The Role of TRPM5 in Dietary Fat Preference, Intake, and Body Composition

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THE ROLE OF TRPM5 IN DIETARY FAT PREFERENCE, INTAKE, AND BODY COMPOSITION

by

Dulce M. Minaya

A thesis submitted in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE in

Biology

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UTAH STATE UNIVERSITY
Logan, Utah

2014
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ABSTRACT

The Role of Trpm5 in Dietary Fat Preference, Intake, and Body Composition

by

Dulce M. Minaya, Master of Science
Utah State University, 2014

Major Professor: Timothy A. Gilbertson
Department: Biology

We recently showed a critical role of Trpm5 in the transduction pathway for long chain polyunsaturated fatty acids. In the present study, I have begun to investigate dietary fat preference and the propensity to develop dietary-induced obesity in Trpm5\(^{-/}\) mice. My preliminary data shows that in male mice placed on a high fat diet, Trpm5\(^{-/}\) mice did not enhance their caloric intake as observed in wild type mice. Most surprisingly, however, was that I did not observe the same differences in between female mice, which posits a potential gender effect of this pathway on dietary fat intake. Also, I show that the preference for dietary fat is not disrupted in Trpm5\(^{-/}\) mice since there is no difference in dietary fat preference between Trpm5\(^{-/}\) and wild type mice. Wild type and Trpm5\(^{-/}\) mice both have a strong preference for the high fat diet, as demonstrated by the fact that they solely consumed the high fat diet. Consistent with our original hypothesis that these responses are specific for high fat feeding, I did not observe any differences in caloric intake in male mice on a high sucrose diet. Again, gender differences were observed, with Trpm5\(^{-/}\) female mice displaying a higher caloric intake than wild type female mice.
Furthermore, I used a paired-feeding approach via oral gavage to delve further into whether the effect of Trpm5 disruption was due to pre- or post-ingestive effects. The results from this experiment show that all animals have a reduction in body weight and body fat with no significant difference between wild type and Trpm5$^{-/-}$ mice. This result suggests that the expression of Trpm5 in the oral cavity is necessary for the changes in body weight and composition observed during ad libitum feeding. Also, the fact that Trpm5$^{-/-}$ mice lost body weight and fat mass is contrary to our previous observations. When these animals consume the roughly same number of calories on a high fat diet ad libitum, we observe an increase in body weight and fat mass. This suggests that there might be another mechanism accountable for the response observed in Trpm5$^{-/-}$ mice when fed ad libitum. In conclusion, the results from these experiments suggest a link between dietary fat consumption and development of adiposity.

(79 pages)
PUBLIC ABSTRACT

The Role of Trpm5 in Dietary Fat Preference, Intake, and Body Composition

Dulce M. Minaya

We recently showed the protein Trpm5 plays a critical role in the transduction pathway for long chain polyunsaturated fatty acids. In the present study, I have begun to investigate the dietary fat preference and propensity to develop dietary-induced obesity in a mouse model that does not express this protein -Trpm5<sup>/−</sup> mice-. My preliminary data shows that in male mice that are feed a high fat diet, Trpm5<sup>/−</sup> mice did not enhance their caloric intake as observed in mice that express the protein -wild type mice-. Most surprisingly, however, was that I did not observe the same differences in between female mice, which posits a potential gender effect of this pathway on dietary fat intake. Also, I show that the preference for dietary fat is not disrupted in Trpm5<sup>/−</sup> mice since there is no difference in dietary fat preference between Trpm5<sup>/−</sup> and wild type mice. Wild type and Trpm5<sup>/−</sup> mice both have a strong preference for the high fat diet, as demonstrated by the fact that they solely consumed the high fat diet. Consistent with our original hypothesis that these responses are specific for high fat feeding, I did not observe any differences in caloric intake in male mice on a sweet (high sucrose) diet. Again, gender differences were observed, with Trpm5<sup>/−</sup> female mice displaying a higher caloric intake than wild type female mice.

Furthermore, I restricted the caloric intake of wild type and Trpm5<sup>/−</sup> mice so that all animals were consuming the same amounts of calories daily via oral gavage to delve further into whether the effect of lack of expression of Trpm5 was due to pre- or post-
ingestive effects. The results from this experiment show that all animals have a reduction in body weight and body fat with no significant difference between wild type and Trpm5\(^{-/-}\) mice. This result suggests that the expression of Trpm5 in the oral cavity is necessary for the changes in body weight and body composition observed during free feeding. Also, the fact that Trpm5\(^{-/-}\) mice lost body weight and fat mass is contrary to our previous observations. When these animals consume roughly the same number of calories of a high fat diet during free feed, we observe an increase in body weight and fat mass. This suggests that there might be another mechanism accountable for the response observed in Trpm5\(^{-/-}\) mice when free fed. In conclusion, the results from these experiments suggest a link between dietary fat consumption and development of adiposity.
DEDICATION

This work is dedicated to the best mother in the world, Francia Caba, for in spite of the distance, she has been with me every step of the way, always with a supporting word, always encouraging me to continue, always believing in me even when I didn’t believe in myself. I also dedicate this to my aunt, Adalgisa Reyes, for being my role model and my support throughout this journey. And last, this is dedicated to my cousin, Isaac Jimenez, whom I love like a son and is my greatest source of happiness and motivation, the reason why I strive to give the best of me every day.
I want to thank my advisor, Dr. Timothy Gilbertson, first for believing in me and allowing me to join his laboratory and foremost, for providing me with all the guidance and support that has made this journey possible. I am grateful for having the opportunity to work with him and learn from his vast knowledge. I am also grateful to Dr. David York, for always taking the time to provide me with his guidance and advice when I needed it. I also thank Dr. Rashid, for all his support and guidance throughout this journey. Special thanks to Dr. Abby Benninghoff for all her advice and support and also for her input and help running the statistical analyses on my data.

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And last, I thank my family, for despite the distance, they have always been there for me, to celebrate with me during good times and to support me when things did not go so well. For always believing in me and encouraging me to dream big and follow those dreams.

Dulce M. Minaya
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LIST OF ABBREVIATIONS AND SYMBOLS

Abbreviations

ATP  Adenosine-5'-triphosphate

\([\text{Ca}^{2+}]_{\text{in}}\)  Intracellular calcium concentration

CCK  Cholecystokinin

CHO  Chinese Hamster Ovary

CD36  Cluster of differentiation 36

DAG  Diacylglycerol

DRK  Delayed-rectifying potassium

ENaC  Epithelial sodium channel

GLAST  Glutamate transporter

GPCR  G protein-coupled receptor

GTP  Guanosine diphosphate

IP\textsubscript{3}  Insitol 1,4,5-triphosphate

KCl  Potassium chloride

LA  Linoleic acid

P2X  Pannexin 2 receptor

PIP\textsubscript{2}  Phosphatidylinositol 4,5-bisphosphate

PKD1L2  Polycystic kidney disease 1-like protein 2

PLC\textbeta{}2  Phospholipase C \textbeta{}2 isoform

S.D  Standard deviation

STC-1  Enteroendocrine type 1 cells

TASK-1  Leak-potassium channel
TALK-1  Leak-potassium channel
TRC    Taste receptor cell
T1R    Taste receptor type 1
T2R    Taste receptor type 2
Trp    Transient receptor potential
Trpm4  Transient receptor potential melastatin member 4
Trpm5  Transient receptor potential melastatin member 5

Symbols

\begin{tabular}{ll}
g & Grams \\
\textit{kcal} & Kilocalories \\
\textit{mL} & milliliters \\
\end{tabular}
CHAPTER I

INTRODUCTION

The increase in the prevalence of obesity has become a major concern worldwide. Given its negative impact on overall public health, understanding the underlying mechanisms in the development of obesity is of great importance.

Researchers have suggested that there is a link between food consumption and the obesity trend. In the last few decades, concurrent with the increase in the incidence of obesity, a transition in general nutrition has been observed starting in the Western world and spreading more recently worldwide. Most societies have entered what is now called the “Nutrition Transition” (Popkin, 2006). We have shifted from hunter/gatherers, where the diet mostly consisted of plants and animal products, to a more sedentary life style where the diet includes high quantities of fat (specifically animal), refined sugars (often in the form of soft drinks) (O'Connor et al., 2006; Astrup et al., 2008) and less dietary fiber. Along with these nutritional changes, physical activity has also decreased. Societies have diverged from labor-intensive jobs like farming to jobs that require less physical activity and thus less energy expenditure, reinforcing sedentarism (French et al., 2001).

Given the importance of these nutritional and lifestyle changes on health, it is critical that we have a better understanding of the mechanisms that are associated with a change to a higher fat and higher processed carbohydrate diet. In this regard, until very recently we have had little idea how the body recognizes and responds to dietary fat.

It has been the general consensus that taste was not a major factor in the sensory recognition of dietary fat. Initially, fat was thought to be detected by its texture rather
than by its taste (Rolls et al., 1999) and thus research was focused on these textural cues with triglycerides as the primary sensory stimulus. Furthermore, the spontaneous preference shown in rats and mice (Tsuruta et al., 1999; Takeda et al., 2001) for lipids was attributable mainly to post-ingestive signals involved in long-term preference and reinforcing effects (Suzuki et al., 2003) and olfactory cues (Ramirez, 1993; Kinney and Antill, 1996). However, in the last decade there has been accumulating evidence to support the idea that there is a taste for fat. Gilbertson et al. provided the first direct evidence that ion channels in TRCs were sensitive to free fatty acids via inhibition of delayed-rectifying $K^+$ channels. However, given that only a small number of DRK channels are open at resting membrane potential, it was hypothesized that there must be an upstream mechanism providing the necessary depolarization to open the remaining DRK channels (Liu et al., 2005; Gilbertson et al., 2010).

During the quest for potential receptors for free fatty acids, several GPCRs and the membrane receptor Cluster of Differentiation 36, CD36, were proposed as plausible candidates. CD36 is expressed in a wide range of tissue cells including erythrocytes, platelets, monocytes, hepatocytes, adipocytes, megakaryocytes and responds to a wide variety of ligands, including collagen, thrombospodin, and low density lipoproteins, amongst others (Berger et al., 1993; Podrez et al., 2000; Febbraio et al., 2001). CD36 has been implicated in phagocytosis, hemostasis, thrombosis, inflammation, atherogenesis, immunity, and apoptosis (Febbraio et al., 2001). In TRCs, Fukuwatari et al. first observed the expression of CD36 in circumvallate papillae in rats (Fukuwatari et al., 1997). Further evidence for the involvement of CD36 in fat taste was provided by Laugerette et al. They
showed that the expression of CD36 in lingual epithelium was specific for the apical side of taste cells. They also showed that CD36−/− mice do not discriminate between control and linoleic acid-enriched solutions (Laugerette et al., 2005). Recent studies on the function of CD36 in fat taste in our laboratory have yielded valuable information. Electrophysiological and behavioral data supports the idea that CD36 may not act as a receptor for free fatty acids but rather facilitate binding of free fatty acids to the receptor (Xu et al., unpublished data).

Recent data has identified some previous orphan GPCRs as being responsive to different fatty acids. GPR120, expressed on circumvallate and fungiform papillae of the tongue and enteroendocrine cells in mice (Matsumura et al., 2007; Montmayeur et al., 2011), has been reported as the receptor for unsaturated long-chain fatty acids (Fredriksson et al., 2003; Hirasawa et al., 2005; Montmayeur et al., 2011). Cartoni et al. provided behavioral evidence that supports the involvement of GPR120 in fat taste. Using a knockout mouse model of GPR120, they showed that these animals have reduced preference for linoleic acid (Cartoni et al., 2010). Other studies have reported GPR120 as a polyunsaturated fatty acid receptor in the intestines and propose that it may participate in release of glucagon-like peptide-1 and cholecystokinin (Hirasawa et al., 2005; Tanaka et al., 2008; Shah et al., 2012). Also GPR40, and more recently GPR84, have been indicated to be receptors for medium-chain fatty acids (Wang et al., 2006) and short-chain fatty acids are ligands for GPR41 and GPR43. Activation of these receptors is involved in chemotaxis of neutrophils in a variety of diseases (Le Poul et al., 2003). Expression of GPR40, -84, -41, and -43 has been reported in circumvallate and foliate
papillae. However, their exact mechanism of action in fat taste is still unclear (Stewart et al., 2011)

Based on these data, a mechanism has been proposed for the transduction mechanism of fatty acids. Fatty acids bind and activate GPCRs, namely GPR120, which activates PLCβ2. This results in breakdown of PIP2 into IP3 and DAG. Binding of IP3 to IP3R3, results in increased intracellular calcium concentration. The depletion of Ca^{2+} activates transient receptor potential melastatin 5 channels, causing an influx of Na^+. This inward movement of positive current depolarizes the cell membrane and activates DRK channels, while a subset of DRK channels is inhibited by fatty acids, potentiating and prolonging the depolarization. This enhanced depolarization is thought to be necessary for neurotransmitter release (see Fig. 1 on page 15).

Efforts are now being devoted to elucidate the specific role of each of the players in the previously mentioned mechanism. In the present report, I used a behavioral approach to characterize the response of mice lacking Trpm5 (Trpm5^{−/−}) to dietary fat intake. This research is predicated on previous studies in our laboratory which have shown that taste receptor cells isolated from Trpm5^{−/−} mice have diminished responses to linoleic acid, a polyunsaturated fatty acid which is the prototypical stimulus for fat taste (Liu et al., 2011). Also, consistent with the role of these channels in other taste modalities, behavioral studies have reported that Trpm5^{−/−} mice have reduced sensitivity to bitter, sweet, and umami compounds (Damak et al., 2006).

In this report, I show that although the preference for dietary fat is not disrupted in Trpm5^{−/−} mice, these animals reduce their caloric intake when fed a high fat diet. Also, I
report that this response is specific for fat since similar results were not observed with a high sucrose diet. My data strongly suggest that the expression of Trpm5 in the oral cavity is of paramount importance for dietary fat consumption and implies that there must be a compensatory mechanism accountable for the response observed in Trpm5$^{-/-}$ mice during ad libitum feeding.
CHAPTER II

LITERATURE REVIEW

The taste system has two main functions that directly impact our ability to survive. The first is to identify compounds with a high nutrient value and the second is to avoid the ingestion of harmful compounds (Tepper, 2008). While sweet taste represents an energy-rich nutrient, salt taste allows for identification of sources of minerals, umami taste codes for proteins, bitter and sour taste warn us against potentially noxious or poisonous compounds.

How the brain interprets what the tongue is tasting is still a topic of debate. Currently, two theories have been proposed as possible mechanisms for taste interpretation. One is the labeled-line theory, which proposes that taste receptor cells respond to a specific taste modality (e.g. sweet or sour) and send a direct line to the central nervous system. Evidence to support this theory comes from the expression of specific receptors, T1R and T2R families, on subsets of taste cells for different taste stimuli (Scott, 2004; Yarmolinsky et al., 2009). The other theory, across-fiber pattern, proposes the idea that gustatory receptor neurons respond to a wide variety of stimuli and cooperate with other receptor neurons to create a neural pattern for a particular taste (de Brito Sanchez and Giurfa, 2011). Although it is true that subsets of cells express receptors for a particular stimulus, central neurons are more broadly tuned and their response may also be modulated by factors such as stimulus concentration, temperature, learning, and physiological responses. So, these neurons need to integrate this
information and convey not only a taste quality but also how pleasurable the stimulus is (Smith and St John, 1999).

Taste receptor cells (TRCs) are modified epithelial cells responsible for the identification of chemical stimuli in the peripheral gustatory system. These cells are clustered in taste buds - onion-like structures that contain approximately 50-100 cells (Gilbertson et al., 2000). Taste buds are mainly found on the tongue in three structures: fungiform, circumvallate, and foliate papillae, but are also present in the soft palate, epiglottis, pharynx, and larynx (Roper, 2013). Nerve innervation to taste buds is by branches of the facial nerve (cranial nerve VII) and the glossopharyngeal nerve. Branches of the facial nerve, specially chorda tympani and the greater superficial petrosal nerve innervate the fungiform papillae on the anterior tongue and the palate. The glossopharyngeal nerve (cranial nerve IX) innervates the circumvallate papillae on the posterior tongue, and branches from the chorda tympani and the glossopharyngeal nerves innervate the foliate papillae on the lateral sides of the tongue (Gilbertson et al., 2000; Breslin, 2013; Roper, 2013).

TRCs have been characterized into three different groups based on their morphology and expression of specific marker proteins (Finger, 2005). Type I cells are considered glial-like cells because they extend lamellae that surround other cells and express the glutamate transporter GLAST. In the brain, GLAST is expressed in glial cells and is involved in the reuptake of glutamate, where it is recycled (Lawton et al., 2000). Type II cells, also known as receptor cells, express all the proteins involved in taste transduction of sweet, bitter, and umami compounds (GPCRs, T1R, T2R, α-gustducin,
PLCβ, IP3 R3, Trpm5) (Finger, 2005; Perea-Martinez et al., 2013). Type III (presynaptic) cells are regarded as nerve cells because they make synapses with nerve processes and express SNAP-25, a neuron-specific protein involved in docking and fusion of synaptic vesicles and N-CAM, a cell-adhesion molecule normally found in neurons, glial cells, and other cell types (Yang et al., 2000; Yee et al., 2001). All cell types are likely present in the same taste bud and have significant cell-cell communication, making taste buds a very active sensory structure (Finger et al., 2005; Roper, 2013).

Taste responses originate when tastants interact with receptors on the apical membrane of the TRC. Significant efforts have been made to understand the mechanisms TRCs use to relay sensory information onto nerve fibers. Several neurotransmitters, including serotonin, norepinephrine, ATP, glutamate, acetylcholine, among others (Huang et al., 2011) have been proposed to be involved in synaptic transmission between taste cells and the afferent nerve fibers. Huang et al. reported that serotonin is released from type III (presynaptic) cells upon gustatory stimulation and may thus act as a neurotransmitter. Also, using biosensor cells created from Chinese Hamster Ovary (CHO) cells, they demonstrated that serotonin is released from TRCs in response to gustatory stimulation (Huang et al., 2005). Further studies show that serotonin, released by type III (presynaptic) cells, exerts paracrine regulation onto type II (receptor) cells and inhibits release of ATP (Huang et al., 2009).

Additional studies have identified the expression of two Pannexin 2 receptor subunits, P2X2 and P2X3, on afferent nerve fibers, which suggests ATP as a possible neurotransmitter. Type II (receptor) cells release ATP via pannexin 1 channels (Romanov
et al., 2012). Using double knockout mice of the aforementioned receptor subunits, Finger et al. showed that ATP signaling via these receptors is required for the relay of information from the sensory cells to the gustatory nerve fibers. They presented electrophysiological and behavioral evidence of the abolished response to tastant stimulus in this double knockout mouse model (Finger et al., 2005). In addition, immunohistochemistry data presented by Huang et al. (2011) showed that the P2X2-P2X3 double knockout mice, aside from being taste-blind, also secrete significantly less amounts of ATP than WT mice. Based on these findings, they have hypothesized that P2X2-P2X3 may also be expressed in presynaptic cells and exert autocrine regulation, via a positive feedback mechanism, to stimulate ATP secretion.

Given that TRCs must recognize and respond to a wild variety of stimuli, it is not surprising that several transduction mechanisms have been proposed to account for the ability of the gustatory system to recognize the primary taste modalities – sweet, bitter, sour, umami, and salty.

Ion Channel-mediated Taste Transduction Mechanisms

Salty

The taste of salty compounds is a response to the presence of sodium ions, the prototypical salty stimulus. That being said, other alkali metals (Li, K) can also elicit a similar, but not identical, response. Over the past few decades, evidence from several studies has demonstrated that the taste of salt in the oral cavity is mediated by amiloride-sensitive epithelial sodium channels (ENaC). ENaC channels are expressed in epithelial cells in the kidneys and urinary bladder, intestines, lungs, colon, and sweat and salivary
ducts. These channels form heterotrimeric channels and their main role is maintenance of sodium levels, where they contribute to the homeostasis of blood volume and blood pressure by reabsorbing sodium ions. Their expression is regulated by mineralocorticoids (e.g. aldosterone) and their function by the availability of sodium ions (Garty and Palmer, 1997; Masilamani et al. 1999; Loffing et al., 2000). In taste, Heck et al. showed that the chorda tympani response to application of sodium on lingual epithelium can be blocked by exposure to amiloride (Heck et al., 1984). Na$^+$ ions enter TRCs through ENaCs and cause cellular depolarization and neurotransmitter release (Gilbertson et al., 2000).

**Sour**

Since a wide range of protein and ion channels are pH-sensitive, several mechanisms are likely responsible for the detection of acids (Gilbertson et al., 2000). A study by Lin et al. reported electrophysiological and molecular data supporting the expression of several leak-potassium channels (TASK-1, -2 and TALK-1) in rat TRCs and their role in maintaining resting membrane potential and taste of acid compounds. They showed that these channels are voltage-independent and that their current can be blocked by small changes in extracellular pH (Lin et al., 2004).

Huang et al. proposed a polycystic disease-like ion channel –PKD$_2$L$_1$– as a candidate sensor for sour taste in mammals. Using electrophysiological techniques, as well as, in situ hybridization and immunostaining, they localized expression of PKD$_2$L$_1$ to a subset of TRCs and showed that mice with complete removal of that set of TRCs had intact responses to sweet, bitter, and umami compounds, but were unable to respond to sour compounds. Expression of PKD$_2$L$_1$ was also localized to a group of neurons located
along the spinal cord. These neurons are thought to be involved in close monitoring of the pH in cerebrospinal fluid (Huang et al., 2006). Further studies by Kataoka et al. showed that the subset of TRCs expressing PKD$_2$L$_1$ is type III (presynaptic) cells (Kataoka et al., 2008). A study by Huang et al. further confirmed these findings and showed that upon stimulation with acetic acid, that type III (presynaptic) cells respond by releasing the neurotransmitter serotonin (Huang et al., 2008).

**GPCR-mediated Taste Transduction Mechanisms**

**Bitter, Umami, Sweet**

Taste transduction of bitter, sweet, and umami compounds is mediated by G protein-Coupled Receptors (GPCR). Although these compounds activate different GPCRs, they share the same downstream transduction mechanism.

**GPCR:** G protein-Coupled Receptors comprise one of the largest families of membrane proteins. They are divided into five families: rhodopsin, secretin, glutamate, adhesion, and Frizzled/Taste 2. These receptors mediate most of the cellular responses to neurotransmitters and hormones, as well as vision, smell, and taste. At the molecular level, GPCRs are composed of seven transmembrane domains connected by three intracellular loops and three extracellular loops, externally located N-terminus, and internally located C-terminus (Rosenbaum et al. 2009; Peeters et al., 2011). The internal region of these transmembrane receptors is bound to a heterotrimeric guanine nucleotide-binding protein (G protein). Binding of a ligand to the active site of a GPCR causes a conformational change in the receptor that activates the G protein. This, in turn, activates/deactivates further components of the cascade and causes a cellular response
GPCRs important to taste transduction involve the T1R family, which mediates sweet and umami, T2R family, which mediates bitter taste (Chaudhari and Roper, 2010), and GPR40, GPR43, GPR84, GPR120, which have been proposed as receptors for free fatty acids (Miyauchi et al., 2010; Talukdar et al., 2011).

As mentioned above, the G protein is a guanine nucleotide binding protein composed by three distinct subunits (α,β,γ), hence the denomination heterotrimeric. It is the bridge between the extracellular receptor for a stimulus and the cellular machinery that orchestrates a response to such stimulus. In their inactive state, the α,β,γ subunits are tightly bound and GDP binds the α subunit. When activated, GDP is converted to GTP and the α subunit dissociates from the βγ complex (Hepler and Gilman, 1992). McLaughlin et al. reported the G protein α-gustducin as the α subunit expressed in TRCs (Mclaughlin et al., 1992).

Second messengers: Activation of α-gustducin leads to the activation of phospholipase C β2 (PLC-β2). PLC-β2 is the enzyme that catalyzes the conversion of phosphatidylinositol-4,5-bisphosphate (PIP2) into the second messengers inositol triphosphate (IP3) and diacylglycerol (DAG). IP3 binds its receptor, IP3R3, in the endoplasmic reticulum and causes an increase in intracellular calcium, followed by activation of Transient Receptor Potential Melastatin 5 (Trpm5) and subsequent neurotransmitter release and gustatory stimulation (Chaudhari and Roper, 2010).

Fat

Until recently, taste was not believed to be a major factor in the sensory recognition of dietary fat. Initially, fat was thought to be detected by its texture rather
than by its taste (Rolls et al., 1999). Because of this assumption, research was focused on the textural cues, with triglycerides as the primary tastant. Furthermore, the spontaneous preference for lipids observed in rats and mice (Tsuruta et al., 1999; Takeda et al., 2001) was attributable mainly to post-ingestive signals involved in long-term preference and reinforcing effects (Suzuki et al., 2003), and olfactory cues (Ramirez, 1993; Kinney and Antill, 1996).

In the last few decades, researchers have shown that free fatty acids can act as extracellular signals in a number of systems, including smooth and skeletal muscle and cardiac cells through inhibition of delayed rectifying potassium (DRK) channels (Ordway et al., 1991; Nelson and Quayle, 1995; Petrou et al., 1995). Given the importance of fat consumption for survival and based on this information, researchers have hypothesized that the gustatory system should also be able to detect fat. Gilbertson et al. (1997) demonstrated that ion channels in isolated TRCs are sensitive to free fatty acids via inhibition of delayed-rectifying K⁺ (DRK) channels. Electrophysiological recordings have shown that DRK channels is repolarize the cell membrane following activation. Inhibition of DRKs by free fatty acids prolongs and potentiates the initial depolarization. Interestingly, expression of DRK channels has been correlated with fatty acid responsiveness and overall dietary fat preference. Molecular experiments have shown that TRCs from obesity-resistant rats express a greater ratio of fatty acid-sensitive DRK channels than do obesity-prone rats (Gilbertson et al., 2005).

As previously mentioned, the transduction pathway for bitter, sweet, and umami compounds involves activation of GPCRs. Recent data has identified some previously
orphan GPCRs as being responsive to different fatty acids. GPR120, expressed on circumvallate and fungiform papillae of the tongue and enteroendocrine cells in mice (Montmayeur et al., 2011), has been reported as the receptor for unsaturated long-chain fatty acids (Fredriksson et al., 2003; Hirasawa et al., 2005; Montmayeur et al., 2011). GPR40, and more recently GPR84, have been suggested as receptors for medium-chain fatty acids (Wang et al., 2006). Short-chain fatty acids are ligands for GPR41 and GPR43. Activation of GPR41 and GPR43 is also involved in chemotaxis of neutrophils in a variety of diseases (Le Poul et al., 2003).

The proposed general model for fatty acid transduction via taste receptor cells is shown below. Fatty acids bind and activate GPCRs, namely GPR120, which activates PLCβ2 resulting in the breakdown of PIP2 into IP3 and DAG. Binding of IP3 to IP3R3 results in increased intracellular calcium concentration. The depletion of Ca2+ from intracellular stores activates Trpm5 channels, causing an influx of Na+. This inward movement of positive current depolarizes the cell membrane and activates DRK channels, while a subset of DRK channels is inhibited by fatty acids, potentiating and prolonging the depolarization. This enhanced depolarization is thought to be necessary for neurotransmitter release.

Introduction to TRP Channels

Studies on the mechanisms of taste detection and dietary consumption are of critical importance for our understanding of obesity because development of obesity has been linked to recent changes in dietary patterns and physical activity (French et al., 2001; Popkin, 2006). Since one of the main components of several of the taste
transduction pathways belongs to the Transient Receptor Potential (TRP) superfamily, it is of great importance that we understand the general characteristics of these channels. The first TRP channel discovered was in the light transduction pathway of Drosophila melanogaster. Nilius et al., in their attempt to elucidate the components of this pathway, discovered the trp-mutated fly strain. Observations were made that these flies had a disruption in Ca\(^{2+}\) influx channels (Nilius et al., 2007). Since then, close to 30 mammalian TRP channel isoforms have been discovered and they have been divided into 6 subfamilies: ankyrin, canonical, vanilloid, melastatin, polycystin, and mucolipin (Nilius et al., 2007; Venkatachalam and Montell, 2007). Placement of individual TRP channels into superfamilies is determined largely based on amino acid homology.

The core structure of TRP channels is very similar to the structure of voltage-gated K\(^+\), Na\(^{+}\), and Ca\(^{2+}\) channels, cyclic nucleotide-gated channels, hyperpolarization-activated cyclic-nucleotide-gated channels and the polycystins (Harteneck et al., 2000). All TRP channels are composed of six (6) transmembrane domains, a pore-forming loop
lined by the fifth and sixth domains, and internally located amino- and carboxyl-termini. Several of the TRP subfamilies have ankyrin repeats at the N-terminus. Ankyrin repeats are 33-amino acid long motifs that mediate protein-protein interaction. Although the role of these repeats in TRP channels is still unknown, they are thought to be involved in localization of the channel. Also, these repeats have been highly conserved throughout the evolution of TRP channels, suggesting that they play an essential role in TRP function (Clapham et al., 2001; Minke and Cook, 2002; Gaudet, 2008). Assembly of the subunits into homo- or heterotetramers results in formation of cation-selective channels. Most TRP channels are permeable to Ca$^{2+}$ with the exception of Trpm4 and Trpm5, which are permeable to monovalent cations (e.g. Na$^+$). The events mediated by TRP channels lead to cellular depolarization and signaling (Nilius et al., 2007).

![Figure 2. Structure of TRP channels. Adapted from (Clapham et al., 2001)](image-url)
Mechanism of Activation of TRP Channels

TRP channels are of great importance because of their role in the relay of sensory information and as cellular sensors; they are known to be involved in almost all the transduction pathways in mammals including those that contribute to chemoreception, mechanosensation, thermosensation, and photoreception (Clapham, 2003).

Although the function of TRP channels as Ca\(^{2+}\) channels and monovalent cations channels has been well established, the mechanism of activation of these channels has not been well elucidated because they appear to respond to a variety of stimuli depending on the physiological state of the cell (Ramsey et al., 2006). Based on observations, several mechanisms have been proposed for gating of TRP channels. The first hypothesis proposes that TRP channel activation involves a GPCR. Activation of GPCRs leads to the production of secondary messengers like diacylglycerol (DAG) and inositol triphosphate (IP\(_3\)), both of which are suspected to have a direct involvement in activation of TRP channels (Clapham, 2003; Ramsey et al., 2006). The second hypothesis proposes that TRP channels are activated by ligands; both exogenous and endogenous molecules are considered as possible ligands (Minke and Cook, 2002; Clapham, 2003; Ramsey et al., 2006). The third hypothesis proposes changes in environment as the trigger for TRP activation. These include changes in temperature, pressure, voltage, and other stimuli (Voets et al., 2004; Nilius et al., 2005; Ramsey et al., 2006).

The TRPM subfamily comprises a total of eight (8) channels, all of which present unusual modes of activation and different degrees of selectivity (Hofmann et al., 2003).
They are known to be involved in regulation of magnesium and calcium levels, taste transduction, and thermosensation, specifically cold sensation (Harteneck, 2005).

**The Role of Trpm5 in Taste Transduction**

Transient Receptor Potential Melastatin 5 (Trpm5) is a monovalent-specific, non-selective cation channel involved in the relay of chemosensory information. Although predominantly expressed in TRCs, Trpm5 is also expressed in olfactory epithelium and vomeronasal organ, liver, respiratory and gastrointestinal tissue, testis, brain, pancreas, kidneys, prostate, and the bone marrow (Hofmann et al., 2003; Fleig and Penner, 2004; Bezencon et al., 2007; Kaske et al., 2007; Liman, 2007; Khan and Besnard, 2009). In olfactory epithelium, Trpm5 is expressed in solitary chemosensory cells and is involved in the response to odorous chemical irritants (Lin et al., 2008). In gastrointestinal tissue, Trpm5 is expressed in the proximal small intestine and in Type I enteroendocrine cells (STC-1) (Rozengurt, 2006). In STC-1 cells, Shah et al. showed that disruption of Trpm5 results in a reduced response to linoleic acid as well as reduced release of cholecystokinin (CCK). These observations suggest that Trpm5 is critical for fatty acid signaling for CCK release (Shah et al., 2012).

In TRCs, Trpm5 has been shown to be essential for detection of carbohydrates, amino acids, bitter compounds, and long chain polyunsaturated fatty acids. A study by Damak et al. reported that Trpm5 knockout mice have reduced responses for sweet, bitter and umami compounds (Damak et al., 2006). Furthermore, Liu et al. reported behavioral and electrophysiological data showing that mice lacking Trpm5 show no preference and reduced sensitivity to linoleic acid, a long chain polyunsaturated fatty acid (Liu et al.,
Recent behavioral data from our laboratory shows that male Trpm5 knockout mice appear to be obesity resistant compared to wild type. Trpm5 knockout male mice have a reduced caloric intake, and thus gain less body and body fat.

The goal of this thesis research is to begin to explore the role of Trpm5 in the regulation of dietary fat intake, body weight, and body fat composition. I used a behavioral approach to further characterize the response of Trpm5 knockout mice to high fat feeding. Since Trpm5 is also involved in the taste response to sweet compounds, another goal of this research was to determine whether the response of Trpm5 knockout mice was specific for high fat diets or if similar responses could be observed with high carbohydrate diets.
CHAPTER III

MATERIALS AND METHODS

Animals

Trpm5−/− mice were developed on a C57Bl/6 background at the Mount Sinai School of Medicine (Damak et al., 2006) and bred at the Laboratory Animal Research Center at Utah State University. C57Bl/6 wild type mice, which served as controls, were purchased from The Jackson Laboratory and allowed to acclimate to our animal facility for five days before starting experiments. All animals were maintained on a 12:12 h day/night cycle with lights on at 7:00 AM; regular chow (rodent feed 8604; Harlan Laboratories) and water were provided ad libitum. All procedures were approved by the Institutional Animal Care and Use Committee of Utah State University.

All experimental diets were purchased from Research Diets, Inc. (New Brunswick, NJ). A 10% fat (D07020902 and D07020902L) diet was used as the control diet because it closely matches the composition of the other test diets. A high (60%) fat diet (D12492 and D12492L) and a high (70%) carbohydrate diet (D06062304) were used as experimental diets. All dietary components are listed in Table 1.

Measurements of body composition of fat mass, lean mass, free water, and total water using nuclear magnetic resonance system (Echo Medical Systems, Houston, TX.) This procedure was performed on conscious, minimally restrained mice.
Table 1. Composition of Experimental Rodent Diets

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| Total               | 773.9 | 4057 | 1055 | 4057 | 1055 | 4057 |

Note: Diets were obtained from Research Diets, Inc. with the following product numbers: High fat diet, D12492; run-in control diet, D07020902; high sucrose diet, D06062304.
Experiment 1: Effect of Trpm5 Disruption on Dietary Fat Intake

Seven-to-10-week-old Trpm5<sup>−/−</sup> mice (10 males, 10 females) and C57Bl/6 wild type mice (10 males, 10 females) were singly housed in clear polycarbonate wire bottom cages and provided ad libitum access to the run in diet (D07020902) for 14 days. Body weight and food intake were recorded daily at 9:00 AM throughout the study and fresh food was provided. Body fat composition was measured on days 0, 14, and 70 using an EchoMRI™ 700 system. On day 14, the animals were switched to the high fat diet (D12492) and maintained on it for 56 days. On day 70, mice were euthanized by CO<sub>2</sub> exposure followed by cervical dislocation.

Experiment 2: Effect of Trpm5 Disruption on Intake of a High Carbohydrate Diet

Eight-week-old Trpm5<sup>−/−</sup> mice (10 males, 10 females) and C57Bl/6 wild type mice (10 males, 10 females) were singly housed in clear polycarbonate wire bottom cages and provided ad libitum access to the run in diet (D07020902) for seven days. Body weight and food intake were recorded every other day at 9:00 AM throughout the study and fresh food was provided. Body fat composition was measured on days 0, 7, and 42 using EchoMRI™ 700 system. On day 7, the animals were switched to the high sucrose diet (D06062304) and maintained on it for 35 days. On day 42, mice were euthanized by CO<sub>2</sub> exposure followed by cervical dislocation.

Experiment 3: Effect of Trpm5 Disruption on the Preference for Dietary Fat

Four-to-five-week-old Trpm5<sup>−/−</sup> mice (8 males, 8 females) and C57Bl/6 wild type mice (8 males, 8 females) were singly housed in clear polycarbonate cages with corn cob
bedding and cotton pads. Animals were presented with two choices of food, a low fat diet (D07020902) and a high fat diet (D12492) in individual cups. Body weight and food intake were recorded daily and fresh food was provided at 9:00 AM for 28 days. On day 28, mice were euthanized by CO₂ exposure followed by cervical dislocation.

**Experiment 4: Site of Regulation of Trpm5**

The goal of this experiment was to determine whether the effect of Trpm5 disruption on dietary fat consumption is pre- or post-ingestive using a paired-feeding approach via oral gavage. I first validated the data obtained in our preliminary study using liquid forms of the run-in (D07020902L) and high fat (D12492L) diets. The diets were solubilized, using distilled water, according to the manufacturer’s instructions: to make a 1 kcal/gm solution of each diet, 260 gm of powder of the run-in diet was dissolved in 740 gm of water and 192.3 gm of powder of the high fat diet was dissolved in 807.7 gm of water.

Seven-week-old Trpm5⁻/⁻ mice (6 males, 6 females) and C57Bl/6 wild type mice (6 males, 6 females) were individually housed in clear polycarbonate cages with corn cob bedding and cotton pads. *Ad libitum* access to the run in diet was provided in graduated drinking tubes for seven days. On day 7, the animals were switched to the high fat diet and maintained on it for 38 days. Body weight and food intake were recorded daily at 17:00 PM throughout the study and fresh food was provided. Body fat composition was measured on days 0, 7, and 45 using an EchoMRI™ 700 system. On day 45, mice were euthanized by CO₂ exposure followed by cervical dislocation.
During the course of the study, due to solubility issues with the diet, I was unable to carry out the experiment as planned using the run-in and high fat diets. So, I decided to use corn oil as the source of calories from fat and regular chow to supplement caloric intake. Five-week-old Trpm5<sup>−/−</sup> mice (8 males, 6 females) and C57Bl/6 wild type mice (10 males, 6 females) were individually housed in wire bottom polycarbonate cages and provided *ad libitum* access to the run-in diet in graduated drinking tubes for 18 days. During this time, all animals were acclimated to oral gavage by administering 0.5 ml of water twice daily. Body weight and food intake were recorded daily at 17:00 PM throughout the run-in period and fresh food was provided. On day 19, I began the paired-feeding paradigm. All animals were provided 12 kcal/day determined empirically to be the normal daily *ad libitum* intake based on my preliminary data; 7.2 kcal (0.9 mL) were provided from corn oil (Mazola®) and administered via oral gavage in two meals. The remaining 4.8 kcal were provided from regular chow (rodent feed 8604; Harlan Laboratories) and the animals were allowed to eat them freely. Water was provided *ad libitum*. Body fat composition was measured on day 0, 18, and weekly during the paired-feeding period. On day 47, all animals were euthanized by CO₂ exposure followed by cervical dislocation and a ventral midline incision was performed to collect the retroperitoneal and inguinal fat pads, and the liver to assess absolute (in gm) and relative (in gm/gm body weight) weights.

**Statistical Analysis**

OriginPro 7.0 software (Northampton, Massachusetts) was used for all statistical analyses. Data were analyzed by two-sample t-tests to evaluate the impact of Trmp5
disruption on each parameter measured. Statistical significance was inferred when $p < 0.05$ and was denoted in each figure as follows: *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$ and ****, $p < 0.001$. 
CHAPTER IV

RESULTS

Experiment 1: Effect of Trpm5 Disruption on Dietary fat Intake

Food intake for the duration of the study (Figs. 1A-B) is reported as the average cumulative caloric intake ± SD per day. During the run-in period, there is no difference in caloric intake between wild type and Trpm5\(^{-/-}\) mice. During the high fat feeding period, Trpm5\(^{-/-}\) male mice did not enhance their caloric intake, compared to what was observed in wild type mice, after being on it for 7 days (note change in slope of line on a high fat diet for wild type mice in Fig. 1A). Interestingly, Trpm5\(^{-/-}\) female mice do not show significant changes in caloric intake.

Body weight and body composition data (Figs. 2-4) are reported as change, in grams, ± SD for the run-in and high fat diet period. In Figure 2A we can observe a decrease in body weight, with Trpm5\(^{-/-}\) mice losing significantly more weight than wild type mice. Figure 3A shows that there are no significant changes in body fat composition during the run-in period. Also, although all the animals have a reduction in lean mass, the difference between Trpm5\(^{-/-}\) male mice and wild type is statistically significant (Fig. 4A). During the high fat period, Trpm5\(^{-/-}\) mice gained significantly less body weight than their wild type counterparts. Also, Trpm5\(^{-/-}\) male mice gained less fat mass and slightly more lean mass than wild types (Figs. 2B, 3B, and 4B). Trpm5\(^{-/-}\) female mice gained fat mass, while female wild type mice lost. Lean mass increased in both Trpm5\(^{-/-}\) and wild type female mice, with wild type gaining significantly more.
FIGURE 1. Average cumulative caloric intake in mice on a high fat diet in pellet form. Average cumulative caloric intakes (Kcal) per day ± SD (N = 10) are shown for males (A) and females (B). Results two-sample t-tests to compare Trmp5 wild-type and null mice for each gender are shown in each panel with level of significance indicated as follows: **, p < 0.01.
FIGURE 2. Impact of Trmp5 deletion on change in body weight after treatment with control run-in diet then high fat diet. Values shown are the average percent change in body weight + SD (N=10) for mice fed the control run-in diet for 14 days (A) then the high fat diet for 56 days (B). Results of two-sample t-tests to compare Trmp5 wild-type and null mice for each gender are shown in each panel with level of significance indicated as follows: *, p < 0.05; **, p < 0.01; ***, p < 0.001.

FIGURE 3. Impact of Trmp5 deletion on change in fat mass after treatment with control run-in diet then high fat diet. Values shown are the average change, in grams, in fat mass + SD (N=10) for mice fed the control run-in diet for 14 days (A) then the high fat diet for 56 days (B). Results of two-sample t-tests to compare Trmp5 wild-type and null mice for each gender are shown in each panel with level of significance indicated as follows: *, p < 0.05.
FIGURE 4. Impact of Trmp5 mutation on change in lean mass after treatment with control run-in diet then high fat diet. Values shown are the average change, in grams, in lean mass + SD (N =10) for mice fed the control run-in diet for 14 days (A) then the high fat diet for 56 days (B). Results of two-sample t-tests to compare Trmp5 wild-type and null mice for each gender are shown in each panel with level of significance indicated as follows: ***, p < 0.001; ****, p < 0.0001.

Experiment 2: Effect of Trpm5 Disruption on Intake of a High Carbohydrate Diet

Food intake for the duration of the study (Fig. 5A-B) is reported as the average cumulative caloric intake ± SD per day. During the run-in period, there is no difference in caloric intake between wild type and Trpm5<sup>-/-</sup> mice. During the high sucrose period, Trpm5<sup>-/-</sup> male mice show no difference in caloric intake compared to wild type (Fig. 5A). In females, Trpm5<sup>-/-</sup> mice have a significantly higher caloric intake than wild type during high sucrose diet period (Fig. 5B).

Body weight and body composition data (Fig. 6A-C) are reported as change, in grams, ± SD for the duration of the study. There is an overall increase in body weight and
FIGURE 5. Average cumulative caloric intake in mice on a high sucrose diet in pellet form. Average cumulative caloric intakes (Kcal) per day ± SD (N = 10) are shown for males (A) and females (B). Results of two-sample t-tests (day 45) to compare Trmp5 wild-type and null mice for each gender are shown in each panel with level of significance indicated as follows: ****, p < 0.0001.
fat mass with no significant difference between Trpm5<sup>−/−</sup> and wild type mice (Figs. 6A-B). Interestingly, although Trpm5<sup>−/−</sup> and wild type male mice have no differences in caloric consumption, weight and fat mass gain is slightly lower in Trpm5<sup>−/−</sup> than in wild type mice. Consistent with Trpm5<sup>−/−</sup> female mice higher caloric intake, they have a higher increase in body weight and fat mass than wild type female mice (Fig. 6B). There is an overall increase in lean mass in with no significant difference between Trpm5<sup>−/−</sup> and wild type mice (Fig. 6C).

**Experiment 3: Effect of Trpm5 Disruption on the Preference for Dietary Fat**

Daily food intake per mouse is shown as relative caloric intake in Figures 7A-B. There is no difference in dietary fat preference between Trpm5<sup>−/−</sup> and wild type mice. Both wild type and Trpm5<sup>−/−</sup> mice have a strong preference for the high fat diet, as demonstrated by the fact that they solely consumed the high fat diet. This pattern is also the same in both males and females as well, which demonstrates that there are no gender effects.

**Experiment 4: Site of Regulation of Trpm5**

Food intake for the duration of the study (Figs. 8A-B) is reported as the average cumulative caloric intake ± SD per day. During the run-in period, there is no difference in caloric intake between wild type and Trpm5<sup>−/−</sup> mice. However, during the high fat feeding period, Trpm5<sup>−/−</sup> male mice did not significantly increase their caloric intake, compared to wild type, after being on this diet for 25 days. Trpm5<sup>−/−</sup> female mice do not exhibit similar changes in their feeding behavior.
FIGURE 6. Impact of Trmp5 deletion on change body weight, fat mass and lean mass after treatment with high sucrose diet. Values shown are the average change, in grams, in body weight (A), fat mass (B) and lean mass (C) + SD (N =10) for mice fed the run-in diet for 7 days followed by high sucrose diet for 35 days.
Figure 7A. Caloric consumption in mice presented with two choices of food: a 10% fat and a 60% fat diet. Data is presented as relative caloric intake ± SD for males mice.
Figure 7B. Caloric consumption in mice presented with two choices of food: a 10% fat and a 60% fat diet. Data is presented as relative caloric intake ± SD for females.
FIGURE 8. Average cumulative caloric intake in mice on a high fat diet in liquid form. Average cumulative caloric intakes (Kcal) per day ± SD (N = 6) are shown for males (A) and females (B). Results of two-sample t-tests to compare Trmp5 wild-type and null mice for each gender are shown in each panel with level of significance indicated as follows: *, p < 0.05.
Body weight and body composition data (Figs. 9-11) are reported as change, in grams, ± SD for the run-in and high fat period. The change in fat and lean mass composition was calculated relative to body weight. During the run-in period, there is an overall increase in body weight in both male and female mice, with statistical significance between Trpm5<sup>-/-</sup> and wild type female mice (Fig. 9A). Also, there is a greater increase in fat mass in Trpm5<sup>-/-</sup> female mice than in wild type mice. Changes in lean mass reflect an overall increase for all animals (Fig. 11A). During the high fat period, Trpm5<sup>-/-</sup> mice gained significantly less body weight and fat mass than their wild type counterparts (Figs. 9B-10B). Also, there is an overall increase in lean mass in wild type and Trpm5<sup>-/-</sup> mice, with wild type females gaining significantly more than Trpm5<sup>-/-</sup> (Fig. 11B).

*Paired-feeding via Oral Gavage*

Food intake for the duration of the run-in period (Figs. 12A-B) is reported the average cumulative caloric intake ± SD per day. During this period, Trpm5<sup>-/-</sup> male mice significantly reduced their caloric intake compared wild type. This was unexpected since I had not seen this adjustment in caloric intake on the run-in diet in previous experiments. There is no significant difference in caloric intake between female mice. During the oral gavage feeding period, all animals consumed 12 Kcal per day (7.2 Kcal from corn oil and 4.8 from regular chow). Body weight and body composition (Figs. 13-15) data are presented as change, in grams, ± SD. All animals have a reduction in body weight, fat, mass and lean mass with no significant difference between wild type and
FIGURE 9. Impact of Trmp5 deletion on change in body weight after treatment with control run-in diet then high fat liquid diet. Values shown are the average change, in grams, in body weight ± SD (N =6) for mice fed the control run-in diet for 7 days (A) then the high fat liquid diet for 35 days (B). Results of two-sample t-tests to compare Trmp5 wild-type and null mice for each gender are shown in each panel with level of significance indicated as follows: *, p < 0.05; ***, p < 0.001 ****, p < 0.0001.

FIGURE 10. Impact of Trmp5 deletion on change in fat mass after treatment with control run-in diet then high fat liquid diet. Values shown are the average change, in grams, in fat mass ± SD (N =6) for mice fed the control run-in diet for 7 days (A) then the high fat liquid diet for 35 days (B). Results of two-sample t-tests to compare Trmp5 wild-type and null mice for each gender are shown in each panel with level of significance indicated as follows: *, p < 0.05; ***, p < 0.001.
FIGURE 11. Impact of Trmp5 deletion on change in lean mass after treatment with control run-in diet then high fat liquid diet. Values shown are the average change, in grams, in fat mass ± SD ($N=6$) for mice fed the control run-in diet for 7 days (A) then the high fat liquid diet for 35 days (B). Results of two-sample t-tests to compare Trmp5 wild-type and null mice for each gender are shown in each panel with level of significance indicated as follows: *, $p < 0.05$; **, $p < 0.01$.

Trpm5$^{/-}$ mice (Fig. 13B, 14B, 15B). Weight of retroperitoneal, inguinal, abdominal fat pads and the liver are presented in Figures 16A-B. The retroperitoneal, inguinal, and abdominal fat pads are significantly smaller in Trpm5$^{/-}$ male mice than in wild type males. There is no difference in liver weight (Fig. 16A). In females, retroperitoneal and inguinal fat pads were significantly larger in wild type mice than in Trpm5$^{/-}$ mice. There were no significant differences in abdominal fat pads and liver tissue (Fig. 16B).
FIGURE 12. Caloric intake in mice on run-in diet. Average cumulative caloric intakes (Kcal) per day ± SD are shown for males (A; $N = 10$ for wild-type, 8 for Trmp5 null genotypes) and females (B; $N = 6$ for both genotypes). Results of two-sample t-tests to compare Trmp5 wild-type and null mice for each gender are shown in each panel with level of significance indicated as follows: *, $p < 0.05$. 
FIGURE 13. Impact of Trmp5 deletion on change in body weight after treatment with control run-in diet then chow diet + corn oil. Values shown are the average change, in grams, in body weight ± SD for mice fed the control run-in diet for 18 days (A) then chow diet + corn oil for 28 days (B). Results of two-sample t-tests to compare Trmp5 wild-type and null mice for each gender are shown in each panel with level of significance indicated as follows: **, \( p < 0.01 \); ****, \( p < 0.0001 \).

FIGURE 14. Impact of Trmp5 deletion on change in fat mass after treatment with control run-in diet then chow diet + corn oil. Values shown are the average change, in grams, in body weight ± SD for mice fed the control run-in diet for 18 days (A) then chow diet + corn oil for 28 days (B).
FIGURE 15. Impact of Trmp5 deletion on change in lean mass after treatment with control run-in diet then chow diet + corn oil. Values shown are the average change, in grams, in body weight ± SD for mice fed the control run-in diet for 18 days (A) then chow diet + corn oil for 28 days (B). Results of two-sample t-tests to compare Trmp5 wild-type and null mice for each gender are shown in each panel with level of significance indicated as follows: **, $p < 0.01$. 
FIGURE 16. Impact of Trmp5 deletion on relative weight of fat pads after 28 days on chow diet + corn oil. Values shown are the average weight of each specific fat pad ± SD for males (A; N = 10 for wild-type, 8 for Trmp5 null genotypes) and females (B; N = 6 for both genotypes) mice fed a chow diet + corn oil for 28 days. Results of two-sample t-tests to compare Trmp5 wild-type and null mice for each gender are shown in each panel with level of significance indicated as follows: **, p < 0.01; ****, p < 0.0001.
CHAPTER V

DISCUSSION

With increasing evidence supporting “fat” as a taste modality, greater efforts have been devoted to understanding the molecular mechanism(s) underlying the taste of fat (i.e. fatty acids). Trpm5 has been proposed as a key component in the transduction mechanism for fat taste. Previous behavioral studies have shown that Trpm5\(^{-/-}\) mice have reduced sensitivity to bitter, sweet, and umami compounds (Damak et al., 2006). Electrophysiological data has shown that inhibiting the Trpm5 channel or deleting it from the genome greatly reduces the response of taste receptor cells (TRCs) to linoleic acid, a polyunsaturated fatty acid (Liu et al., 2011). Furthermore, Sclafani et al. showed that Trpm5\(^{-/-}\) mice are indifferent to 0.313 – 2.5% sefa soyate oil and soybean oil emulsions when presented in two-bottle preference test. However, when presented with 2.5% or higher concentrations of an intralipid soybean emulsion, Trpm5\(^{-/-}\) mice display a preference similar to that of wild type mice. Further testing in these experienced mice revealed that after being exposed to the intralipid solutions, they display a preference for these solutions at low and high concentrations (0.039-2.5%). The researchers suggested that this might be due to the post-ingestive reinforcing effects of soybean oil (Sclafani et al., 2007).

Here I report for the first time that Trpm5\(^{-/-}\) male mice did not enhance their caloric intake, compared to what was observed on wild type mice, when fed a high fat diet. This result could possibly indicate that Trpm5\(^{-/-}\) mice have compromised cephalic phase responses during high fat feeding, which could be why they do not increase their
caloric intake as wild type mice do. The cephalic phase responses refer to the pre-absorptive release of enzymes (e.g. pancreatic polypeptide) and hormones (e.g. insulin) triggered by stimulation of the vagus nerve, which are thought to improve the efficiency of digestion, absorption and metabolism (Crystal and Teff, 2006). A previous study in rats reported that fatty acids delivered to the oral cavity result in an increase in the release of pancreatic polypeptide (Fushiki and Kawai, 2005). Another study conducted in humans compared the cephalic response of restrained and unrestrained eaters to high fat and low fat foods, and reported that the release of pancreatic polypeptide was significantly greater after oral stimulation with high fat foods. No significant differences were observed between restrained and unrestrained eaters (Crystal and Teff, 2006).

Interestingly, this difference in caloric intake is not observed in Trpm5<sup>−/−</sup> female mice. This finding was surprising and could potentially be influenced by differences in the discrimination threshold for fats. A study conducted in rats reported that female rats have a lower discrimination threshold to linoleic acid than male rats (Stratford et al., 2006). If this is the case, Trpm5<sup>−/−</sup> females may have cephalic responses that are unaffected or are not as affected as they are in Trpm5<sup>−/−</sup> males.

Also, I have demonstrated that the form in which the diet is provided only affects the overall caloric intake, but does not alter the response of Trpm5<sup>−/−</sup> mice to consumption of a high fat diet. Trpm5<sup>−/−</sup> mice fed a liquid form of the high fat diet have similar consummatory behavior as when fed a high fat diet in pellet form. Interestingly, all mice have a significantly higher caloric intake of the diets in liquid form compared to the diets in pellet form. One plausible explanation for this result is that liquid foods have a weaker
compensatory effect than solid foods. Several reports have shown that the caloric intake of two isocaloric diets is higher or lower based on the physical form of the diet, with liquid diets generally resulting in higher caloric intake than solid diets (DiMeglio and Mattes, 2000; Mourao et al., 2007; Martens et al., 2011). I also show that Trpm5\(^{-/-}\) mice preference for dietary fat is not impaired. Trpm5\(^{-/-}\) mice presented with two choices of food, a low fat and a high fat diet, have a strong preference for the high fat diet, similar to what is observed in wild type mice. However, it is clear in this part of the study that we are dealing with a ceiling effect and that altering the fat content of the diet might have yielded different results. This observation will require further study to validate this finding. Furthermore, it is possible that texture is playing an important role in preference, since it has been well documented that textural cues (e.g. creamy, crispy, and crunchy) contribute to the overall palatability of food (Drewnowski, 1997).

Another parallel system for fat perception may be contributing to the recognition of and preference for dietary fat. Trpm4, a monovalent non-selective cation channel which belongs to the same ion-channel superfamily and possesses similar physical and functional characteristics as Trpm5, is also expressed in TRCs. In a previous study by Liu et al., they tested the possibility that these channels are responsible for the residual linoleic acid-induced current observed in taste cells from Trpm5\(^{-/-}\) mice. They found that chemically blocking Trpm4 in taste cells from Trpm5\(^{-/-}\) mice, technically making them double knockouts, does not reduce the residual current further (Liu et al., 2011). This result suggests that Trpm4 is not involved in taste transduction of fat. However, contrary to my findings, two-bottle preference test results from the same study report that Trpm5\(^{-/-}\)
mice, compared to wild type mice, are indifferent to linoleic acid emulsions. Although these results are not comparable with my results due to the differences in the experimental design, I believe that additional behavioral studies are necessary to further investigate the possible involvement of Trpm4 channels in fat taste.

Previous studies have shown that Trpm5−/− mice display a diminished response to carbohydrates (e.g. sucrose) in two-bottle preference tests (Glendinning et al., 2012). However, here I show that Trpm5−/− mice do not differ from wild type mice in their caloric intake when fed a high sucrose diet. In fact, I observed that Trpm5−/− female mice, but not males, have a higher caloric intake than wild type female mice on the aforementioned diet. This can possibly be attributed to the post-ingestive effects of sucrose. Previous reports have shown that Trpm5−/− mice develop a preference for sucrose-containing solutions not observed when the mice are first presented with the sucrose-containing solution (de Araujo et al., 2008; Ren et al., 2010). Interestingly, although Trpm5−/− female mice have a higher caloric intake, they did not gain more weight than wild type mice. This behavior can possibly be attributed to impaired glucose tolerance resulting in reduced insulin secretion. Several reports have shown that Trpm5 is expressed in insulin-secreting pancreatic β-cells and that Trpm5−/− mice show impaired glucose tolerance and a reduction in the release of insulin and incretin hormones (Brixel et al., 2010; Islam, 2011). Our results further support these findings.

No report have been previously made on the effect of gender on the response of Trpm5−/− mice to sweet, bitter and umami compounds, or fat. The data presented in this report shows that gender has a strong impact on the response of Trpm5−/− mice to dietary
fat and sucrose consumption. Previous studies have proposed that these differences might be influenced by the presence and interaction of androgenic and estrogenic hormones with neuropeptides that regulate food intake (Lovejoy and Sainsbury, 2009; Brown and Clegg, 2010). However, further studies are necessary to evaluate these effects in Trpm5<sup>−/−</sup> mice.

Based on the response of Trpm5<sup>−/−</sup> mice to ad libitum high fat feeding, I used a paired-feeding approach via oral gavage to determine whether this response is due to pre-ingestive effects (i.e. texture, taste and perception of fat) or to post-ingestive effects (e.g. cholecystokinin release). Wild type and Trpm5<sup>−/−</sup> mice were paired-fed an isocaloric diet of 12 Kcal per day, 7.2 Kcal in the form of corn oil via oral gavage and 4.8 from regular chow, to bypass the gustatory sensors in the oral cavity. This experiment shows that all wild type mice lose body weight and fat mass in the same proportions as Trpm5<sup>−/−</sup> mice, which strongly supports the idea that expression of Trpm5 in the oral cavity is necessary for the changes in body weight and composition observed during ad libitum feeding. Furthermore, it may also imply that the orosensory signals through Trpm5 are needed for the initiation of post-ingestive effects. It has been previously shown that expression of Trpm5 in enteroendocrine cells in the intestine is necessary for the release of the hormone cholecystokinin (CCK). This hormone is release in response to fat consumption and aids in its digestion. Low levels of CCK would impair the absorption of dietary fat. The fact that Trpm5<sup>−/−</sup> mice lost body weight and fat mass differs from our previous observations. When these animals consume roughly the same number of calories on a high fat diet ad libitum, we observe an increase in body weight and fat mass. This result suggests that
there might be another mechanism accountable for the response observed in Trpm5−/− mice when fed ad libitum. The results from all this experiments suggest a link between dietary fat consumption and development of adiposity.
CHAPTER VI

SUMMARY AND FUTURE DIRECTIONS

Until recently, taste was not considered to be a major factor in the sensory recognition of dietary fat. Initially, fat was thought to be detected by its texture rather than by its taste (Rolls et al., 1999). Because of this assumption, research was focused on the textural cues of fat, with triglycerides as the primary tastant. However, in 1997, Gilbertson et al. provided the first direct evidence that ion channels in TRCs were sensitive to free fatty acids via inhibition of delayed-rectifying K⁺ channels. Nevertheless, given that only a small number of DRK channels are open at resting membrane potential, it was hypothesized that there must be an upstream mechanism providing the necessary depolarization to open the remaining DRK channels (Liu et al., 2005; Gilbertson et al., 2010).

During the quest for potential receptors for free fatty acids, several GPCRs and the membrane receptor cluster of differentiation 36, CD36, were proposed as plausible candidates. Recent electrophysiological and behavioral studies from our laboratory supports the idea that CD36 may not act as a receptor for free fatty acids but rather facilitate binding of free fatty acids to the receptor (Xu et al., unpublished data). GPR120, expressed on circumvallate and fungiform papillae of the tongue and enteroendocrine cells in mice (Montmayeur et al., 2011), has been reported as the receptor for unsaturated long-chain fatty acids (Fredriksson et al., 2003; Hirasawa et al., 2005; Montmayeur et al., 2011). GPR40, and more recently GPR84, have been suggested as receptors for medium-chain fatty acids (Wang et al., 2006). Short-chain fatty acids are
ligands for GPR41 and GPR43. Activation of GPR41 and GPR43 is also involved in chemotaxis of neutrophils in a variety of diseases (Le Poul et al., 2003).

Based on these data, a mechanism has been proposed for the transduction mechanism of fatty acids. Fatty acids bind and activate GPCRs, namely GPR120, which activates PLCβ2 resulting in the breakdown of PIP2 into IP3 and DAG. Binding of IP3 to IP3R3 results in increased intracellular calcium concentration. The depletion of Ca2+ from intracellular stores activates Trpm5 channels, causing an influx of Na+. This inward movement of positive current depolarizes the cell membrane and activates DRK channels, while a subset of DRK channels is inhibited by fatty acids, potentiating and prolonging the depolarization. This enhanced depolarization is thought to be necessary for neurotransmitter release. This mechanism was proposed fairly recently and the exact action of each of the elements in the cascade are being actively investigated.

In the present report, I used a behavioral approach to characterize the response of mice lacking Trpm5 (Trpm5−/−) to dietary fat intake. This research is predicated on previous studies in our laboratory which have shown that taste receptor cells isolated from Trpm5−/− mice have diminished responses to linoleic acid, a polyunsaturated fatty acid which is the prototypical stimulus for fat taste (Liu et al., 2011). Also, consistent with the role of these channels in other taste modalities, behavioral studies have reported that Trpm5−/− mice have reduced sensitivity to bitter, sweet, and umami compounds (Damak et al., 2006).

In this thesis, my research aimed to answer the following questions: Are these differences between wild type and Trpm5−/− mice maintained under paired-feeding
conditions? Do Trpm5^{−/−} mice display different macronutrient preferences from wild type mice? Given that Trpm5^{−/−} is involved in the taste of carbohydrates, are there also differences in food intake and body weight between wild type and Trpm5^{−/−} mice under high carbohydrate (sweet) feeding? To answer these questions, I performed a series of behavioral studies.

To determine if the differences between wild type and Trpm5^{−/−} mice were observed under high carbohydrate (sweet) feeding, I provided wild type and Trpm5^{−/−} mice with a high carbohydrate where most of the corn starch had been replaced with sucrose. I did not observe any changes in food intake in male mice. In fact, Trpm5^{−/−} female mice had a significantly higher caloric intake than wild type female mice.

To determine macronutrient preference, I presented wild type and Trpm5^{−/−} mice with two choices of food, a low fat and a high fat diet, and all animals displayed a strong preference for the high fat diet. However, using the same diets where we saw differences in feeding behavior, it became apparent that we saw a ceiling effect and further testing with fat modifications to the diet (different fat content, different forms of fat) are necessary to validate this result. This was beyond the scope of this thesis research.

Next, I used a paired-feeding approach via oral gavage to determine if bypassing the orosensory cues would yield similar results as during ad libitum feeding. Wild type and Trpm5^{−/−} mice were paired-fed an isocaloric diet of 12 Kcal per day, 7.2 Kcal in the form of corn oil via oral gavage and 4.8 from regular chow. This experiment shows that all wild type mice lose body weight and fat mass in the same proportions as Trpm5^{−/−} mice, which strongly supports the idea that expression of Trpm5 in the oral cavity is
necessary for consumption of dietary fat. Furthermore, it may also imply that the orosensory signals are needed for the initiation of post-ingestive effects. It is also possible that due to the dietary restrictions to which these animals were subjected, they had other deficiencies (vitamins, minerals) that interfered with their growth, development, and ability to process other macronutrients (fat).

Based on these results, my conclusions from this research are: 1) Expression of Trpm5 in the oral cavity is a contributor to the control of dietary fat intake; 2) The effects seen in Trpm5−/− mice are limited to high fat feeding; 3) Trpm5 disruption seems to have no effect on the preference for dietary fat. However, a number of questions remain. Are these results still observed with a diet that more closely matches the composition of a typical diet? Also, a more controlled intragastric feeding study would be necessary to confidently tease apart the pre- and post-ingestive effects. Furthermore, additional experiments are needed to evaluate the gender differences observed between Trpm5−/− male and female mice both under high fat and high carbohydrate (sweet) feeding.
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