (Chavez-Jauregui et al., 2010; Mattes, 2001 and 2010; Petit et al., 2007). In a study by
Laugerette et al., (2005) they showed that this response did not occur in mice lacking CD36, an element in the TrpM5 transduction pathway. CD36<sup>-/-</sup> mice did not show increases in pancreatic secretions needed for fat digestion after oral exposure to LCFAs (Laugerette et al., 2005). Disrupting this signaling pathway, by knocking out TrpM5, leads to a decreased signal being sent from the oral cavity; fatty acids are not able to be detected as thoroughly, if at all, and therefore a lesser signal could be sent from the oral cavity to the gustatory cortex, and from the gustatory cortex to the gut- leading to a lesser physiological response to fat. This helps underscore the importance of the oral detection of fatty acids in preparing for lipid digestion and metabolism.

In my current study, through collected fecal samples of male and female, WT and KO mice, I have shown that TrpM5 does alter some aspect of lipid metabolism, which is verified by significant differences in lipid content of feces. Since TrpM5<sup>-/-</sup> mice gained significantly less weight than WT mice, I hypothesized that the KOs would excrete more lipids, possibly indicating that lipids were not processed correctly and more lipids were simply being excreted out in the feces opposed to being utilized and stored properly. Contrary to what was hypothesized, however, TrpM5 KO mice excreted significantly less lipids than the WT mice. This same result was seen in all three diets, in both males and females, despite differences in caloric intake trends in males and females.

One possible explanation for this could be due to differences in serum triglyceride levels. In a study by Drover et al., they found that mice lacking CD36 have significantly higher serum triglyceride levels compared to WT mice. Blood triglyceride is largely determined by clearance levels of chylomicrons. Chylomicrons are responsible for
carrying lipids and other molecules from the intestines, and through the blood to their target destinations, i.e., fat storage areas such as white and brown adipose tissue. In the study by Drover et al., they found that plasma lipase activity and chylomicron clearance were affected. These together lead to more triglycerides being in the blood in mice lacking CD36 (Drover et al., 2005).

Although these studies were done in CD36 deficient mice, it is plausible that a similar effect could be happening in mice lacking TrpM5. CD36 is an important element in the TrpM5 transduction pathway, in that it is a possible receptor for LCPUFAs. The net effect, in terms of fatty acid detection, should be similar if either CD36 or TrpM5 is missing; the result of this being decreased detection of fatty acids, and decreased intestinal response to ingested fatty acids. Indeed, male mice lacking CD36 show similar, though less extreme, reductions in fat intake compared to TrpM5\textsuperscript{-/-} mice on a high fat diet (Minaya, unpublished data). Because there were less fatty acids being excreted in mice lacking TrpM5, determining if they have higher plasma lipid levels could help explain the lower weight gain with lower lipids being excreted.

After determining that TrpM5 plays a role in downstream processes and affects weight gain, I aimed to test if TrpM5 played any role in weight loss. In males, there were no significant differences in rate of weight loss between KOs and WT mice. This was unexpected; I hypothesized that TrpM5 deficient mice would be less susceptible to weight loss than their wildtype counterparts since they were impervious to weight gain and seemed to maintain overall more stable measures in terms of body composition.
However, this was not shown. Both WT and TrpM5\(^{-/-}\) males lost weight at similar rates, regardless of what their diet had been prior to the deprivation period.

In females, there were significant differences in rate of weight loss, but only in females who had been on a high fat diet prior to deprivation. These KO females lost weight at a significantly slower rate, taking almost twice as long as the WT females to lose 20% of their body weight. This result was not seen in the females who had been on a control diet before the deprivation period. The KO females maintained a consistent percentage of body fat throughout the entire study, despite losing weight. Their body fat percentage was at 10% prior to starting the study, and this percentage was maintained during the high fat feeding period as well as during the deprivation period. This phenomenon was not seen in any other group.

While interesting, these results have many limitations. The deprivation was quite severe, allowing animal’s access to only 60% of their normal caloric intake for the entire deprivation period. Due to this, all mice lost weight very quickly. If the deprivation had been less significant, maybe allowing access to 75% of their normal calorie intake, more definitive results may have been obtained. There are apparent differences in the slope of the weight loss between WT and KO males, however due to the short duration of the study, these results were not significant. By extending the weight loss period, more distinctions and patterns might appear between the wildtypes and the knockouts, allowing more insight into the possible role of TrpM5 in weight loss.

TrpM5 has previously been shown to be involved in the sweet taste transduction pathway (Chandrashekar et al., 2006). Studies done previously have also shown that mice
lacking TrpM5 are unable to form an aversion to sweet tastants and have a decreased preference for sweet stimuli (Damak et al., 2006; Sclafani et al., 2007; Zhang et al., 2003). Here, however, I show that knocking out TrpM5 does not play a significant role in calorie intake on a high sucrose diet over a six-week period. This is in agreement with data showing that there might be another pathway also involved in detecting sweet stimuli in the oral cavity (Damak et al., 2003; Eddy et al., 2012).

Another theory is that the TrpM5 pathway might be responsible for taste detection of sweet stimuli, but there might be another pathway responsible for the intake of sweet stimuli, something that is responsive to the caloric load found in sweet foods. A number of glucose transporters, homologous to the receptors found in the pancreas, have been shown to be expressed in the enteroendocrine cells in the intestines as well as in taste receptor cells in the oral cavity (Kojima and Nakagawa, 2011; Merigo et al., 2011). Yee et al., also found similar results: several glucose transporters, like GLUT2, are expressed in taste cells. They also discovered that ATP-gated $K^+$ ($K_{ATP}$) channels are also expressed in select taste cells. These $K_{ATP}$ channels are components of a metabolic sensor and play a significant role in maintaining glucose homeostasis in other parts of the body. While the exact role of these glucose sensors in the oral cavity is still debated, it is highly suggestive of a role in regulation and intake of sweet stimuli (Yee et al., 2011). These glucose sensors and transporters could explain why mice lacking TrpM5 do not show the decreased calorie intake on the high sucrose diet. Expression of glucose sensors in the taste receptor cells could provide another means of carbohydrate (sweet) detection in the oral cavity.
In summary, TrpM5 has proven to play a significant role in oral fatty acid detection as well as in downstream processing of fatty acids, possibly affecting lipid metabolism and mobilization. Body composition and weight status are influenced by TrpM5 and its role in both the oral cavity as well as in the enteroendocrine cells in the gut. Although further research is needed, TrpM5 appears to play a role in rate of weight loss, primarily in the female KOs. This leads to the assumption that TrpM5 might play different roles, or be of differing importance in the sexes. However, complete elucidation of TrpM5’s differential role in the sexes still remains a necessary area of research. Additionally, I’ve shown that TrpM5 is primarily involved in the detection of long-chain polyunsaturated fatty acids. It doesn’t appear to be involved in the transduction pathway of saturated fatty acids.

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Coding of sweet, bitter, and umami tastes: Different receptor cells sharing similar

CHAPTER III

SUMMARY AND FUTURE DIRECTIONS

In my current work, I have verified that TrpM5 is crucial in long chain polyunsaturated fatty acid taste detection in the oral cavity, and it also may contribute downstream to body composition and lipid excretion. This pathway, both in the oral cavity as well as in the enteroendocrine cells is primarily involved in long-chain polyunsaturated fatty acid transduction and does not play a significant role in the taste transduction of saturated fatty acids. Additionally, I’ve shown that the TrpM5 pathway might not be the only taste transduction or intake pathway for sweet stimuli. Finally, I have shown that there is a sex-difference associated with TrpM5.

In this study, I aimed to determine the role of TrpM5 in both weight loss and weight gain using mice who lacked the TrpM5 cation channel. The results show that TrpM5 plays a definite role in weight gain; all mice lacking TrpM5 gain significantly less weight than wild-type mice, independent of calorie intake. This was seen in both males and females. In terms of weight loss, the question of the exact role of TrpM5 still remains. In males, no significant difference in rate of weight loss was seen, though the slopes of the lines do allude to differences. In females, a significant difference was observed in TrpM5−/− females who had previously been on the high fat diet, with the KOs losing weight significantly slower than the wild-type females.

Due to these ambiguous results, determining the exact role TrpM5 may or may not play in weight loss remains elusive. Further studies need to examine this possible
phenomenon more thoroughly. By including a less severe deprivation, of around 75% normal calorie intake, and therefore extending the weight loss period, more definitive results might be obtained. If this period is longer, more distinctions between the groups might occur and the exact role, if any, of TrpM5 in weight loss could be elucidated.

While TrpM5 does appear to play a role in weight gain, it is still uncertain what the exact mechanism is for this observation. I hypothesized that TrpM5 being absent was affecting the processing and storing of ingested lipids, (specifically fatty acids). To test this, I collected feces and extracted lipids to measure and compare total lipid content in the KOs and WTs, both males and females. There is a significant difference in the amount of lipids being excreted, however it was contrary to my hypothesis. TrpM5\(-/-\) mice, both male and female, excreted significantly less lipids than their WT counterparts.

To determine where this discrepancy in lipids is coming from, a number of studies could be performed in the future. These could include a more thorough analysis of fecal lipid content, determining the fatty acid profile found in these excreted lipids. In my study, I only analyzed total lipid content, which includes fatty acids, sterols, phospholipids and more. Deciphering the exact content of the excreted lipids would be beneficial in understanding differences in processing and excretion of fatty acids in mice with or without TrpM5.

Additionally, analyzing blood lipid levels could help elucidate these differences in lipid processing as well. Collecting blood samples to measure lipid levels at baseline as well as postprandial lipid levels could provide answers. This could be done by measuring the amount of Vitamin A in the blood at any given time. Vitamin A is a fat soluble
vitamin and is therefore a good measure of how much lipid is present in the blood. Blood lipid levels are a very common measure to determine lipid metabolism and could provide needed answers for the role of TrpM5 in lipid processing.

Throughout all of the studies I have done, it has been clear that there are significant sex differences present in the females. Metabolically, we see very similar responses in males and females lacking TrpM5: they both gain less weight while being on a high fat diet. The only real differences come into play when calorie intake is considered. Females lacking TrpM5 do not show the decreased caloric intake seen in males; female KOs eat the same amount as female WTs. Despite this, female TrpM5−/− mice still gain significantly less weight than the wildtypes.

This leads to a myriad of further questions. Determining what is different in the females from the males, which causes the matched caloric intake of the wildtypes, yet still results in decreased weight gain is imperative. I previously hypothesized that the matched calorie intake in females might be due to an unrelated pathway that monitors calorie intake, which is unrelated to the taste of fat. Additionally, I hypothesized that this could be due to an increased expression of TrpM4 in female mice, which could act as a secondary protective mechanism for fat intake. While these hypotheses potentially explain this sex-specific phenotypic difference in calorie intake, an explanation for how TrpM5−/− females eat an isocaloric meal, but gain significantly less weight than the WT females is still needed. This alludes to the idea that TrpM5 is altering something downstream, (such as lipid processing), and is responsible for the differences in weight
gain. This mechanism would be similar in both males and females, while still allowing for the discrepancies seen in calorie intake between the sexes and their WT counterparts.

To validate what mechanism is actually at play and is responsible for these sex-differences, much further research needs to be done in the future. Some possible studies could include *in-vitro* assays of female taste-cells, taken from mice, to measure the cellular response to fatty acids in comparison to males. Additionally, evaluating gene expression to quantitatively measure expression levels of TrpM4 in both males and females could provide insight. If females do show increased expression of TrpM4 compared to males, studying the exact role of TrpM4 in the taste system would be crucial. This could be addressed through further *in-vitro* assays, such as patch clamping, to monitor how TrpM4 contributes to a cellular response to fatty acids. Lastly, another approach could be examining the role of sex-specific hormones on weight gain and calorie intake. Determining the role of these hormones, such as estrogen, and how differing levels of hormones, dependent on a female’s cycle, contribute to intake, weight gain, and overall body composition will be necessary. All of these approaches could provide needed insight to better understand all the physiological differences observed that are due to TrpM5, sex, and any interactions between the two.

While much has been elucidated about the role and specificity of TrpM5 in this current study, there are many more studies and ideas that need to be verified to fully quantify the role of TrpM5 in the fatty acid taste transduction pathway in both the gut and the oral cavity.