Oral and post-oral factors controlling energy balance in GF rodents

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Thesis for a Ph.D. in Physiology and Pathophysiology

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Defense:

June 18, 2012

INRA (Institut National de la Recherche Agronomique), Jouy-en-Josas, FRANCE

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Abstract

The present studies examine intestinal, metabolic, and behavioral alterations in germ-free (GF) animals. The first set of experiments examine preference and acceptance for sweet solutions, sucrose and saccharin, in GF C57Bl/6J mice with associated changes in expression of lingual and intestinal nutrient-sensing sweet taste receptors, T1R2 and T1R3, and the glucose transporter, SGLT1. It demonstrates that GF mice consumed more of the highest concentration of sucrose relative to NORM controls, with an increased expression of intestinal T1R3 and SGLT1. The second set of studies examine if findings of increased sucrose intake extend to fat, and whether the GF mice display alterations in lingual and intestinal fat sensors as well as intestinal satiety peptides. We found that GF mice display increased intake and preference of fat at high and low concentrations, respectively. Additionally GF mice display decreased fatty-acid GPRs and satiety peptides in the intestine, decreased circulating gut peptide levels, increased lingual fat detecting receptors, and increased markers of fatty-acid metabolism, all of which are adaptive effects to the chronically depleted energy state of the GF mice. The final succession of experiments was to determine if the GF state, with its associated decreases in adiposity and chronic fasting state in mice, is present in the GF rat model. Interestingly, we found that GF rats display similar or increased levels of body adiposity, with decreased markers of liver lipogenesis, yet increased lipogenesis in adipose tissue associated with adipocyte hypertrophy. Overall, these data demonstrate that absence of gut microbiota in mice leads to increased energy consumption of sugars and fats associated with alterations in oral and intestinal nutrient sensors while the gut microbiota in the F344 does not play a pivotal role in adiposity.

Mes études ont pour but d'examiner les altérations métaboliques et comportementales dans des modèles de souris axéniques. Nous avons démontré que les souris axéniques présentent une augmentation de préférence et d'acceptation des solutions sucrées. Cette augmentation est corrélée à des changements des niveaux d'expression des récepteurs du goût sucré au niveau de l'épithélium lingual et la muqueuse intestinale; T1R2, T1R3, et le transporteur de glucose SGLT-1. De plus, elles ont une préférence pour des fortes concentrations de saccharose comparées aux souris normales. Cet effet est associé à une augmentation des niveaux d'expression de T1R3 et SGLT-1 dans l'intestin. Nous avons étudié si cette augmentation de consommation de sucre était similaire à celle de acide gras, étayé les effets d'une consommation des lipides sur les niveaux d'expression des récepteurs des acides gras "CD36" au niveau de l'épithélium lingual et la muqueuse intestinale ainsi que les niveaux d'expression et de sécrétion des peptides intestinales à vocation satiétogène chez les souris axéniques comparées aux souris normales. En effet, nous avons démontré que les souris axéniques affichent une consommation accrue et une préférence pour les acides gras à des fortes et faibles concentrations respectivement. Ces changements étaient associés à une diminution des niveaux d'expression des détecteurs gustatifs de gras (GPRs), des faibles taux d'expression et de sécrétion des peptides intestinales, une augmentation d'expression du récepteur des acides gras au niveau de l'épithélium lingual et une augmentation des taux circulants des acides gras. Ces modifications peuvent constituer des mécanismes d'adaptation à l'état énergétique appauvri des souris axéniques. Nous avons essayé de savoir si ces altérations étaient présentes chez le rat dépourvu axénique. En effet, nous avons constaté que les rats axéniques présentent un niveau similaire ou élevé de la masse grasse, avec une diminution de la lipogenèse et une augmentation de l'adipogenèse expliquant l'hyperphagie du tissu adipeux. En résumé, nous avons démontré que l'absence du microbiote intestinal chez la souris conduit à une augmentation de l'apport énergétique en augmentant la consommation de sucres et de gras. Ces effets sont associés à des altérations orales et post-orales des niveaux d'expressions des détecteurs gustatifs tandis que le microbiote intestinal du rat F344 ne joue pas un rôle central dans l'adiposité.

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1. Introduction

1.1. Obesity and healthcare costs

Currently, the obesity epidemic afflicting the global population spans numerous countries and over 300 million people. While the United States is a large contributor to this epidemic, with approximately 33% of its population obese [1], over 115 million people in developing countries are currently obese as well [2]. Furthermore, obesity is a risk factor for other life-threatening co morbidities, such as diabetes mellitus, cardiovascular disease, hypertension, and certain cancers. In response to the myriad of health problems associated with obesity, national governments and global organizations have implemented funding systems in an attempt to curb the growth of this disease, which has been rampant for the past 40 years. Recently, the annual direct and indirect costs of obesity in the United States are estimated to be higher than $147 billion [3]. In France, the 1992 obesity-related healthcare costs were approximately 2% of healthcare costs [4] with no recent indications of its cost. However, despite France having one of the lowest rates of obesity in Europe and lowest average body mass index (BMI); nearly 50% of the French male population is overweight and over 11% obese [5]. While these data demonstrate that obesity is prevalent globally, with an increased prevalence in developed countries, the etiology of obesity remains unclear.

The causes of obesity are complex and include genetic deficits or alterations in central homeostatic signaling, involving mutations in hormone sequences or hormone receptors that govern feeding behavior and regulate metabolism. Evidence of these alterations in obesity comes from studies demonstrating that replacement with exogenous leptin in leptin-deficient individuals results in normalization of body weight

and adiposity [6]. More recently, an array of data has demonstrated that mutations in the fat mass and obesity-associated protein (FTO) results in obesity as well [7-9]. Despite these evidences clearly linking genetic alterations with obesity, the prevalence of obesity has risen dramatically in the past 50 years while genetic mutations occur over much longer periods of time. Thus, the current obesity epidemic is hypothesized to occur due to an interaction of genetics and environmental factors, such as obesigenic diets. Obesigenic diets promote increased body weight and adiposity in the presence [10, 11] and absence of hyperphagia [12, 13]. The cause of obesity induced by these diets is unclear; however, both peripheral and central homeostatic signaling deficits are implicated. For example, diet-induced obese (DIO) rats display decreased sensitivity to leptin [14, 15] while displaying decreased capacity for peripheral fatty acid oxidation [16, 17]. Additionally, DIO rodent models display decreased sensitivity to intestinal satiety peptides, such as cholecystokinin [18]. Evidence for peripheral satiety signals in the treatment of obesity comes from data demonstrating that administration of exogenous satiety peptides normalizes food intake, body weight, and metabolism of obese rodent models [19, 20]. In addition to intestinal satiety peptides influencing energy regulation, recent evidence suggests microbes lining the GI tract, collectively referred to as the gut microbiota, contribute to the obese state [21, 22]. *Accordingly, this thesis aims to focus on the gut microbiota, its contribution to alterations in food intake, nutrient detection, and metabolism, and how energy balance is associated with these changes.*

1.2 The digestive system

1.2.1 Oral cavity

1.2.1.1 Taste

The most elementary evidence of taste arising from the tongue predates scientific publications and comes from the ingrained response that application of various substances on the tongue results in the stimulation of further intake or avoidance of a tasted substance. Alternatively, injury to the tongue or surface of the tongue results in loss of sensation to taste or total inability to taste substances, denoting the importance of this organ, its sensory cells, and innervations in taste function [23]. The specific contribution of the lingual epithelium to taste had begun over 120 years ago as the identification of specific taste bud locations, types, and their sensory pathways had taken place at the beginning of the 20th century [24]. While animals generally can taste a variety of chemicals and nutrients, which together play a pivotal role in the perception of a meal, taste can be generalized to five categories: sweet, bitter, sour, salty, and umami. While these tastes have been well established and have withstood numerous scientific rigors, more recently, substantial evidence has demonstrated that fat should be added to this list, prompting many taste researchers to recognize the newly coined "fat taste."

The lingual epithelium contains distinct regional papillae that house taste buds, which are comprised of 100 – 150 single taste receptor cells (TRCs) that directly sense incoming nutrients and chemicals (Figure 1) [25]. Taste receptor cells are grouped into three categories: Type I, Type II, and Type III. Briefly, the function of Type I TRCs is the sensing of salt [26]; Type II TRCs are responsible for sweet, bitter, and umami taste

[25]; Type III TRCs sense sour taste [27]. The focus of this thesis will be on Type II TRCs as they express the receptors for sweet, and possibly fat taste; however, the possible function of Type III receptors will also be addressed briefly. In total, there are four afferent nerves that innervate the oral cavity, but the majority of taste transduction involves the chorda tympani (CT) nerve that innervates the anterior lingual epithelium containing the fungiform papillae, and the glossopharyngeal (GP) nerve that innervates the posterior epithelium containing the vallate and circumvallate papillae. While at the most basic level researchers hypothesized that specific taste buds respond to distinct taste stimuli, provoking unique responses upon activation. However, the predominant hypothesis, which has prevailed for the last 30 years, is that taste buds are capable of responding to a variety of taste [28]. This has led to the connotation that taste buds are generalists rather than specialists. As such, each area of the tongue is hypothesized as capable of detecting every taste, with varying thresholds and sensitivity to each specific taste. Despite this, the specific TRCs, with individually expressed taste receptors, are relatively tuned to sense specific taste stimuli. More recently, the identification of multiple receptor configurations, belonging to the appropriately named taste receptor family, has allowed researchers to better understand the role of taste cell populations.

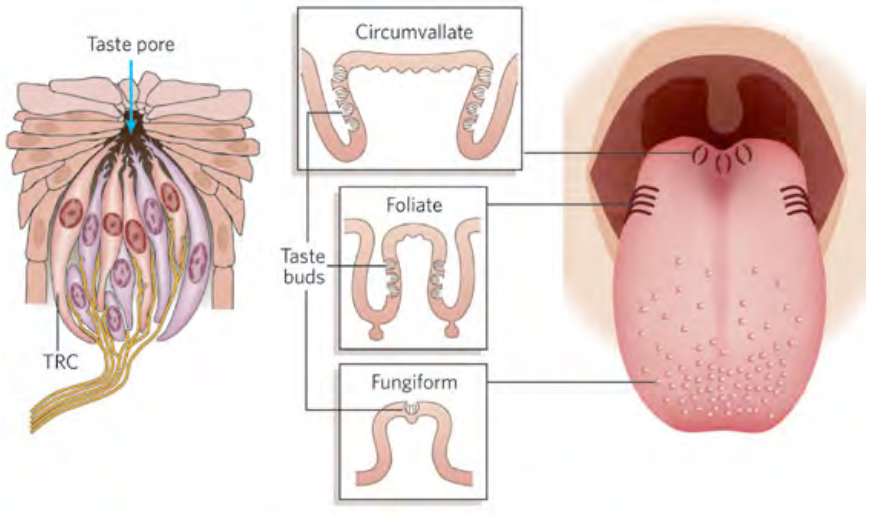


Figure 1: Taste papillae and structure of taste buds comprised of TRCs. Lingual taste papillae located in the anterior (fungiform), lateral (foliate), and poster (circumvallate) contain apical membrane TRCs that are positioned to sense stimuli and transduce taste signaling to afferent nerve fibers [25].

1.2.1.2 Sweet taste

1.2.1.2.1 *Sweet taste and feeding behavior*

Sweet taste has long been known to evoke pleasure and stimulate intake of sugar-laden foods. Laboratory animals generally prefer sweet tasting solutions and foods, even in the absence of nutritive value [29]. The strength of sweet taste in altering food intake was first shown by studies employing the sham feeding technique. Sham feeding, which typically is performed by placing a fistula in the stomach so that contents drain from the stomach before entering the intestine, is largely influenced by oral factors as the intestine receives very little of the ingested substance and thus cannot provide sufficient chemosensory feedback [30] (Figure 2). During sham feeding of sweet solutions, animals will drink copious amounts of fluid generally in a monotonic function of concentration [31]. While animals sham-feed sweet solutions continuously, previous

exposure to the sweet solution is also important in determining fluid intake via conditioned “oral satiation,” which is probably due to the lingual sensing of sweet substances [32]. In addition to sham feeding experiments, 24-h two-bottle tests and brief access tests have revealed the role of sweet taste in feeding behavior. In 24-h two-bottle tests, animals are presented with varying concentrations of sweet solutions in one bottle and water in the other bottle. Through this method, researchers can determine the threshold of detection for sweet stimuli, the overall intake of sweet solution, as well as the preference of the solution relative to water. However, with the nutritive value of most sweet solutions, post-oral feedback can significantly alter these responses [33]. Thus, brief access tests in which animals are given access to taste solutions for brief periods (seconds) has better assessed the role of oral sweet sensing in determining intake. Similarly to sham feeding, animals exhibit increased oral acceptance of sweet solutions during brief-access tests, with variations between animal models and strain used [29, 34]. These observed variations have been found to be due to the *Sac* locus, discovered 40 years ago, which houses the genetic material that encodes for sweet taste receptor proteins [35].

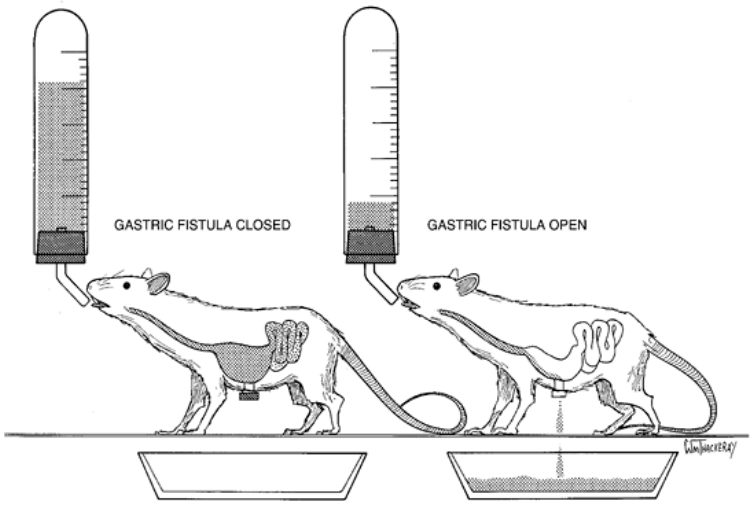


Figure 2: Depiction of sham feeding in the rat. ***Left:*** **A surgically placed fistula with a screw cap intact allows for the passage of ingesta into the intestine.** ***Right:*** **When the screw cap is removed, ingested fluids in the stomach drain into a reservoir, allowing for the examination of oral infuences in the absence of intestinal feedback.**

1.2.1.2.2 *Mechanisms of oral sweet detection*

While highly complex and integrative central pathways in reward signaling may be largely responsible for driving consumption of palatable sugary foods (discussed below), the sensing of sweet taste is initially detected in the lingual epithelium and mediated predominantly by two apical membrane g-protein-coupled receptors (GPR) belonging to the taste receptor type 1 (T1) family: T1R2, and T1R3 [35-39]. The relative expression of these two receptors throughout the lingual epithelium is broad with T1R2 being most commonly expressed in the posterior circumvallate and foliate papillae in both rats and mice; however, expression of T1R2 in the anterior fungiform papillae is sparse in rats while mice express high levels of fungiform T1R2 [36, 40]. This could explain the relative weakness of sweet stimuli to activate the CT nerve in rats while mice exhibit strong activation of the CT nerve in response to sweet stimuli [41, 42]. Similarly to

T1R2 in the mouse, T1R3 is highly expressed in the fungiform and circumvallate papillae [35, 37, 40, 43]. Both homodimeric or heterdimeric assembly of these proteins results in a functional sweet taste receptor [44], and this is consistent with expression patterns of T1R2 and T1R3 [35]. For example, a large degree of T1R2 and T1R3 are co-expressed in both the fungiform and circumvallate papillae, with the former papillae expressing T1R3-only taste receptor cells as well [35]. Electrophysiological recordings from the CT nerve in T1R knock-out (KO) animals have revealed the functional role of these receptors in taste. For example, KO of T1R2 or T1R3 in the mouse results in abolishment of non-nutritive sweetener-induced activation of the CT nerve [45, 46]. However, both of these models display weak responses to nutritive sweet substances, such as glucose, fructose, and sucrose, especially at higher concentrations [42, 45, 46]. It should also be noted that in addition to the heterodimeric T1R2+3 receptor, the homodimeric T1R3 complex is responsible for high nutritive sweet taste responses [35]. While these findings in KO models suggest the possibility of another receptor mediating sweet taste, double KO of T1R2 and T1R3 abolishes nutritive sweet mediated CT and GP nerve responses [46]. Together this demonstrates that T1R2 and T1R3 are responsible for the detection of sweet taste.

While the previously mentioned data demonstrate a strong role of these receptors to mediate neural activation in response to sweet tastants, the behavioral role has been established using both 24-h and brief access tests. During 24-h tests, T1R3 KO mice display no preference for non-nutritive sweeteners over water compared to control animals that display a preference for sweeteners [35, 45, 46]. However, when exposed to nutritive sweet solutions, such as sucrose, T1R3 KO mice display

preferences at higher concentrations when naïve and low and high concentrations with previous exposure, suggesting an involvement of post-oral learning [42]. Similarly, when subjected to brief access tests, both T1R2 KO and T1R3 KO animals display no response to nonnutritive sweeteners and only a mild response to highly concentrated nutritive sweeteners [35, 45, 46]. Double KO of these proteins, however, abolishes all preferences to both nutritive and nonnutritive sweeteners [46]. Polymorphisms of the T1R3 receptor also alter sweet taste responsiveness as mice with variants of the T1R3 gene display altered sensitivity to sweet substances [47]. Despite these behavioral and electrophysiological data demonstrating the role of T1R2+3 in sweet taste, administration of a T1R3 antagonist in rodents does not affect the ability of animals to discriminate sweet tastants from water [48]. Interestingly, the functionality of T1R2 and T1R3 are not confined to laboratory animals alone as felines, which do not respond to sweet substances, maintain a naturally occurring mutation in the T1R2 gene rendering the receptor nonfunctional, further signifying the importance of these receptors in sweet taste [49].

The sensing of sweet substances in the oral cavity is also species specific and an important determinant in the detection of various sweet tastants. For example, humans, but not mice can distinguish aspartame from water, and insertion of the human variant of T1R2 in mice results in an ability of mice to taste aspartame [50-52]. The observed differences and the finding that T1Rs respond to a variety of sweet stimuli, denotes an importance of the ligand binding domains in sweet sensing. The differences in domains observed even between the T1R2 and T1R3 receptors in the same species explains the broad perceived sweet taste across a variety of substances. For both

T1R2 and T1R3, the N-terminus is responsible for binding multiple sweet taste molecules [52] while the transmembrane and cysteine rich domain of the T1R3 receptor is also responsible for mediating sweet taste [51]. However, for some sweet substances, such as glucose, sucrose, and the sucrose derivative sucralose, the N-terminus of both T1R2 and T1R3 is necessary for the binding of these ligands [53].

Both T1R2 and T1R3 belong to the g-protein coupled receptor superfamily in which intracellular signaling is dependent upon second messenger g-proteins. The specific intracellular signaling mechanisms responsible for sweet taste signaling involve cyclic adenosine monophosphate (cAMP) signaling [54-57], as well as phospholipase-C β2 pathways (PLCβ2) [25, 58, 59] (Figure 3). The latter is thought to be the predominant pathway of sweet taste recognition as ablation of PLCβ2 results in decreased electrophysiological and behavioral sweet taste responses [58]. Additionally, for T1R2+3, α-gustducin may be the specific second messenger subunit. Behaviorally, this comes from the finding that α-gustducin animals display an abolished preference for sweet solutions [60]. Immunohistolochemical studies also have determined that the T1R2+3 complex is co-expressed with α-gustducin [43] while electrophysiological results have demonstrated a decreased CT or GP nerve response in α-gustducin KO animals in response to sweet stimuli [61]. Despite this, application of either nonnutritive saccharin or sucrose to cells expressing T1R2+3 does not activate gustducin [62]. Interestingly, similar to ligand binding domains, the activation of intracellular signaling mechanisms differs between non-nutritive and nutritive sweet T1R2+3 activation. For example, upon binding to the sweet taste receptor, nonnutritive stimuli are hypothesized to activate a PLCβ2 pathway, increasing intracellular Ca^{+2} stores. Intracellular Ca^{+2}

then binds to transient receptor potential melastatin-5 channel (TRPM5), which is a selectively permeable Na^+ and K^+ ion channel and responsible for cellular depolarization and eventual exocytosis of neurotransmitters from TRCs that activate the CT and GP nerves [63]. In support of this mechanism involving TRPM5 is that TRPM5 KO mice exhibit abolished behavioral and electrophysiological responses to sweet tastants [58]. Additionally, nutritive sweeteners may signal via adenyl cyclase, serving to increase intracellular cAMP activing Protein Kinase A, which inhibits basolateral potassium channels leading to taste cell depolarization, increased intracellular Ca^{+2} and subsequent release of adenosine triphosphate (ATP), which may interact with Type III TRCs to stimulate afferent nerve fibers, or possibly involved in direct activation of nerve fibers via connexins [64-66].

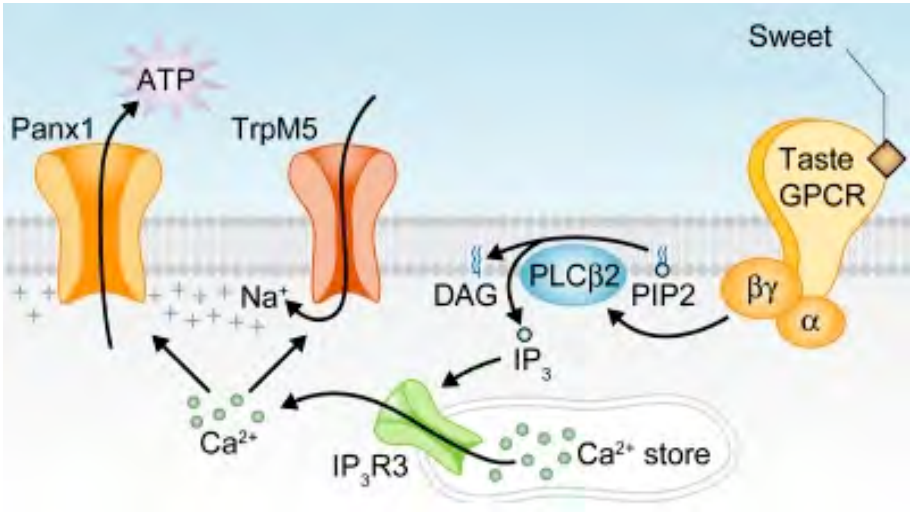


Figure 3: Intracellular sweet taste transduction pathway. A sweet stimulus is bound to the extracellular domain of T1R2+3 (Taste GPCR). The secondary messenger system for sweet taste involves PLCβ2, leading to release of Ca^{+2} and an increase in intracellular Ca^{+2}. Elevated Ca^{+2} levels allow for the depolarization of the TRCs via TRPM5-dependent influxes of Na^+ [67].

1.2.1.3 Fat taste

Relatively recent findings demonstrate that in addition to the five tastes that have been known for many years, both rodents and humans can detect fatty-acids in the oral cavity. The sensing of fat is indeed thought to be considered a fat “taste” as it occurs across a large range and classification of fatty acids and does not occur with similarly textured non-nutritive oils and is not blocked when olfactory cues are removed [51, 68-70]. Although lingual lipase is found in extremely low concentrations in adult human saliva, and its physiological role is still debated, there is a sufficient concentration of naturally occurring free-fatty acids in oils and other food products to elicit oral sensory feedback [71].

1.2.1.3.1 *Fat taste and feeding behavior*

The dissection of factors involving fat intake leading to alterations in feeding behavior has been a topic of research for over 60 years. The first finding that oral properties of fat alter food consumption was that addition lard, stimulates food intake in rodents [72]. In general, laboratory rodents prefer HF foods relative to a standard low-fat diet [73-75]. In one study examining the general propensity of laboratory mice to prefer HF foods, it was demonstrated that 10 out of a total 13 mouse strains examined prefered HF foods to low-fat foods [76]. Using two-bottle preference tests, it has also been demonstrated that rodents prefer oil emulsions with varying degrees of fat relative to water or non-nutritive control solutions with similar texture [77]. To minimize post-oral feedback, which has a potent influence on stimulating food intake following previous exposures with nutritive solution, a majority of these experiments have been conducted using brief

access to the test preference. In addition to brief access tests, research also demonstrates that animals subjected to the sham feeding paradigm freely drink nutritive corn oil or non-nutritive mineral oil emulsions during one-bottle feeding tests [77, 78]. However, when presented with two bottles in a choice preference test with one bottle containing corn oil and the other mineral oil, animals always prefer corn oil to mineral oil [77]. With the current discovery of fatty-acid receptors on the lingual epithelium, this effect can likely be attributed to the free fatty acids found in corn oil, which activate lingual taste receptor cells and are not present in mineral oil [79]. Finally, demonstrating the ability of fat taste to alter energy consumption, sham feeding fats stimulates increased intake of a standard rat diet following fat exposure [80]. Together, these data exemplify the profound role that oral fat detection can have on influencing feeding behavior.

1.2.1.3.2 *Mechanisms of oral fat detection*

The location of oral fat detection is thought to occur almost solely in the posterior lingual epithelium, specifically, in the taste receptor cells of the circumvallate papillae [81, 82]. Lingual fat sensing involves several receptors expressed on taste receptor cells such as g-protein coupled receptor 40 (GPR40) [83] and GPR120 [84, 85] as well as the fatty acid translocase CD36 [81] and delayed-rectifying potassium channels [86]. Similarly to sweet taste, fat detection is transduced via gustatory nerves that innervate the lingual epithelium, which transmit signals to higher brain centers ultimately controlling food intake [79].

Fat activates taste receptor cells via an apical sensor, such as voltage-gated ion channels that contribute to neurotransmitter release on the basolateral portion of the cell [87]. For example, patch-clamp recordings on isolated fungiform taste cells demonstrate that free-fatty acids inhibit delayed rectifying potassium channels (DRKs) [88]. This is specific to poly-unsaturated fatty-acids that are applied extracellularly, denoting these channels may establish oral fat preferences. Various DRKs have been found in the fungiform papillae [89], but the specific Kv1.5 channel that is found in cardiac tissue and inhibited by fatty acids [90] is present in rodent lingual epithelium [86]. The proposed mechanism of activation of this channel is via a direct binding between fatty acids and a domain of the Kv1.5, which has been reported in cardiomyocytes [90]. Furthermore, DRKs may contribute to taste modulation by enhancing perceived intensity of taste, which is in agreeance with findings that fatty acids enhances perceived intensity of various tastes [86].

G-protein coupled receptors on the lingual epithelium may also be responsible for the oral detection of fat. Normally localized on intestinal epithelium enteroendocrine cells, GPRs respond to various lengths of free fatty acids and mediate the release of gut hormones CCK [91, 92] and GLP-1 [93]. Recently, researchers have identified GPR120, which responds to long chain fatty acids, in the sensory fungiform and circumvallate papillae, whereas GPR40, which responds to medium and long-chain fatty acids, was absent from the lingual epithelium [85]. Interestingly, the enteroendocrine cell model, STC-1, expresses GPR120 and activation of this receptor induces cellular depolarization via a PLCβ2, Ca^{+2}-dependent mechanism similar to the activation of taste receptor cells by a taste stimulus [93, 94]. Furthermore, genetic ablation of

GPR120 results in reduced fatty-acid preference and loss of fatty acid-induced activation of nerves innervating the lingual epithelium [83]. Together, these data exemplify a role of GPR120 in the detection of oral fats.

In addition to DRKs and GPRs, the fatty acid translocase, CD36, may play the most pivotal role in oral fat detection as suggested by research over the past 10 years [81, 82]. Originally discovered as the main mechanism of fatty-acid uptake in adipocytes [95], CD36 is also significantly expressed in the lingual epithelium, specifically in the circumvallate papillae [81, 82]. Located on the apical membrane of taste cells [82] and conservatively expressed across multiple species [82, 96], CD36 is positioned to bind extracellular fatty acids in the oral cavity. Furthermore, taste receptor cells that express CD36 are located in close proximity to a lipid-rich environment near the Von Ebner's glands that secrete lingual lipase [97] (Figure 4). The extracellular structure of CD36, with a large hydrophobic pocket, also illustrates its role in binding fatty acids [98]. Indeed, CD36 reversibly binds fatty acids, specifically in the nanomolar range [99, 100]. Experiments using CD36 KO mice demonstrate the significant physiological role of this receptor in fat taste [82, 101]. The integral function of CD36 in fat detection was established by Laugerette et al, who demonstrated that deletion of CD36 in mice results in abolished spontaneous fat preference and an absence of oral fatty-acid induced neural activation [82]. Together, it is hypothesized that GPR120 and CD36 may mediate fat taste; however, due to its relative infancy in relation to sweet taste, further clarification is needed to examine the exact mechanisms of fat taste.

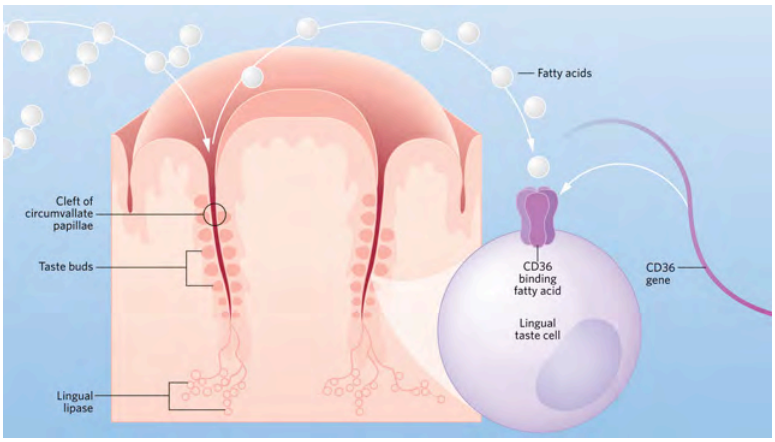


Figure 4: Localization of CD36 on the apical portion of the circumvallate papillae TRCs. Taste buds containing TRCs expressing CD36 are juxtaposed to the Von Ebner's gland that secretes lingual lipase, which hydrolyzes triglycerides into free fatty acids in the mouth [102].

Experiments examining fatty acid-induced activation of TRCs have demonstrated the great dependence of these cells on CD36 in mediating this response. Isolated taste receptor cells expressing CD36 display large increases in cellular activation in response to fatty acid application [79]. This is specifically dependent upon CD36 as blockade of fatty acid binding inhibits increases in intracellular calcium [79]. The second messenger system which mediates CD36-induced activation of taste receptor cells may be PLCβ2 as taste cells express inositol-triphosphate [59] and CD36 and PLCβ2 are co-localized in other cells of the mammalian system [103]. Together, these findings demonstrate that the intracellular signaling mechanisms for sweet and fat taste may be extremely similar. For example, increases in intracellular calcium activate TRPM5 channels that induce an influx of Na^+ that leads to cell depolarization [104]. Taste receptor cells expressing CD36 indeed depolarize in response to fatty acids making a case for this protein as a proposed mechanism in oral fat detection [79]. Furthermore, the finding that TRPM5 KO mice display no preference for oils implicates this protein in mediating

CD36-dependent fatty-acid signaling and taste cell depolarization [105]. While it is hypothesized that CD36 is localized on Type II TRCs due similar intracellular signaling markers as sweet taste, this has yet to be examined. Rapid influxes in intracellular calcium also serve to induce neurotransmitter via cellular depolarization, which is observed in CD36-positive TRCs [106]. Some of the proposed neurotransmitters responsible for taste cell signaling to afferent nerve fibers include acetylcholine, norepinephrine, serotonin, and glutamate. Specifically, serotonin and noradrenaline may be the primary neurotransmitters released by CD36-positive taste receptor cells as the transcript for two vital enzymes in the production of these neurotransmitters is present in CD36-positive cells [106]. As such, serotonin and noradrenaline are both released from fatty-acid activated taste receptor cells; however whether this is directly by CD36 expressing cells or indirectly via other TRCs is unknown [106]. Thus, despite the wealth of data illustrating the role for CD36 mediating the oral detection of fats, intriguing questions such as the exact pathways in TRCs that are responsible for fat taste transduction as well as the TRC type that expresses CD36 remain unanswered.

1.2.1.4 Taste transduction, central signaling, and reward

Regardless of the taste detected, TRCs directly or indirectly release various neurotransmitters [107-112] or peptide hormones [113-117] that activate the CT and GP nerve fibers. The specific mechanism is not completely understood; however, this may involve direct TRC to nerve connections, in which Type II cells generate ATP that interacts via connexins to nerve fibers [65, 66]. A second mechanism is that indirect signaling from TRC Type II cells that sense tastants, release ATP, which then

stimulates Type III TRCs to release neurotransmitters that activate afferent nerves. Support of this latter hypothesis comes from the finding that Type III cells are the only TRCs that express voltage gated calcium channels and neurotransmitters, such as serotonin [108, 109]. Additionally, the release of ATP from Type II cells can bind to P2X receptors [64], which are ion channels that open in response to extracellular ATP and expressed by Type III TRCs (Figure 5). While this intriguing and yet unestablished transduction mechanism is currently under investigation, the afferent CT and GP fibers indeed receive input from taste bud complexes and signal upstream to the nucleus of the solitary tract (NTS) of the caudal brainstem.

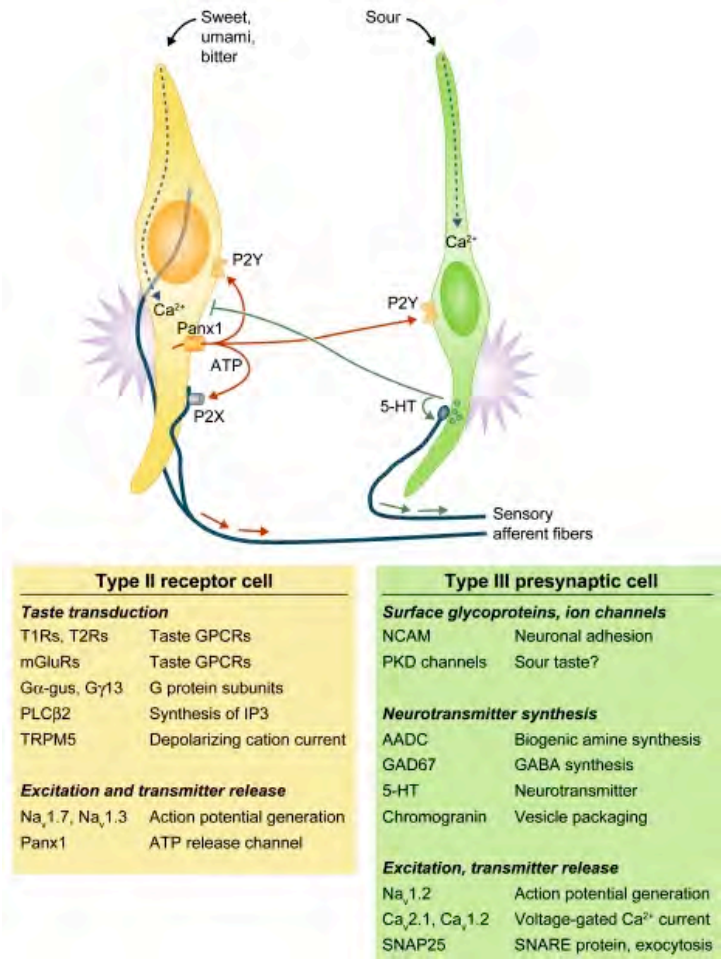


Figure 5: Proposed mechanism of cell-to-cell communication for taste transduction. Type II TRCs express the receptors for a variety of tastants, most importantly, T1R2+3. Type II cells relay taste information either directly via cell-afferent nerve connections or indirectly via Type III cell-nerve synapses which repond to extracellular ATP released by Type II cells. Afferent nerves then relay information to the hindbrain for taste coding [118].

Somewhat similar to the taste buds, in which taste receptive fields are reasonably broad, NTS neurons can respond to a broad number of stimuli [119]. Despite this, specific populations of NTS neurons are more responsive to specific taste stimuli [120-122]. This has been shown by various electrophysiological methods, such

as single neuron recordings, which has led to the identification of “tuned” neurons. For example, neurons that are highly activated by sweet stimuli evoke a specific neural activation pattern and can be differentiated from neurons responding to other tastes such as umami, sour, or bitter [123]. Furthermore, for each taste, different stimuli evoke specific neural firing patterns. For example, a nonnutritive sweetener, such as saccharin, can be differentiated from a nutritive sweet stimuli, such as sucrose based on neural firing [124]. Using single neuron recordings, researchers have demonstrated that T1R3 is vital in the activation of sweet responsive NTS neurons, providing evidence of this receptor in the central processing of sweet stimuli [125]. Upstream, neurons from the NTS project to the pontine Parabrachial Nucleus (PBN). From the PBN, neurons project in two directions, the first is the gustatory cortex (GC), which is responsible for encoding taste, mechanical, and visceral stimuli. The second pathway consists of PBN neurons projecting to the limbic system, ultimately terminating in the ventral striatum, involving reward function. The gustatory cortex is best viewed as the brain region that assigns hedonic value to taste stimuli. For example, stimuli with similar hedonic values activate similar regions of the GC and differing hedonic values activate more distinct regions [126-128]. Additionally, the GC is also associated with conditioned taste aversion (CTA), in which an animal avoids a normally preferred taste stimuli (such as saccharin) paired with an aversive stimuli (such as lithium chloride (LiCl)) [129, 130]. In this manner, the GC exhibits relative plasticity as saccharin will typically activate specific neuron subsets of the GC, but after pairing with LiCl, a different neural activation patterning occurs [131]. Furthermore, due to the plasticity of this brain region, this effect is fully reversible. Although these pieces of evidence may demonstrate the functional

role of the GC in determining taste, an overwhelming amount of evidence places a role of the PBN limbic projection to the ventral striatum in hedonics and controlling reward function of palatable stimuli.

Central reward pathways upstream of the NTS and PBN are strong contributors to both sweet and fat taste. Behavioral evidence of this comes from the finding of reinforcement studies demonstrating the rewarding values of sweet and fat stimuli. For example, reinforcement designs are used in which an animal must work more to obtain the same reward (palatable food). When given access to sucrose or fat, in the absence of post-oral feedback, rats work harder for continued access to these stimuli, demonstrating the behaviorally rewarding value of sweet or fat taste [132, 133]. As previously mentioned, neurons of the PBN extend to the ventral striatum [134]. Using microdialysis, immunohistochemistry, and brain lesioning, several collective studies demonstrated that the PBN-striatal projections are responsible for the stimulation of sweet solution intake and subsequent sucrose-induced influxes of dopamine (DA) in the Nucleus of Accumbens (NAcc), a nuclei in the ventral striatum involved in reward function [135]. While the NAcc consists of two parts, a core and shell, the latter of the two is vital in tracking reward. Thus, in addition to the view that the GC tracks hedonic value, release of DA from the striatum, which contains the NAcc, is associated with this perception as well [136, 137]. For example, influxes of DA in the NAcc are a direct function of oral reward and the motivational state to obtain a reward, such as sweet stimuli [138, 139]. The first initial evidence linking sweet taste to reward function was the finding that drinking saccharin led to increases in NAcc DA, and is associated with learned behavioral processes [138]. As well as saccharin, the consumption of sucrose

leads to increases of DA in the NAcc [140]. However, this latter finding could be influenced by the nutritive value of sucrose reaching the intestine. Thus, through a variety of controlled experiments using fixed and non-fixed volumes of low and high concentrations of sucrose during sham-feeding, it was demonstrated that sweet taste alone is sufficient for increasing NAcc DA [141]. In addition to sweet taste, sham feeding of nutritive oils has also demonstrated fat taste leading to increases in DA in the NAcc relative to water [142]. Therefore, although detection of taste arises from the tongue involving apical membrane bound receptors, afferent signals relayed from TRCs indirectly provide input to central brain areas, such as the NAcc, which signal sweet and fat taste as rewarding, and lead to stimulation of intake for these palatable stimuli.

1.2.1.5 Obesity and taste

While the varying detection for sweet and fat taste influences short-term intake of these stimuli, the role of oral sensitivity to sweets and its relation to obesity is less clear. In general, obese animals over consume sweet foods, with evidence suggesting this may be mediated by taste functions. Genetic rodent models of obesity display an inability to detect low concentrations of sweet solutions with an accompanying increased consumption of highly concentrated sweet solutions [143, 144]. Thus, obese animals are typically described as having a decreased oral sensitivity to sweet solutions. Decreased oral detection of sweets could contribute to over consumption as more sucrose would be needed to stimulate lingual sweet receptors and release sufficient DA to signify reward [145]. The mechanism responsible for the observed detection of sweet stimuli in obese rodents may be due in part to the fact that the sweet taste heterodimer

is co-localized with the leptin receptor, and loss of leptin receptor function leads to diminished oral sensitivity to sweet substances [146]. Thus, the observed leptin resistance in obese rodent models due to increased adiposity denotes that energy status in these models is a major determinant for these behavioral findings. Furthermore, lean or food deprived animals, where normal or enhanced leptin receptor sensitivity is present, exhibit in an increased sensitivity to oral sweet stimuli [147]. Both of these findings, however, could also be attributed to central reward signaling controlling feeding behavior and guiding taste function as obese animals display increased motivational states [145, 148, 149] demonstrating the complexity of taste signaling in energy homeostasis. While obesity clearly is correlated with impaired sweet taste signaling in animal models already obese, studies examining variations in the sweet taste receptor before the onset of obesity have been less promising. For example, the genotype of T1R3 does not predict sucrose- or fructose-induced hyperphagia [47]. However, this does not necessarily rule out altered taste in the pre-obese state as animals prone to obesity display increases in consumption of palatable sweet stimuli before the onset of obesity [145, 150]. Furthermore, in humans, a polymorphism in the T1R2 gene has been shown to correlate with an overweight BMI and increased consumption of carbohydrates [151]. Whether individuals displaying the T1R2 polymorphism exhibit altered sensitivity to sweets, which would lead to increased carbohydrate consumption is unclear from this study alone. In general, data concerning sweet taste sensitivity in obesity is conflicting, and little data has assessed the contribution of taste to human obesity. Initial data demonstrated that obese individuals do not differ in sweet taste sensitivity relative to normal weight individuals [152-155] with

formerly obese individuals displaying increases in sweet taste responsiveness [156]. More recent evidence, however, reveals obese individuals maintain a higher affinity for sweet stimuli relative to lean individuals [157-160]. The observed differences in these studies are explained by improvements in the assessment of sweet taste function [158]. Interestingly, in animals, while obesity is associated with alterations in sweet taste function, more recent evidence indicates that taste is vital in influencing short-term intake of sweet solutions and post-oral feedback contributes to long-term intake [161].

In addition to oral sweet sensitivity, obese animals also display altered oral sensitivity to fats. For example, the Otsuka Long-Evans Tokushima Fatty (OLETF) rat, which is hyperphagic and subsequently becomes obese due its overeating consumes more of high corn oil concentrations when sham feeding than lean controls during the fed state. Furthermore, at a relatively low concentration of corn oil, OLETF rats exhibit increased intake compared to lean animals when food deprived [162]. While this genetic model of obesity clearly displays alteration in oral fat sensitivity leading to increased consumption of fat, high-fat (HF) feeding in rats or mice, which results in obesity, is associated with decreases in lingual expression of CD36 [163]. Thus, it is hypothesized that by expressing less CD36, these animals are unable to detect lingual fats as well as lean or chow-fed controls and increase fat consumption as a response. However, the effect of HF feeding on lingual CD36 expression in the absence of obesity has not been examined. This is extremely important as HF-feeding influences oral fat sensitivity regardless of the obese state as HF-fed non-obese animals display increased acceptance for fats relative to low-fat (LF) fed controls [164]. Nevertheless, together these findings exemplify a decreased ability of obese rodents to detect oral fats, which

is associated with increased fat intake. Despite these data, one study in inbred obese rats, which prefer HF foods over LF foods, shows that these rats exhibit increased sensitivity to linoleic acid relative to obese resistant animals during LF-feeding [165]. However this effect is in conflict with previous data demonstrating that the same obese prone animals exhibited decreased inhibition of delayed-rectifying K^+ channels, which would result in decreased activation of taste receptor cells compared to the lean controls [166]. Surprisingly, lingual CD36 in this model has yet to be examined, and may play a significant role in these behavioral findings. Unlike sweet taste, obesity in humans is well correlated with increased affinity for fats [156, 167, 168], with the analyses of possible genetic contributions currently under investigation. While initial data from a European population suggested no differences in BMI or oral fat sensitivity in individuals displaying CD36 polymporphisms [71], more recent data demonstrates that polymorphisms in CD36 are associated with fat perception and BMI [169, 170].

1.2.2 Stomach

1.2.2.1 Gastric distention

While oral detection of sweet and fat are thought to be largely stimulatory in regards to food intake, post-oral detection of nutrients, involving predominantly gastric and intestinal feedback typically serve to terminate a meal. The temporary distention of the gastric wall observed upon the entrance of ingesta in the stomach may be the most important means of the stomach to regulate food intake. For example, humans with naturally occurring gastric fistulas remain hungry following a meal [171]. As well, rats with man-made esophageal [172] or gastric [173] fistulas consume food continuously

when food drains from a cannula. However, in the same animals, closing the cannula to allow passage of ingesta into the stomach and intestine rapidly reduces food intake [174]. While these data do not exclude the contribution of intestinal satiation, experiments that prevent gastric using an inflatable pyloric cuff have shown that gastric loads produce volume-related suppression of liquid diet intake [175, 176]. Thus, the distention of the stomach alone is sufficient for the termination of a meal. Furthermore, the finding that this reduction in intake is extraneous to nutritive value as intragastric delivery of non-nutritive loads suppresses food intake similarly to nutritive loads of the same volume [176] demonstrates the mechanical, rather than chemical nature of this process in inducing satiation.

Vagal nerve mechanoreceptors are the mediators of gastric distention-induced satiation, and this comes from the finding that vagotomy abolishes the effect of a gastric preload to inhibit food intake [177]. The stomach is lined with various nerve endings, which consist of two morphologically distinct nerve endings: intraganglionic laminar endings (IGLEs), as well as intramuscular arrays (IMAs). Intraganglionic laminar endings that are comprised of sensory fibers that are positioned to adjacent myenteric neurons [178, 179] and IMAs are nerve fibers located in the longitudinal and circular muscle layers that run parallel to the muscle fibers [180]. The former of these two is thought to respond predominantly to the stretch reflex and tension produced by incoming ingesta, inducing satiation. The support of this hypothesis comes from the finding that stimulation of vagal afferents via mechanical stretching of the stomach is associated with IGLEs, and not IMAs [181, 182]. Furthermore, despite the apparent physiological function of IGLEs in gastric distention, both fibers are hypothesized to

respond to distention or stretching of the stomach as IMAs are activated by large forces of distention and stretch. This piece of evidence may be important in behavioral experiment as although gastric distention reduces food intake in the absence of intestinal signals, animals with an occluded pylorus display increased gastric volume, and thus increased gastric distention, compared to freely feeding animals [183], which most likely denotes that post-gastric signals are also important in inducing satiation. Thus, despite findings that tension may activate vagal endings synapsing with the stomach, signals from the stomach do not autonomously induce satiation. For example, while draining of ingesta from the stomach via a fistula results in increased intake of nutritive liquids, the observed increase could be due to lack of distention from the stomach as well as lack of nutrients entering the intestine, the major site of nutrient absorption. Furthermore, while gastric loads indeed induce distention of the gastric cavity, nutrients empty from the stomach and provoke intestinal stimulation. As such, previous research indicates that approximately 40% of a liquid meal may empty from the stomach into the intestine during a meal [184]. In addition to its mechanical role in reducing food intake, the stomach also serves an endocrine organ that is responsible for controlling energy balance.

1.2.2.2 Endocrine function

In addition to the stomach serving as an endocrine organ secreting gastrin and somatostatin, which influence gastric acid secretion, the stomach releases at least two signals, leptin and ghrelin, which may be integral for intestinal sensing and absorption of nutrients, as well as the control of energy intake. Furthermore, while the stomach lacks

chemosensory properties, more recent evidence suggests a role of gastric nutrients in altering endocrine secretions although this is still debated.

1.2.2.2.1 *Gastric Leptin*

Leptin, a 16 kDa protein that is normally secreted from adipose tissue and reflective of energy status, also is secreted by epithelial cells of the stomach [185-188] and thought to play a role in gastrointestinal signaling [189]. Gastric leptin secretion is influenced by feeding [190], neurotransmitter release [191], as well as other gastrointestinal hormones [189]. Furthermore, the identification of leptin receptors (LepR) on the cell bodies of vagal afferents [192, 193] and application of leptin increases activation of neurons in the vagus [194], which implies that gastric leptin may play a role in feeding behavior. Indeed, leptin and CCK synergistically activate vagal neurons [195-197], and produce synergistic reductions in food intake [198, 199]. It is thought that gastric leptin, rather than circulating leptin is responsible for leptin activation of the vagal afferents. Evidence of this comes from the finding that gastric, but not circulating leptin quickly rises after ingestion of a meal [190] and circulating hormones typically do not utilize a vagally mediated pathway [200]. Also, despite the acidic pH of the stomach, leptin remains stable, and enters the intestinal lumen [189]. This, together with the identification of leptin receptors on the brush border of the intestinal lumen denotes the possibility of gastric leptin influencing intestinal function [201].

Interestingly, application of leptin to an enterocyte cell model or intestinal infusion of leptin increases enterocyte absorption of amino acids [202]. The effects of intestinal luminal leptin on sugar transport are less clear. For example, luminal leptin has an

adverse effect on glucose absorption, decreasing expression of the active glucose transporter SGLT1 [201, 203]. However, *in vitro* and *in vivo*, luminal leptin increases expression of the passive sugar transporters GLUT2 and GLUT5 [204]. Additionally, luminal leptin may inhibit secretion of intestinal triglyceride processing via inhibition of apolipoprotein A-IV [205, 206]. In addition to its effects on nutrient transport, leptin activates enteroendocrine cells of the duodenum evoking the release of CCK. Interestingly, luminal leptin alone sufficiently increases plasma CCK concentrations similar to that of a meal [189]. This mediation of CCK secretion by gastric leptin is thought to be a positive regulatory loop as intestinally released CCK stimulates secretion of gastric leptin as well [189]. Despite these findings, the relative contribution of gastric leptin to food intake has yet to be elucidated, but the finding that vagal afferents contain leptin receptors [193] denotes a possible role in this gastric peptide in the regulation of food intake.

1.2.2.2.2 *Ghrelin*

Of all the hormones and peptides secreted from the gastrointestinal tract, only one discovered thus far has been shown to promote food consumption. Discovered in nearly 15 years ago, ghrelin, the potent orexigen, is a 28 amino acid peptide released mainly from specialized endocrine X/A-type cells [207] of the stomach. It exerts its physiological effects on stimulating eating and growth hormone (GH) secretion by binding to the growth hormone (GH) secretagogue receptor-1a (GHS-R1a). Compared to other peptides released from the GI tract, ghrelin is unique due to its increased circulating levels during fasting, which together with its ability to increase food intake,

indicates the possibility of a role in initiating a meal. In addition to its direct effects in stimulating food intake, ghrelin also decreases energy expenditure and promotes the storage of fatty acids in adipocytes, denoting its potential importance in pathological conditions, such as obesity.

Ghrelin is the endogenous ligand for the GHS-R1a receptor or recently renamed, "ghrelin receptor." Both ghrelin and its receptor are located in peripheral and central tissues. Within the central nervous system (CNS), ghrelin is localized in hypothalamic and pituitary nuclei of the forebrain that are heavily implicated in the control of food intake or growth hormone secretion. Despite the initial finding that ghrelin stimulates GH secretion [208], the most potent biological function of the peptide is stimulation of food intake through a GH-independent mechanism [209]. The arcuate nucleus (ARC) of the hypothalamus, which is involved in controlling food intake, expresses the highest concentration of centrally distributed ghrelin [210]. Although ghrelin is distributed throughout the central nervous system, peripheral ghrelin from the GI tract, most notably the stomach, is thought to be the primary site for ghrelin secretion and circulating ghrelin [211]. In support of this, partial or complete, gastrectomy (removal of the stomach) markedly reduces circulating ghrelin levels by approximately 70% [212]. Ghrelin is produced in the mucosal layer of the stomach by the endocrine X/A-type cells [207], which are distributed throughout the stomach, but are highly concentrated in the gastric fundus [213]. Gastric X/A-type cells increase in number throughout the fetal period, reaching a maximum during infancy. Likewise, ghrelin levels in the stomach are low during development. One month following birth, however, ghrelin concentrations reach a peak and show no further increase. While the stomach is the main site of

ghrelin secretion, ghrelin is detected throughout all layers of the GI tract, salivary glands and alimentary organs, such as the pancreas [214], all of which contribute to the remaining 30% of circulating ghrelin. In the circulation, ghrelin is represented by two forms: des-acyl ghrelin and acyl ghrelin (*n*-octanoyl-modified ghrelin) [215]. The former version is 5 to 10 times more abundant in plasma than the latter; however, the less common acyl-peptide is thought to be the active form in nearly all physiological, behavioral, and endocrine processes, including food intake. To yield the biologically active acyl-ghrelin, the enzyme, gastric O-acyl transferase (GOAT), cleaves the pre-proghrelin peptide [216]. While the finding of two forms or ghrelin as well as the enzyme responsible for yielding the active form of ghrelin have been recent in respect to the discovery of ghrelin, both total ghrelin and active ghrelin plasma concentrations have been shown to be highly correlative following experimental manipulations [217]. A variety of factors control ghrelin secretion from the stomach into the peripheral circulation with energy and macronutrient content of a meal the main contributors.

Ghrelin is the only known potent peripheral peptide hormone that is an orexigenic. This is supported by several pieces of evidence. First, both central and peripheral administration of ghrelin results in increased food intake and associated appetitive ratings [218-220]. In rodents, exogenous ghrelin induces food intake during the light cycle, a period associated with minimal food consumption [220]. Second, administration of either a ghrelin receptor antagonist or an anti-ghrelin immunoglobulin (IgG), which inactivates biologically active ghrelin, causes a decrease in food intake in several feeding paradigms [221, 222]. Furthermore, ghrelin decreases latency to eat and increases meal number. Interestingly, both humans and rodent models retain sensitivity

to the peptide and repeated administration results in exponential increase in cumulative food intake leading to increased bodyweight and adiposity [218, 220, 223] (Table 3). Altogether, these findings support the hypothesis that ghrelin is an orexigenic signal controlling food intake.

Endogenous ghrelin levels are consistent between sexes and across age groups; however, age-related anorexic rats' plasma ghrelin levels fail to increase after 72 hours fasting [224]. While this could implicate age as a factor in modulating ghrelin secretion, it more likely underscores the importance of food components regulating secretion of the peptide. As such, during periods of food deprivation, across a large range of ages, plasma ghrelin is substantially elevated while refeeding or recovery from food deprivation rapidly blunts elevated circulating levels [218, 225]. Furthermore, increased levels of plasma ghrelin during these deprivation challenges are associated with up-regulation of ghrelin receptors leading to increased food intake [226].

Ghrelin levels are also influenced by the timing of a meal. In schedule-fed rats, rising ghrelin levels coincide with pre-prandial period or onset of a meal [227]. Similarly, in humans, ghrelin levels substantially increase before the onset of a meal and adjust according to meal times [228]. Long-term markers of altered energy homeostasis, such as adiposity, also correlate with circulating ghrelin concentrations. Obesity, a pathological state characterized by increased body mass, is characterized by an alteration of secretion and circulating ghrelin concentrations. Specifically, an increased body mass index (BMI) directly correlates with decreased plasma ghrelin levels [225]. Because obesity is associated with suppressed ghrelin levels, it is not surprising then that anorexic patients with significantly lower BMIs exhibit chronically elevated

circulating ghrelin concentrations [229]. The decreased concentration of circulating ghrelin in obese individuals is thought to be due to excess energy intake; whereas in anorexic patients, a constant caloric deficit causes elevated plasma ghrelin concentrations, in attempt to restore proper energy balance. In both pathological states, however, ghrelin levels begin to return to normal concentrations when the individual is nearing normal body weight [230]. Furthermore, in addition to food intake and energy balance, the composition of an ingested meal is an important regulator of ghrelin secretion.

Despite ghrelin being released from the stomach, an organ sensitive to mechanical rather than chemical signals (volume vs. specific nutrients of a meal), and macronutrient content of the meal has a significant effect on modulating ghrelin release. Nutrients from all three major macronutrient classes suppress ghrelin secretion; however, carbohydrates and proteins are most potent inhibitors. Specifically, in humans, a carbohydrate solution significantly decreases ghrelin secretion in a biphasic manner, while protein suppresses circulating ghrelin significantly more 40-min post-prandially than an equicaloric and equivolumetric lipid drink [231]. In rodents, gastric infusions of glucose more potently inhibit ghrelin secretion than infusions of fatty acids and amino acids. These effects, however, are dependent upon intestinal absorption of nutrients as gastrically infused glucose solutions fail to suppress circulating ghrelin concentrations when the gastric pylorus is occluded [232]. Furthermore, treatment with orlistat, a potent lipase inhibitor, abolishes long-chain fatty acid induced reduction of plasma ghrelin levels [233]. Together, these pieces of evidence demonstrate the importance of short- and long-term food intake as well as macronutrient content of a meal to regulate

ghrelin secretion. The exact detection of nutrients that regulates ghrelin secretion is thought to occur predominantly in the intestine, as it rapidly receives ingesta that is emptied from the stomach.

1.2.3 Intestine

1.2.3.1 *Intestinal nutrients and satiation*

The small intestine of the GI tract serves as a portal for digesting, sensing, and absorbing nutrients, which all contribute to intestinal nutrient satiation. Infusion of a liquid diet into the intestine results in inhibition of food intake [234-237]. While the effects of intestinal nutrient infusions on the suppression of food intake may be long-lasting and extend to multiple hours, termination of the meal begins rapidly, normally seconds after commencing of a nutrient infusion [235, 238]. Thus, it is hypothesized that the sensing, rather than absorption of nutrients is of importance in intestinal nutrients reducing food intake. Furthermore, while intestinal nutrient infusions inhibit feeding in animals, intestinal nutrients surely reduce the rate of gastric emptying, thus increasing distention of the stomach, which could be one way by which nutrients reduce food intake [239]. However, intestinal nutrient infusions also inhibit feeding in animals with gastric fistulas, where gastric distention is absent [235, 240] denoting the importance of intestinal factors in autonomously controlling meal size. Ingesta entering the intestine from the gastric cavity are typically hyperosmotic, and contain a variety of nutrients, all of which influence feeding responses. In animals, the infusion of hyperosmotic loads indeed decreases food intake in freely feeding animals [241]. Despite this, the hyperosmotic and complete nutritive nature of chyme is not the main

contributor to intestinal satiation. For example, intestinal infusion of specific macronutrients, such as oligosaccharides [242] or long-chain fatty acids [243] reduces food intake, even in hypotonic or isotonic concentrations.

1.2.3.2 Intestinal carbohydrates

1.2.3.2.1 *Intestinal carbohydrates and feeding behavior*

Intestinal carbohydrates and sugars have been reported to reduce food intake in both sham- and real-feeding animals [243, 244], and increasing the length of the oligosaccharide used for an intestinal infusion results in a greater reduction of food intake than a simple sugar [245]. Additionally, the digestion of oligosaccharides to simple sugars is nearly essential in reducing food intake as administration of the oligosaccharidase inhibitor, acarbose, results in attenuation of oligosaccharide-induced reduction in food intake [246]. Specifically, the ability of carbohydrates to reduce food intake may require glucose, at least when low concentrations are present as glucose infusions will significantly reduce food intake, but low concentrations of fructose has no effect on feeding [247]. Collectively, these data implicate carbohydrate-induced satiation, and most likely, the detection of glucose in influencing food intake.

The specific intestinal mechanism responsible for glucose-induced satiation is still not completely understood. It is well known that hydrolysis of oligosaccharides produces monosaccharides, which are predominantly absorbed via active transport on the apical membrane of the intestinal epithelium. For example, luminal glucose is largely transported by the active glucose transporter SGLT1 while other monosaccharides, such as fructose, require the passive transporter, GLUT5, for

absorption. Interestingly, intestinal infusions of glucose isomers, which are substrates for SGLT1 reduce food intake [248] while the aforementioned fructose does not. However, the findings that intravenous glucose is mostly ineffective at reducing food intake compared to intestinal infusions of glucose, and that glucose in the lumen is inversely related to blood glucose levels exemplifies that a pre-absorptive mechanism is responsible for glucose-induced satiation [245]. This is probably independent of SGLT1 as well because inhibition of SGLT1 activity does not attenuate intestinal glucose-induced satiation [249]. Therefore, the hydrolysis of oligosaccharides to glucose is at least somewhat necessary for carbohydrate-induced reductions in food intake, but the absorption of glucose is not. Recent evidence suggests that luminal intestinal epithelial receptors, similar to those located in the lingual epithelium, may a possible pathway in detecting intestinal glucose, and subsequently regulating dietary absorption of carbohydrates.

1.2.3.2.2 *Mechanisms of intestinal carbohydrate detection*

The transport of luminal glucose in the intestine is carried out by the active glucose transporter SGLT1. To facilitate this process, SGLT1 localized on the apical epithelium of enterocytes transports both glucose and sodium, which is driven by the increased glucose concentration in the intestinal lumen and decreased intracellular Na^+ levels due to the basolateral Na^+-ATPase [250] (Figure 6). The other major dietary monosaccharide, fructose, is transported into the enterocyte via the passive GLUT5 transporter. Once localized in the enterocyte, glucose exits the cell and enters the hepatic portal circulation via the basolaterally expressed passive transporter, GLUT2

[250]. The general expression pattern for SGLT1 is greatest in the proximal intestine, and more specifically, the duodenum [251]. While the basolaterally expressed GLUT2 may also be expressed on the apical membrane of enterocytes, and has been hypothesized to play a role in post-prandial glucose transport [252-254], an animal model displaying a GLUT2 mutation does not exhibit defects in intestinal glucose absorption [255]. As such, it is hypothesized that SGLT1 is the principal mediator of intestinal glucose absorption.

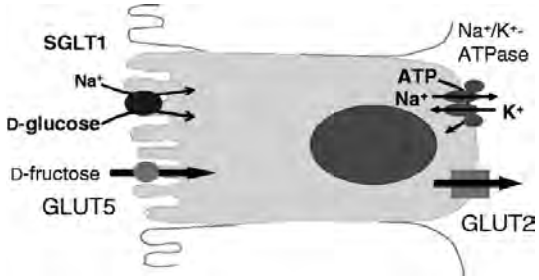


Figure 6: Luminal and basolateral enterocyte monosaccharide transport. Luminal glucose is absorbed via the active transporter SGLT1 while fructose is absorbed via the passive transporter GLUT5. SGLT1 function is dependent upon basolateral Na+/K+-ATPase, which decreases intracellular Na^+. To transporter absorbed sugars into the blood stream, both dietary glucose and fructose utilize the passive transporter, GLUT2, on the basolateral membrane [256].

The intestine can alter its ability to absorb glucose through the induction of SGLT1 expression [257-260], independent of increases in intestinal surface area [261] or metabolism of glucose [257, 259] . As such, intestinal infusions of both metabolically active and inactive substrates of SGLT1 up-regulates the expression of SGLT1 in the intestinal brush border [262]. Furthermore, the finding that glucose analogues incapable of being transported via SGLT1 increases expression of this transporter and

is independent of SGLT1 activity suggests that a SGLT1-independent mechanism senses luminal glucose concentration to up-regulate SLGT1. The transfection of cells with glucose sensing elements further reveals that SGLT1 expression regulation is dependent upon G-protein coupled receptor signaling [263]. In addition to its localization in the taste buds, the sweet taste receptor dimer, comprised of T1R2 and T1R3, coupled with α-gustducin, is found throughout all levels of the gastrointestinal tract of many animals [264-267] as well as enteroendocrine cell lines [268]. Both proteins, however, are largely expressed in the proximal intestine, specifically in the duodenum and jejunum, where SGLT1 expression is highest, with only marginal expression occurring in the distal ileum and colon [269].

It is hypothesized that the T1R2+3 complex, identical to that found in the lingual epithelium (Figure 7), coupled to α-gustducin, is the mediator of glucose-induced SGLT1 up-regulation [265]. The definitive importance of these receptors in regulating glucose absorption and glucose sensing comes from studies that show absence of T1R3 or α-gustducin results in an inability of animals to increase expression of SGLT1 during high carbohydrate feeding [265, 270]. In cats, which lack a functional T1R2 receptor [49], and are relatively insensitive to sweet taste, SGLT1 expression is not up-regulated in response to carbohydrate feeding as well [264]. Similarly, in chickens, which lack expression of T1R2 [271], intestinal infusion of carbohydrates does not up-regulate SGLT1 expression [272]. Finally, feeding wild-type mice a high-carbohydrate diet or standard laboratory chow diet with sucralose added to drinking water enhances expression of SGLT1, an effect absent from T1R3 KO animals. The contribution of T1R3 in up-regulating SGLT1 expression is not necessary; however, as T1R3 KO mice

fed a low carbohydrate diet express similar SGLT1 levels as wild type animals [265]. Thus, there is constitutive expression of SGLT1, and the sweet receptor taste complex is hypothesized to have a role in up-regulating SGLT1 expression when dietary carbohydrates are increased. In support of this is the finding in pigs that the feeding of low and moderate levels of dietary carbohydrates results in similar SGLT1 expression ; however, increasing dietary carbohydrates further increases expression of SGLT1 [266]. Recent evidence also suggests that T1R3 may play a crucial role in increasing GLUT2 expression [273], which is not necessarily mutually exclusive from T1R2+3 increasing SGLT1 as these receptors function in coordination to deliver luminal glucose to the enterocyte and into the circulation.

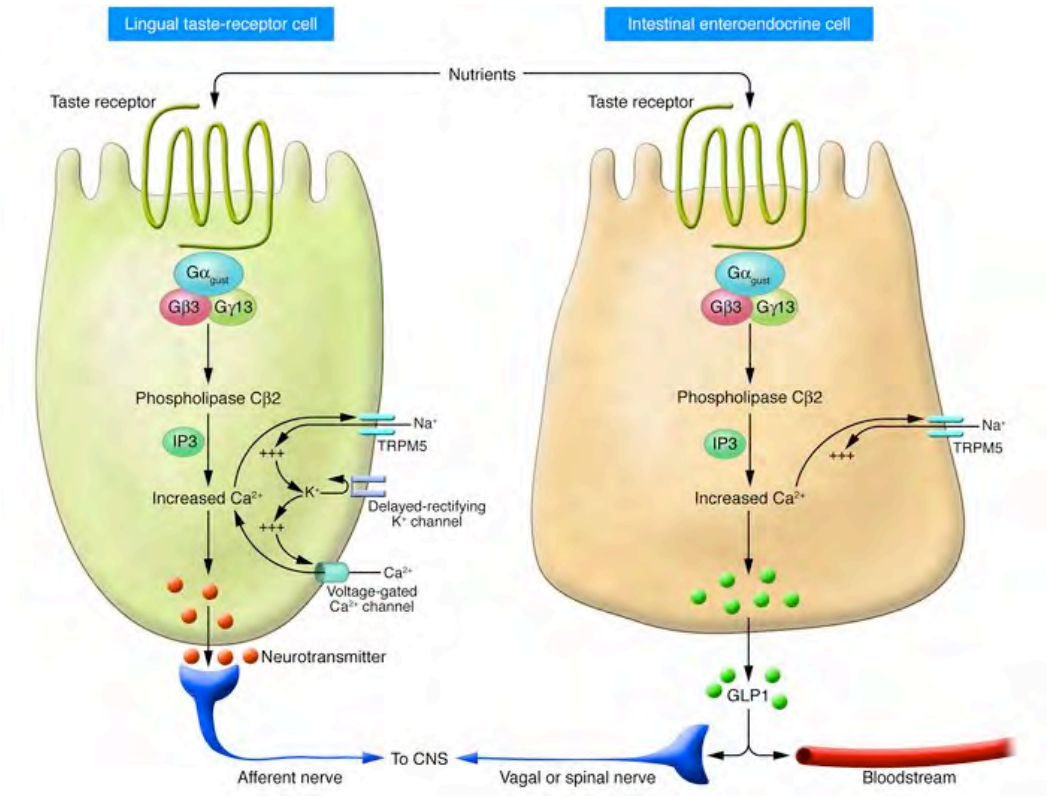


Figure 7: Similarities between lingual TRCs and intestinal enteroendocrine cells. Recent evidence demonstrates that numerous receptors located on TRCs in the mouth are also located on enteroendocrine cells in the intestine and display similar second messenger pathways.

The exact mechanisms of T1R2+3 mediated up-regulation of SGLT1 in response to dietary carbohydrates are still unclear; however, it is thought to be dependent upon intestinal peptide release. The initial hypothesis was that T1R2+3 stimulation, occurs on the apical membrane of the enteroendocrine cells results in secretion of gut incretin peptides GLP-1, GLP-2, or GIP via a Ca^{+2}-dependent mechanism identical to that of intracellular taste signaling described previously [274]. Briefly, this pathway utilizes PLCβ2 to increase intracellular Ca^{+2} stores, which bind to TRPM5, and serve to depolarize the cell (Figure 8). However, further research in humans and rats has yielded conflicting results of T1R2+3 stimulation leading to gut peptide release [275,

276]. Despite this, T1R3 KO mice display decreased circulating levels of GLP-1 during the fasting and post-prandial state [277]. The exact mechanism thought to induced SGLT1 up-regulation involves the ENS and GLP-2 signaling [277-281]. As such, GLP-2 is localized in enteroendocrine cells expressing T1R2+3 [269] and the GLP-2R is located on enteric neurons [282, 283]. Furthermore, application of GLP-2 stimulates enteric neurons innervating the small intestine, and activation of these neurons induces responsiveness of enterocytes [283]. As well, use of electric field stimulation up-regulates SGLT1 expression in enterocytes. In support of a glucose-mediated mechanism involving the ENS regulating SGLT1 expression is luminal glucose-mediated increases in SGLT1 is abolished when enterocytes are isolated from the intestinal mucosa [284]. Together, these pieces of evidence demonstrate the ENS, involving GLP-2 signaling as a likely candidate to increase SGLT1 after stimulation of T1R2+3 on enteroendocrine cells. In the enterocyte, the specific intracellular signaling for the up-regulation of SGLT1 is dependent upon adenyl cyclase signaling mechanisms and cAMP concentrations [285-287] as mutations in PKA results in impaired SGLT1 expression [288] and increases in cAMP result in increased SGLT1 expression. [289]

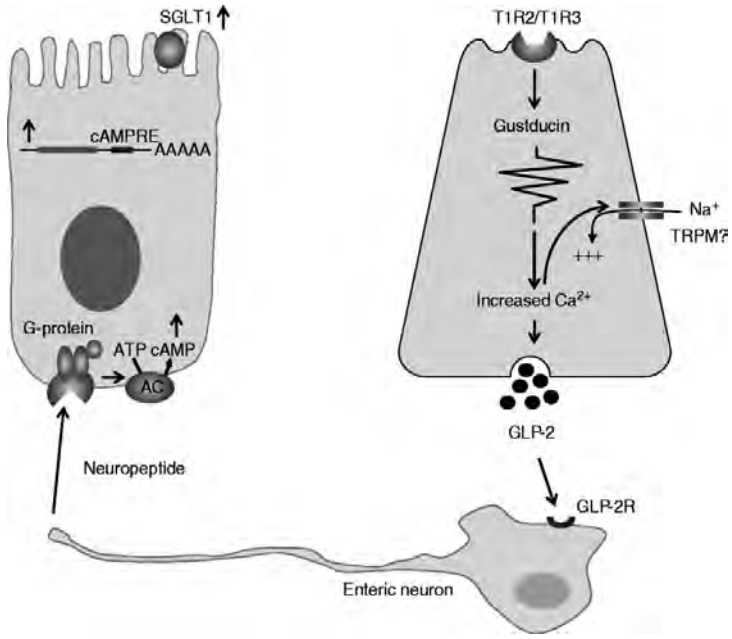


Figure 8: Hypothesized mechanisms for the up-regulation of SGLT1 in response to dietary carbohydrates. Stimulation of intestinal T1R2+3 located on enteroendocrine cells results in secretion of intestinal peptides, specifically GLP-2 that binds to neurons on the enteric nervous system, which release a neurotransmitter that activates a GPR neurotransmitter receptor located on the enterocyte. The neurotransmitter receptor utilizes cAMP signaling, which enhances genetic transcription of SGLT1 that results in the up-regulation of luminal SGLT1 [256].

1.2.3.3 Intestinal fats

1.2.3.3.1 *Intestinal fats and feeding behavior*

In addition to carbohydrates, fats and their products of digestion are potent inhibitors of feeding when infused into the small intestine. Similar to carbohydrates, the composition of the infusate is extremely important as products of fat digestion reduce food intake more than non-digested fat products [290]. This data, coupled with the finding that inhibition of pancreatic lipase via orlistat administration attenuates intestinal fat-induced reductions in food intake denote the vital role of fat digestion in controlling intestinal fat

satiation [291]. In addition to this, the carbon chain length of fatty acids is important as intestinally infused oleic acid, comprised of an 18 carbon chain reduces food intake while infusion of the 8 carbon length octenoic acid does not [242]. These findings may be due to the digesting and processing of longer chain fatty acids into chylomicrons as long-chain fatty acids are suspended in micellar emulsions in the intestinal lumen, and then processed into chylomicrons, and secreted to the lymph [292, 293]. Short- and medium-chain fatty acids, in contrast, are absorbed directly into the hepatic-portal duct. The importance for chylomicron formation in fat-induced satiation is further supported by the finding that administration of Pluronic L-81, a chemical that blocks fat absorption via inhibition of chylomicron formation [294], attenuates reductions in food intake induced by intestinal fat infusion [291, 295].

1.2.3.3.2 *Mechanisms of intestinal fat detection*

Similar to the detection of carbohydrates, receptors expressed in TRCs that are responsive to fatty acids are also expressed on the apical membrane of gut enterocytes and enteroendocrine cells positioned to sense intestinal lipids [296]. The fatty acid translocase CD36 is necessary for proximal intestine lipid absorption [297] and chylomicron formation [298]. For example, CD36 KO mice exhibit decreased proximal, but not distal intestine chylomicron formation [298] and cholesterol and fatty acid absorption [299]. The binding of CD36 is almost exclusive to long-chain fatty-acids (LCFA) as there is almost no effect of CD36 KO on short-chain fatty-acid (SCFA) or medium-chain fatty-acid (MCFA) absorption. While the specific mechanism by which CD36 mediates LCFA absorption is unclear, one hypothesis is that it is part of a larger

complex assisting in the absorption, which is further dependent upon fatty acid transporter 4 or simple diffusion. In support of CD36 mediating chylomicron formation is that isolated enterocytes from CD36 KO mice display decreased triglyceride secretion [300]. Similar to glucose transporters, the intestine also increases production of CD36 in response to increasing dietary fat [301]. As such mice fed a HF diet exhibit up-regulation of CD36. Furthermore, CD36 KO animals display decreased intake and preference of fat emulsions [82]; however, the contribution of CD36 lingual fat detect (discussed previously), as well as the possible role of neurons utilizing CD36 for lipid sensing [302] blur the contribution of intestinal CD36 in these findings. Interestingly, CD36 KO mice display decreased reductions in food intake in response to intestinal lipid infusions [303]. Despite this wealth of data demonstrating a distinct role in lipid sensing, absorption, and short-term satiation, the specific contribution to intestinal CD36 to long-term feeding behavior is uncertain

. In addition to LCFA, energy status mediated signals, such as peroxisome proliferative factor-α (PPAR-α), are responsible for mediation of CD36 [303]. The endogenously produced fatty acid ethanolamide, oleoylethanolamide (OEA), present in the proximal small intestine is also a crucial mediator of CD36. For example, administration or over-expression of OEA results in decreased expression of CD36 and PPAR-α, with subsequent reductions in food intake [304]. Endogenous OEA is produced in response to the presence of intestinal fat. Hence, with intestinal fat infusion, OEA synthesis is increased and food deprivation inhibits OEA synthesis. Synthesis of OEA is accompanied by increases in intestinally produced oleic acid as well, denoting this pathway as a crucial pathway of intestinal fat satiation [303]. Thus, in the presence

of intestinally infused fat, OEA production increases, leading to up-regulation of CD36, which allows for further lipid absorption and enhanced satiety [305]. In support of this evidence is that administration of OEA leads to reductions in food intake as well as increases in intermeal intervals [306]. Similarly to intestinal fat infusions, which require vagal sensory afferents to induced satiation [242], application of capsaicin results in attenuation of OEA-induced satiation [306]. Therefore, these findings provide the substantial evidence that intestinal fat satiation may be mediated, predominantly by CD36 and OEA (Figure 9).

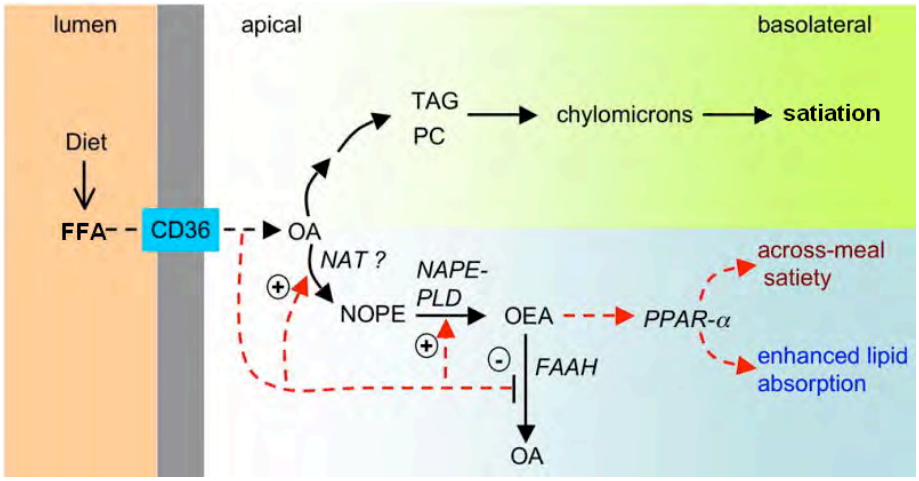


Figure 9: Schematic diagram demonstrating how CD36 and OEA mediate satiation and satiety. Free-fatty acids absorbed from the intestinal lumen via a CD36-dependent mechanism lead to the production of OEA and chylomicrons. Production of OEA stimulates PPAR-alpha enhancing satiety while chylomicron production is necessary for the reduction of food intake by intestinal fats via the release of intestinal satiety peptides [303].

The direct sensing of luminal fats in the intestine by apical transmembrane GPRs may also be of importance as well in fat-induced satiation. Satiety peptides released from the intestine in response to nutrients, such as fat, elicit satiation (discussed below). Specifically, GPR40, which is expressed in the intestinal epithelium [91], is activated by

fatty acids with 6 or more carbons [307], mediates CCK secretion [91]. Additionally, GPR40 is co-localized with CCK expressing cells of the intestine and KO of GPR40 in mice results in attenuation of lipid-induced CCK secretion. Isolated enteroendocrine cells from these animals also display an abolish response of LCFA-induced stimulation of intracellular Ca^{+2} level increases and are unable to secrete CCK in response to LCFA [91]. Despite the clear role of GPR40 in enteroendocrine cell activation and CCK secretion as well as the finding that GPR40 KO animals consume more standard chow than wild-type animals, HF-feeding, a potent stimulus for CCK secretion, results in similar daily food intake and body weight between GPR40 KO and wild-type mice [308]. Short-chain fatty acids activate GPR41 and 43 [309], which are found in both the proximal and distal intestine [310, 311], and are linked to PYY [312] and 5-HT secretion [313]. In cell models, stimulation of either receptor with SCFA results in PYY secretion and both GPR41 and 43 are co-localized with PYY [313]. As well, SCFA in the distal intestine, including the colon, induce PYY and serotonin secretion. Despite this, to date, only GPR41 is known to mediate PYY secretion *in vivo* as KO of this receptor leads to decreases in circulating PYY [312]. Furthermore, GPR41 KO animals display increased intestinal transit, which is most likely secondary to the decreased secretion in PYY, which serves to inhibit intestinal motility [312]. As well, GPR120, which is activated by long-chain fatty acids, is responsible for GLP-1 and possibly CCK secretion as knockdown of GPR120 results in attenuation of GLP-1 [93] and CCK [91] secretion from enteroendocrine cells lines. Finally, KO of GPR120 results in increased body weight and deleterious effects on blood glucose metabolism during HF-feeding relative to control animals [314]. However, the fact that GPR120 is found on various metabolically

active tissues [315], such as the pancreas [316] and adipose tissue [317] has confounded the relative contribution of GPR120 in feeding behavior. Thus, despite the few *in vivo* studies demonstrating a role of these receptors in intestinal satiety peptide secretion, the majority of research demonstrating GPR stimulation inducing gut-peptide release has been performed in cell models, and it remains unclear how these receptors regulate feeding behavior.

The specific intracellular pathway that each GPR utilizes to induce gut peptide secretion is under investigation, and has been found to encompass second messenger pathways similar to TRCs. After the binding of fatty acids to GPRs on the extracellular N-terminus, coupled second messenger proteins dissociate and stimulate downstream kinases and lipases. The first, which is nearly identical to that of T1R2+3, is that second messenger g-proteins activate PLCβ2, leading to increases in intracellular Ca^{+2} signaling resulting in exocytosis of gut peptides via a Ca^{+2}-dependent mechanism [318]. Additionally, these GPRs also utilize a second messenger pathway stimulating Protein Kinase B via an extracellular receptor kinase (ERK) [319], which leads to secretion of gut peptides. As such, stimulation of enteroendocrine cells lines with nutrients results in a rapid increase of intracellular Ca^{+2} as well as activation of ERK and concomitant gut peptide secretion.

1.2.3.4 Intestinal nutrients, conditioned preferences, and reward

In addition to intestinal nutrients playing a profound role in terminating a meal, the exposure of nutrients to the intestine is vital in establish learned food preferences, a term coined “post-oral conditioning.” The evidence of post-oral conditioning influencing

feeding behavior comes from studies where intake of a flavored nonnutritive solution is paired with gastric or intestinal infusion of a specific macronutrient, typically carbohydrates that are strong enforcers of post-oral conditioning. For example, rats that are trained alternating days to drink a specific flavor (e.g. grape) paired with a gastric infusion of glucose or another flavor (e.g. cherry) paired with a gastric infusion of water display a profound preference for the grape flavored solution over the cherry solution when given concomitant access to both flavors and in the absence of gastric infusion [320]. In these studies, animals typically "self-infuse" the infusate by starting to drink the flavored solution and the infusion beginning almost simultaneously [321]. However, post-oral infusions of glucose also can also stimulate consumption of a flavored solution when paired 1-h post consumption [322]. The effects of post-oral glucose conditioning flavor preferences also is long lasting, denoting a probable reward pathway controlling this behavior [323-325]. As well as increases in flavor preference, post-oral conditioning with intestinal carbohydrates increases overall intake of the flavored solution [326-328]. The site of action for this phenomenon is the proximal intestine as occluding the stomach prevents gastric infusions from conditioning flavor preferences [329] and duodenal or jejunal, but not ileal [329, 330], or intravascular infusions of sugars [331] results in post-oral flavor conditioning . However, changes in the feeding paradigm, such as water and food deprivation, coupled with a flavored nutritive solution can result in intravascular glucose to condition flavor preferences [332].

While glucose is an extremely strong enforcer of conditioned flavor preferences, other nutritive sweet substances, such as sucrose and fructose can have the same effect [333-336]. However, for the latter, animals require long infusions (>20-h) in

conditioning flavor preferences paired with gastric infusions [336]. In contrast to this, nutritive sweet lactose and galactose [320, 337, 338] as well as nonnutritive sweet stimuli, such as sacharrin or sucralose [339, 340] do not condition flavor preferences. The former two may be explained by the inability of an animal to fully digest these nutrients while the latter two are explained by the nonnutritive value. In addition to these findings, research demonstrating that T1R3 KO mice maintain normal post-oral conditioned flavor preferences [340] lends notion that a mechanism other than sweet receptors, such as SGLT1 or SGLT3 activity [341], may be responsible for these findings. Furthermore, in the absence of post-intestinal signaling, such as vagal deafferentation via mechanical [342, 343] or chemical [344] means, animals continue to display conditioned flavor preferences with glucose infusions. Additionally, intestinal satiety peptides, which are released in response to intestinal nutrients, control nutrient-induced satiation, but do not control glucose-induced conditioned flavor preferences [335, 345, 346]. Collectively these findings demonstrate that a pre-absorptive mechanism, localized in the intestine, is responsible for post-oral conditioned flavor preferences induced by glucose.

Similarly to glucose and sucrose, intestinal infusions of fat can condition flavor preferences rapidly [347] with a resistance to extinction [348]. The length and composition of the fatty acid is important as well in influencing this finding as medium chain fatty acids or saturated fatty acids do not condition flavor preferences as strongly as polyunsaturated, long chain fatty acids do [349]. Further, although the caloric value of intestinal fat infusions is important to condition flavor preferences, and inhibition of fat digestion with orlistat partially blocks fat conditioned flavor preferences [350], it is not

the major determinant of this effect. For example, an oil emulsion that has a higher caloric density than a glucose solution, conditions flavor preferences less than glucose [339]. However, the relatively long time course for the digestion of fat is hypothesized to play an integral role in its conditioned effects as more trials are needed to condition flavor preferences than those observed with glucose [325, 333], but once established, animals consume more of the fat-paired flavor over one hour [351] . Currently, the origin and mechanisms of intestinal fat conditioned flavor preferences are unknown, but involve a post-gastric site [344]. Post-intestinal, intravascular infusions of fat have not been examined, and CD36 KO mice display relatively strong fat conditioned preferences [101] while GPR KO animals have not been tested under this paradigm. Despite this relative uncertainty, vagal afferents are not necessary for fat conditioned flavor preferences [344], but peripheral inhibition of fat oxidation reduces reinforcing properties and preference of fat [352]. Thus, it is probable that the oxidation of fat, and not an intestinal factor, is responsible for fat conditioned preferences.

Similarly to taste signaling, post-oral nutrient conditioned flavor preferences and the enhancement of solution intake is hypothesized to be controlled via reward centers in the brain [332, 353]. However, in contrast to central taste processing and via afferent pathways, which are better established, the knowledge of post-oral signals conditioning intake are less clear. Intestinal and intravascular glucose is a documented stimulator of DA release in the NAcc [332, 353], and a metabolic, rather than central glucose sensing mechanism is thought to induce this release as hepatic-portal infusions stimulate more DA release than jugular infusions [332]. Additionally, intestinal infusion of glucose also increases neuronal activation of the NAcc, and is unchanged following vagotomy [354,

355], similar to behavioral findings of conditioned flavor preferences [343, 344]. The role of endogenous DA stimulating intestinal glucose-induced conditioned preferences comes from the finding that application of a DA1 receptor antagonist in the NAcc blocks glucose conditioned flavor preferences [356]. Similarly to taste, this pathway may involve neurons of the PBN as lesions of this nucleus results in impairment of carbohydrate induced flavor learning [357]. The effect of post-oral fat on reward centers are even less known. Intestinal infusions of fat stimulate DA release from the NAcc in the absence of oral stimuli. As well, post-oral infusions of fat active neurons in reward nuclei, such as the NAcc ventral tegmental area and amgydala with neural activation occurring before fat absorption [358]. Despite this, peripheral administration of DA receptor antagonists does not prevent fat conditioned flavor preferences in rats [359], but blocks fat-induced place preference [360] and operant licking for gastrically infused oil emulsions in mice [361]. Together, this relative scantiness of data with the fact that in humans, post-oral feedback from foods likely contributes to the over consumption of high calorie and palatable foods, demonstrates the need for further research in examining how post-oral nutritive feedback stimulates feeding behavior and its relationship with reward pathways.

1.2.3.5 Obesity and intestinal nutrient satiation

Studies examining the effect of obesity on intestinal sensitivity to nutrients show that obese rodent models display decreases in nutrient-induced satiation, which likely contributes to their over consumption. For example, carbohydrate or fat infusions in genetically obese OLETF rats suppress food intake less than in lean controls [143, 362].

Similarly, the obese prone Osborne-Mendel (OM) rat displays decreases in intestinal fat satiation [363]. Also, the Zucker fatty rat, which displays impaired leptin signaling, exhibits decreased intestinal-induced satiation relative to lean controls, but increased protein-induced satiation. While the OLETF model most likely exhibits a broad decreased sensitivity to intestinal nutrients due to lack of functional CCK signaling [364], the mechanism responsible for the decreased reduction of food intake following nutrient loads in the OM and Zucker rat are less clear. For example, the OM rat displays modestly enhanced sensitivity to endogenously CCK [365], which is largely released in response to intestinal fats [238]. However, blockade of endogenous CCK, which mediates intestinal fat satiation [238] was not examined and could provide conclusive evidence for the mechanism of decreased intestinal fat satiation observed in this model. The Zucker fatty rat, on the other hand, displays impaired leptin signaling, and given that leptin interacts with numerous signals inhibiting feeding, including CCK [199, 366], this may be the main contributor to decreased intestinal fat satiation in this model. More recent evidence in the outbred DIO model, which becomes obese during HF-feeding, demonstrates that these animals exhibit decreases in gastrointestinal fat induced satiation relative to DIO resistant (DR) animals fed the same diet [10]. In humans, this data is less clear, with virtually no studies examining intestinal expression of carbohydrate and fatty acid sensing receptors in the intestine between obese and normal individuals. However, the diabetic state is not associated with any differential expression of intestinal T1R2+3 relative to normal patients [367]. Despite this, a majority of studies in humans that have examined circulating levels of intestinal satiety

peptides following a meal have demonstrated levels are decreased in obese individuals, and this is reversed during weight loss [368-370].

1.2.3.6 Vagal afferents and intestinal nutrient satiation

Signals generated from the intestine in response to the presence of intestinal nutrients are transmitted through the vagus nerve to the caudal brainstem, which receives and process visceral information that then relays information to upstream forebrain nuclei that regulate long-term energy homeostasis. However, forebrain structures are not necessary in regulating food intake as decerebrate animals, with only the brainstem intact, terminate a meal normally [371-373]. As such, vagal afferents mediate a majority of nutrient [242, 243] and nutrient-induced satiation signaling [374] arising from the small intestine. This is thought to be due predominantly to sensory vagal afferents, because while vagotomy abolishes reduction in food intake from both carbohydrate and fat infusions [243], peripheral or central administration of capsaicin, a potent neurotoxin that selectively destroys vagal sensory fibers only, abolishes carbohydrate and fat-induced satiation as well [242]. Furthermore, the coeliac vagotomy, which denervates the main branch of the vagus that provides innervation to the duodenum, abolishes carbohydrate or fat induced satiation [247, 375]. This, along with behavioral evidence also demonstrates the duodenum being a major site for nutrient-induced satiation. For example, infusions in the proximal intestine suppress food intake more than distal intestinal infusions [248]. However, some recent data still demonstrate a significant role of ileal nutrient infusions reducing food intake and elevating plasma intestinal satiety peptide levels [376].

The specific layer of the intestine that underlies vagal mediated signals inducing satiation is unknown, however, evidence suggests it is the intestinal mucosa [377]. The intestinal mucosa is highly innervated by the mucosal vagal terminals predominantly located in the duodenum, and these nerve fibers are relatively unresponsive to distention and highly responsive to mechanical changes of the mucosa, which occurs in the presence of intestinal nutrients [378-381]. Mucosal afferents penetrate through the ENS and muscular layers of the intestinal wall supplying innervations from the lamina propia to the intestinal villi. Despite their relative proximity to enterocytes that readily absorb nutrients, and enteroendocrine cells that release intestinal satiety peptide release, no experimental evidence has demonstrated that the mucosal endings maintain synapses with these intestinal epithelial cell types. However, in relative lack of this anatomical evidence, capsaicin infusions into the intestine, resulting in denervation of the submucosal plexus containing the mucosal nerve endings, but not myenteric plexus, which contains IGLEs, results in abolishment of intestinal fat-induced satiation [377]. Thus, the mucosal afferents are a main contributing pathway to intestinal nutrient satiation. Furthermore, because vagal afferents do not come into direct contact with intestine luminal contents, but are in close proximity to epithelial cells, the main hypothesis is that absorbed nutrients or more likely chemical signals, released in response to nutrients, mediate intestinal nutrient satiation between the intestinal lumen and vagal afferents.

1.2.3.7 Intestinal satiety peptides

1.2.3.7.1 *Cholecystokinin (CCK)*

Cholecystokinin (CCK) was the first discovered gastrointestinal peptide implicated in the control of food intake and subsequently coined “satiation signal”. Detected for the first time in 1928 by Ivy and Oldberg and later characterized by Jorpes and Mutt [382], it was not until 36 years ago when Gibbs et al. published the landmark paper showing that the biologically active, synthetic, CCK octapeptide (CCK-8) reduced food intake in the rat [383]. Since then, the investigation into the role of CCK on food intake continued unabated making CCK one of the most intensely studied gut peptide. Consequently, its suppressive effects on food intake have been demonstrated in several species including humans [246]. CCK controls food intake by coordinating visceral functions to optimize digestion and absorption and by interacting with other short- and long-term meal-related signals. CCK may also contribute to satiation by reducing caloric consumption, thus exerting its role in the control or regulation of other systems, such as body adiposity.

CCK is released from discrete enteroendocrine I-cells concentrated primarily along the proximal duodenal and jejunal mucosa. The apical surface of the CCK cells comes in contact with food components triggering a series of intracellular events resulting in the peptide release from the basolateral cell membrane into the circulation [384]. Several molecular forms of CCK have been identified and they are derived from the 95 amino acid pro-CCK (CCK-5 to CCK-83) with CCK-8 and CCK-58 being the most biologically potent in suppression of food intake [385].

Reduction of food intake by intraintestinal nutrient infusions is thought to exercise controls of food intake, which normally are activated when components of a meal enter

the duodenum from the stomach. In the rat and other mammals, plasma CCK concentrations are elevated in response to intraintestinal products of fat digestion, unhydrolyzed protein, and inhibitors of pancreatic trypsin [386-388]. Some, but not all, intestinal nutrients stimulate secretion of the gut peptide, cholecystokinin. For example, carbohydrates and amino acids do not release CCK in the rat [386] although in humans, CCK is released in response to both l-phenylalanine [389] and glucose [390]. CCK reduces food intake by acting at CCK-1Rs, located on small unmyelinated vagal sensory neurons [391], indicating that the substrate that mediates CCK-induced satiation is similar, if not identical, to that which mediates reduction of food intake by intestinal nutrients. Participation of CCK-1Rs in the reduction of food intake by intestinal nutrients is well supported by the fact that CCK-1R antagonists attenuate or abolish reduction of food intake by intraintestinally infused triglycerides [392], long chain fatty acids [238], oligosaccharides [393], and protein [394]. In addition to reversing the reduction of food intake observed following exogenous CCK [395] injection or intestinal nutrient infusion, CCK-1R antagonists increase food intake when they are administered alone denoting an important role of endogenous CCK in controlling meal size [395, 396]. Taken together, these results suggest a direct relationship between CCK-1Rs and control of food intake by intestinal nutrients.

There is accumulating convincing evidence indicating that CCK mediates nutrient-suppression of food intake mainly through a paracrine rather than an endocrine mode of action. For example, both suppression of food intake and inhibition of gastric emptying by intraintestinal carbohydrate infusions, which do not elevate plasma CCK, are attenuated by a CCK-1R antagonist [386]. On the other hand, infusions of proteins

that markedly increase plasma CCK concentrations have a marginal effect on reduction of sham feeding [386]. Furthermore, administration of a CCK-1R antagonist impermeable to the blood-brain barrier attenuates the satiating effects of CCK and intestinal nutrients [395] and increases food intake when given alone [395]. Finally, the endocrine source of CCK must undergo hepatic portal degradation which renders most of the biologically active CCK ineffective in suppressing food intake [397]. Consistent with this, very low concentrations of CCK (1-5 picomolar range) is detectable in the blood circulation and the only endocrine form of CCK found in the rat is CCK-58 [397]. Together, these data overwhelmingly point to a peripheral site of action whereby a local source of CCK acting in proximity of vagal afferent fibers may be sufficient to mediate reduction of food intake. This is also supported by studies showing that low doses of intraperitoneal, that likely mimic the paracrine mode of action on vagal afferents [398], or near-arterial administration of CCK-8 reduces food intake more than higher doses when administered intravenously [399]. Whether sufficient amounts of systemically administered CCK are able to penetrate the lamina propria and elicit a physiological and behavioral effect is unknown. Thus, the exact identity of this source of CCK outside the enteroendocrine cells remains to be located. Studies examining receptor affinity indicate a relationship between local concentrations of CCK in the GI tract and CCK-1R affinity. It is thought that low affinity receptors are localized near areas with high CCK concentration while high affinity receptors are in areas with low CCK levels [400, 401]. For example, administration of an agonist of high affinity CCK-1Rs does not decrease food intake [402]. Additionally, work done in vagal afferent preparations shows the majority of CCK-1Rs expressed on vagal afferent neurons are thought to be low affinity

CCK-1Rs [403]. Together, these data suggest that gastrointestinal CCK acting locally on vagal afferent sensory fibers is responsible for nutrient induced satiation.

1.2.3.7.2 *Glucagon-like Peptide-1 (GLP-1)*

A number of glucagon-like peptides have been identified with similar biological activities that are representative of the proglucagon family. One such peptide is glucagon-like peptide-1 (GLP-1). GLP-1 exerts physiological and behavioral effects by binding to the GLP-1 receptor (GLP-1R), which is expressed by central and peripheral nervous systems as well as alimentary organs, such as the pancreas. In addition to the L-cells of the GI tract, neurons of the CNS produce GLP-1. To decrease food intake, GLP-1 acts through paracrine and endocrine pathways, which stimulate neuronal nuclei involved in the control of food intake.

Intestinal L cells located mainly in the distal ileum and colon secrete GLP-1 in response to a variety of nutrient, neural, and endocrine factors. Initially, due to the kinetics of meal-induced GLP-1 release, the primary mechanism of GLP-1 secretion was thought to be through an indirect, neuro-humoral reflex. Specifically, GLP-1 secretion is highest ten minutes post meal ingestion when nutrients are not yet thought to make contact with GLP-1 secreting L-cells [404]. While this mechanism induces secretion of GLP-1, nutrients are also thought to directly regulate release of the peptide [405]. Two pieces of indirect evidence support this. First, infusion of glucose directly into the proximal small intestine results in elevated circulatory GLP-1 levels comparable to distal small intestine infusion of glucose [406]. Secondly, recent advances in the ability to tag and detect L-cells throughout the GI tract have identified large populations

of GLP-1 secreting cells that are also present in the proximal intestinal tissues that most likely mediate this rapid release of the peptide [407].

The L-cells of the GI tract are open-type endocrine cells, with the apical surface strategically positioned to sense intraluminal intestinal nutrients. Binding of nutrients to apical surface membrane proteins is thought to stimulate release of GLP-1. Some of the candidate receptors thought to induce GLP-1 release are gustducin and T1R family receptor subtypes [408]. Inside the cells, a vesicular transport system shuttles GLP-1 to the basolateral portion of the cell and releases the peptide into the circulation or lymph. Thus, GLP-1-secreting cells are in an excellent position to release the hormone in response to mixed meals or single nutrients. Indeed, ingested nutrients cause secretion of GLP-1 from the GI tract. Specifically, peptone or products of protein digestion, such as amino-acids, result in secretion of GLP-1 both *in vitro* and *in vivo* [409, 410]. Fatty acids, triglycerides, and carbohydrates are also potent stimuli for GLP-1 secretion [411]. GLP-1 is secreted from cell models via mechanisms that are normally dependent on glucose, including sodium-glucose transporters, and potassium-ATP sensitive pumps [412]. The increase of circulatory GLP-1 is prolonged in response to complex carbohydrates and fiber as well, which can be attributed to the slow digestion of these nutrients allowing for greater intestinal transit time, and increasing exposure of nutrients to GLP-1 secreting L-cells. While fasting typically results in low levels of GLP-1, blockade of enzymatic GLP-1 degradation results in increased circulatory levels, denoting some constitutive release of the peptide [413]. Altogether, these data show that nutrients stimulate GLP-1 secretion, thus demonstrating the role of GLP-1 in control of food intake.

Administration of GLP-1 into either the periphery or central tissues results in a subsequent dose-dependent decrease of food intake in several species [414-416]. Similar to injections of other peripheral satiety agents, such as CCK, GLP-1 limits food intake by decreasing meal size; however, at high doses, GLP-1 also decreases meal number. Furthermore, chronic administration of the GLP-1R agonist, exendin-4, results in reduced bodyweight gain and adiposity [417, 418]. Conversely, blockade by exendin-9 in fed rats increases food intake [419]. In humans, peripheral administration of GLP-1 reduces food intake and gastric emptying. However, the role of endogenous GLP-1 in control of food intake in humans is not entirely clear since no studies thus far examined the effect of exendin-9 on energy intake. In contrast, mice lacking the GLP-1R exhibit normal eating behavior and are of normal body weight [420]. This has been attributed to compensatory up-regulation of other satiation signals derived from the gastrointestinal tract, especially hormones co-secreted with GLP-1 from the intestinal L-cells. Together, these data provide strong support that GLP-1 has an important role in energy balance.

1.2.3.7.2 Peptide YY (PYY)

The intestinal peptide PPY, a member of the pancreatic polypeptide-fold family of peptides is synthesized by the L-cells and contains tyrosine residues at its terminus. The cleavage of two amino acid residues by DPP-IV produces the major circulating form of the hormone, PYY3-36 [421, 422]. PYY mediates inhibition of gastric emptying [423], intestinal motility [424], gallbladder contractions [425], and is co-localized in L-cells with GLP-1 [426]. Peptide YY is released predominantly in the ileum and colon [424]; however, the jejunum also exhibits significant PYY activity [427]. Additionally,

circulating PYY, which may be a result of both nutrient- and neural-humoral reflex-induced secretion, rises shortly following a meal, and levels are highest following carbohydrates and protein while fats are also a potent secretagogue of PYY. Caloric content of a meal is also an important determinant of PYY release. Specifically, circulating PYY rises 15 minutes following a meal, peaks approximately between 1 to 2-h post feeding, and remains elevated for another 4 – 5-h. The findings that PYY levels remain elevated for hours following a meal suggest that it may be a satiety factor, promoting a longer intermeal interval rather than decreasing meal size.

The finding that peripheral administration of PYY leads to decreases in food intake in a variety of species has further lent notion that it can modulate feeding behavior. Effects of injected PYY occur via the Y2 receptor, for which PYY maintains a high affinity. Both the peripheral and central nervous system express the Y2 receptor. Specifically, nodose ganglia cell bodies of the vagal afferents express Y2, as does the arcuate nucleus. The main upstream effects of PYY are mediated by the hypothalamus, specifically the ARC; however the mode of transmission may occur via two pathways. Found in relatively high circulating concentrations, PYY can easily diffuse across the median eminence that lacks a functional blood barrier, and bind to ARC Y2 receptors. By binding Y2 receptors, PYY down-regulates the orexigenic signals NPY and AgRP that are localized in the ARC as well as up-regulating POMC and CART expression in neurons. A second paracrine mode, involving vagal afferents has also been suggested to mediate PYY's effects on the hypothalamus and eating behavior as vagotomy abolishes PYY-induced down-regulation of NPY and AgRP as well as its feeding suppressive effects. While an endogenous role of PYY in controlling food intake is not

completely clear, several pieces of evidence lend notion to the fact that it plays a role in food intake and energy balance.

While PYY is elevated in response to macronutrients, and caloric value of a meal, fasting results in suppression below typically observed basal levels of circulating PYY. Additionally HP feeding results in significantly increased circulating levels of PYY, and in humans, feeding a meal high in protein resulted in higher circulating PYY compared to a HF or HC meal. Long-term energy homeostasis also regulates PYY secretion as obese individuals display significantly decreased levels of PYY. For example, fasting and post-prandial PYY is negatively correlated with BMI, which may be one reason for increased energy intake in this population. Similarly to humans, DIO rats display decreases in peripheral PYY. Interestingly, surgical interventions for obesity results in weight loss and subsequent increases in circulating PYY. In addition to the obese state reducing circulating PYY levels, chronic administration of PYY results in reductions in food intake and body weight in animal models. The most evident endogenous role for PYY in the control of body weight and food intake is that PYY deficiency leads to hyperphagia and obesity with reversal occurring following PYY treatment. Despite this finding in an animal model, human trials examining the efficacy of PYY to reduce body weight have been unfruitful. However, together, with the use of other peptide hormone treatments, PYY may have a place in the curbing of obesity rates.

1.2.4. Liver

1.2.4.1 *Hepatic nutrients and feeding behavior*

The liver maintains a wealth of responsibilities that are integral to the functions of many systems such metabolizing, detoxifying, and inactivating endogenous hormones as well as exogenous chemicals, such as drugs. Juxtaposed to the intestine and connected via the hepatic portal vein, the liver also processes absorbed nutrients, ultimately sensing and controlling peripheral metabolism. The hepatic regulation of food intake was initially demonstrated over 50 years ago when infusions of glucose into the liver of dogs reduced food intake while infusions of systemic glucose did not [428]. Subsequently, this finding was limited by further research that yielded conflicted results, but eventually was supported that hepatic portal vein infusions of glucose reduced intake in rats during normal feeding [429]. Furthermore, the finding that administration of 2-deoxy-D-glucose (2-DG), a competitive inhibitor of glucose uptake and glycolysis, stimulates food intake when infused into the liver [430] supported that the hepatic sensing of glucose is partially responsible for controlling food intake. The ability of glucose hepatic portal vein infusions to reduce food intake lasts for an extended time following infusion, and is determined by the presence of glucose itself, rather than the amount of glucose [431, 432]. As well as glucose, hepatic infusions of fructose, which is exclusively processed in the liver, and cannot enter the CNS, also reduces food intake [433]. The effects observed with carbohydrates and portal vein infusions reducing food intake in rats also extend to medium to long chain fatty acids [434, 435]. Additionally, inhibition of liver fatty acid oxidation increases food intake in rodents [436]. Despite these evidences of hepatic nutrients controlling food intake, similar to intestinal nutrient-induced satiation, it

the exact events that are responsible for reductions of food intake observed following hepatic glucose infusions are unclear. Similarly to the stomach and small intestine, the liver is high innervated by the vagus nerve. The presence of nutrients in the liver and their effects on food intake are mediated at least partially via the vagus. For example, infusion of nutrients in the hepatic portal vein increases vagal firing [437, 438], and the increase in food intake following administration of 2-DG is diminished by hepatic branch vagotomy or capsaicin application [439, 440]. Additionally, administration of inhibitors of metabolism increase neural activation in the caudal brainstem, where vagal afferents synapse [439]. Finally, deafferentation of the hepatic vagal branch also increases daytime food intake in male rats, resulting in increased daily food intake and subsequent weight gain [441].

1.2.4.2 *Hepatic metabolism and feeding behavior*

Although nutrient infusions into the hepatic portal vein decrease food intake, the majority of evidence linking the liver to controlling food intake suggests that the energy status of the liver is more important than the nutrient itself in regulating feeding. In the normal physiological state, fasting, which stimulates food intake, is associated with decreases in liver energy status [442]. The specific evidence that hepatic energy status is important in controlling food intake comes from administration of metabolic inhibitors. For example, infusion of the fructose analogue, 2,5-anhydro-D-mannitol increases food intake in rats [443]. In the liver, the end step of processing this analogue is phosphorylation, which decreases free phosphate levels in hepatocytes [444]. The decrease in free phosphates in the liver leads to decreases in phosphorylation of ADP

to ATP, leading to negative energy balance signaling at the cellular level as evidenced by the ADP:ATP ratio [445]. In this case, the energy status induced by the fructose analogue is the primary cause of increasing food intake as treatment with sodium phosphate increases liver ATP levels and prevents the feeding behavior effects of 2,5-anhydro-D-mannitol [446]. Further evidence of hepatic cellular energy levels being responsible for the hepatic control of food intake comes from the finding that decreasing ATP via a phosphate-independent mechanism increases food intake [447]. Collectively, these data demonstrate that hepatic energy status is influential in feeding behavior.

1.2.4.3 *Obesity and hepatic metabolism*

The obese state is associated with decreased liver catabolism, and reduced fatty acid oxidation specifically plays an important role in the development of obesity. For example, while animals are lean and feeding a low-fat diet, fatty acid oxidation is correlated with weight gain during subsequent HF diet feeding [448]. Specifically, a lower rate of fatty acid oxidation in obesity prone animals during LF feeding is correlated with increased weight gain on a HF-diet. Furthermore, when lean and fed a LF diet, obesity prone animals oxidize less fat than obesity resistant animals [16]. The specific mechanisms for the decreased fatty acid oxidation in these animals is not clear; however, it is associated with decreased expression of the rate limiting step of fatty acid oxidation [16]. An endogenous role for the decreased expression of this rate-limiting enzyme partially in DIO comes from the finding that administration of fenofibrate, which attenuates hyperphagia and body weight gain in obesity prone animals fed a HF-diet results in up-regulation of fatty acid oxidation [449]. Further direct evidence of fatty acid

oxidation leading to perturbed energy balance is the finding that inhibition of oxidation leads to increases in food intake and body weight in lean animals [450, 451]. Thus, liver energy status, due to decreased fatty acid oxidation, is hypothesized to contribute to obesity. As such, basal hepatic ATP concentrations in humans are inversely correlated with BMI [452]. Additionally, the inability to increase hepatic ATP in response to hepatic ATP depletion is correlated with obesity [453]. Collectively, these results demonstrate that the obese state is associated with decreased fatty acid oxidation and may be one underlying factor contributing to the obese state.

1.3. Microbiota

The mammalian associated microbiota is comprised of three life domains (Bacteria, Archaea, and Eukarya), and the term for this population was first introduced in the middle of the 20^{th} century [454]. While the collective term “microbiota” refers to all the microorganisms inhabiting a eukaryotic organism, the focus from here forth will focus solely on the gut microbiota, which is found throughout the gastrointestinal tract, but predominantly the distal small intestine and colon. The gut microbiota consists of both indigenous and non-indigenous microbes [455], which are responsible in maintaining intestinal function, and at first, were greatly underestimated in number. The gut microbiota is an enormous population, containing 10^{14} organisms, which outnumbers the host cell number 100 to 1 [456]. Of interest in intestinal function and energy homeostasis are the two largest phyla of the gut microbiota: Bacteroides and Firmicutes, which account for approximately 10 - 20 and 60 - 80% of the dominant bacterial population, respectively [457]. The gut microbiota of an organism is acquired at birth

[458], and varies throughout age [459] and exposure to environmental factors, such as diet [460].

1.3.1 Microbiota and energy harvest

The majority of nutrients ingested from a typical diet are digested via enzymes that are produced by the mammalian host. However, ingested dietary fiber, which contains acetal β linkages, is unable to be digested by mammalian produced enzymes. Interestingly, the gut microbiota displays high activity of various glycosylhydrolases, which are capable of digesting β linkages found in dietary fibers [461]. The result of this hydrolysis, combined with the lack of oxygen in the distal intestine, results in the production of SCFA and gases in the distal intestine. The SCFAs that are produced in the distal intestine are predominantly acetate, propionate, and butyrate [462, 463], which are absorbed in the colon. The resulting SCFAs from fiber digestion supply energy for the colonic epithelium [464], or transported to various tissues, such as the liver, cardiac and skeletal muscle [465], or adipocytes [466]. Furthermore, increasing the amount of dietary fiber can increase SCFA production, and energy derived from dietary fiber intake has been estimated to be approximately 10% of the daily total energy intake [21, 465]. While this may seem relatively meager to the total daily energy intake, initial studies have shown that conventional animals with an intact gut microbiota can eat 30% less food daily than germ-free (GF) animals, which lack the gut microbiota, and maintain similar body weights [467].

1.3.2 Microbiota and intestinal morphology

Animals bred to be GF are especially useful in determining the contribution of the gut microbiota to a variety of behavioral, physiological, endocrine, and metabolic functions. Preliminarily, the contribution of the gut microbiota to overall energy balance was not as apparent as its contribution to intestinal morphology and physiology. Perhaps the greatest visual difference between GF and CV animals is the substantially increased colon size and contents [468]. At the microscopic level, the morphology of the intestine in GF animals is distinctively different from the CV animals as GF rats display significantly smaller mucosal surface area, which is marked by decreases in intestinal crypt cell depth [469]. These findings are most likely due to proliferative factors as epithelial cell renewal rate is decreased in GF animals. Specifically, the transit time from crypt to villus peak is twice as long in GF mice relative to CV controls [470]. This is accompanied by an increase in the time for generating duodenal crypt cells, which is 2-h longer in the GF mouse [471]. This is also found in the colonic epithelium as well, which is marked by significant decreases in cell proliferation in GF rats, which is fully reversed by the inoculation of GF animals with microbiota [472].

Gastrointestinal physiology is also slightly altered in the GF animal, which is perhaps a function to compensate for morphological and digestive changes observed. Most studies examining gastric emptying in GF animals have found a slower rate of emptying [312, 473]. As well, intestinal transit time measurements have shown that both GF rats and mice display slower intestinal transit of a radioactively labeled meal [474, 475]. In addition to this effect of the microbiota on the intestine morphology and physiology, the absence of the gut microbiota leads to significant increases in

disaccharidase levels in the proximal small intestine as well as the distal intestine [476, 477]. While the apparent reasons for the increase in the proximal small intestine are unknown, the increase in distal intestinal activity to disaccharidases may be due to lack of bacterial inactivation of these enzymes [478]. Despite these changes, the absence of the gut microbiota does not lead to any other significant alterations in digestive machinery compared to the CV animal [479]. The effects of the microbiota on intestinal absorption are less known, however, recent evidence suggests impaired glucose absorption is present in GF animals [21].

1.3.3 Microbiota and intestinal endocrine function

While studies examining nutrient digestion in GF animals are relatively scant, the gut microbiota has an influential role on GI endocrine function. For example, in the small intestine GF mice display decreased CCK immunoreactivity of gut peptides compared to control animals [480]. This effect is also present in the colon as well; however, colonic immunoreactivity of CCK is lower than in the small intestine [481]. Examinations of enteroglucagon, the precursor for GLP-1, and PYY levels in GF and CV animals, however, have demonstrated that both peptides are increased in GF rats, yet decreased in the GF mouse. The first study, examining the effects of various fiber products on circulating enteroglucagon and PYY levels during refeeding demonstrated GF rats display increased circulating levels of both PYY and enteroglucagon relative to CV controls during refeeding [482]. Consecutive studies from another laboratory demonstrated that GF rats display increased enteroglucagon immunoreactivity in the proximal compared to CV controls and density of PYY and enteroglucagon reactive cell

number was significantly increased in GF animals as well [483, 484]. Finally, a more recent study has shown that GF mice display decreases in circulating PYY, an effect attributed to the lack of SCFA normally present from microbial fermentation [312]. Collectively, the effect of these alterations in intestinal satiety peptides in the GF model on food intake is not clear as relatively few studies have examined feeding behavior in this model. Despite this, the absence of the microbiota clearly alters the level of intestinal satiety peptides.

Dietary supplementation with soluble fibers and starches, which are collectively referred to as “prebiotics,” has recently shown promise as a means of manipulating gut peptides levels and potentially food intake as well. Typical prebiotic fibers include dietary resistant starch and inulin, both of which are hydrolyzed by the wealth of bacterial glycohydrolases in the distal intestinal. The addition of prebiotics to various diets, spanning from low-fat to HF and even high-protein have demonstrated an increase in GLP-1 levels relative to the respective control diet following this supplementation [485, 486]. Surprisingly, this occurs in obese animals that display insulin resistance or impaired glucose metabolism, which would signify the ability of prebiotics to ameliorate metabolic impairments. In addition to GLP-1, dietary prebiotic supplementation also increases intestinal and circulating PYY [487]. Interestingly, as well as increasing circulating GLP-1 and PYY, the addition of dietary prebiotics up-regulates arcuate nucleus expression of POMC, a potent central peptide that reduces food intake, as well as decreases in plasma leptin [488]. Whether this effect is mediated by the alterations in circulating GLP-1, PYY, or leptin is unknown given all of these peptides influence on central POMC expression; however, it is independent of

vagal signaling [488]. Interestingly, reduced food intake and adiposity in rats fed prebiotics may be an effect of ameliorating the inflammatory state associated with obesity as well.

1.3.4 Microbiota and inflammation

The obese state and other metabolic pathological states are associated with low-grade inflammation, which is marked by increased secretion of adipokines and cytokines from various tissues [489, 490]. The term “cytokine” is typically used to refer to immunomodulating factors, which comprises a large group of molecules emcompassing interleukins and interferons. Originally, cytokines were thought to be produced predominantly or exclusively by macrophages, which play an integral role in immune function; however 15 years ago the discovery that adipocytes secrete cytokines (predominantly TNF-alpha and IL-6) that impair metabolism reevaluated the role of adipose tissue on metabolism and the obesity [490]. Furthermore, increases in adiposity are associated with increased macrophage infiltration of adipocytes [491, 492]. While macrophages and adipocytes share similar pathways to differentiation, and macrophages may be locally produced in adipose tissue, isolated macrophages from adipose tissue display bone marrow lineage. As well as obesity itself, nutritional regulation of obesity may also occur with and high fat meals inducing post-prandial inflammation [493]; however, the ability to separate obesity from diet and its role on systemic inflammation is relatively difficult as rodent models fed high sugar or HF diets are typically obese [494]. Recently, the systemic inflammation that occurs during HF feeding and obesity has been attributed to the gut microbiota [495].

Intestinal epithelial cells that come into contact with the intestinal microbiota express various receptors that display affinity for bacteria-related products. For example, intestinal cells express an abundance of toll-like receptors (TLRs), which respond to foreign molecules and trigger the inflammatory process [496]. During HF-feeding, the circulating levels of the bacteria-produced inflammatory molecule lipopolysaccharide (LPS) are increased. As such, this display of increased systemic LPS is defined as “endotoxemia” [497] (Figure 10). Through various efforts, LPS was shown to be responsible for the accompanying metabolic derangements displayed during HF-feeding as knockout of LPS-related receptors prevented impaired metabolism and obesity [498, 499]. Furthermore, the intestinal bacteria is most likely the cause of the increased circulating LPS as LPS is an integral component to the cellular membrane in Gram-negative bacteria and increases in the ratio of Gram-negative to Gram-positive bacteria are associated with HF feeding [497]. Additionally, while HF-feeding increases plasma LPS, treatment against Gram-negative bacteria with antibiotics in the water of mice, which induces little effects systemically, ameliorates increases in plasma LPS and associated pro-inflammatory markers. Furthermore, this treatment decreases insulin resistance as well as visceral adiposity despite increases in food intake [500]. More recently, the role of HF-diet induced increases in bacterial LPS has also been extended to influencing vagal signaling. Specifically, HF-diet fed obese, but not non-obese, animals display increases in intestinal and circulating LPS [460]. Furthermore, vagal afferent neurons of these animals are resistant to leptin, which may be a cause of their hyperphagia [501]. This effect is may be due primarily to LPS as vagal afferents display TLR4 [502], which is a co-receptor for LPS, and application of LPS to vagal afferent

neurons results in leptin resistance [501]. Thus, while the gut microbiota clearly contributes to systemic inflammation, this effect may alter pathways that control food intake while promoting metabolic impairment leading to pathological states, such as obesity and/or diabetes.

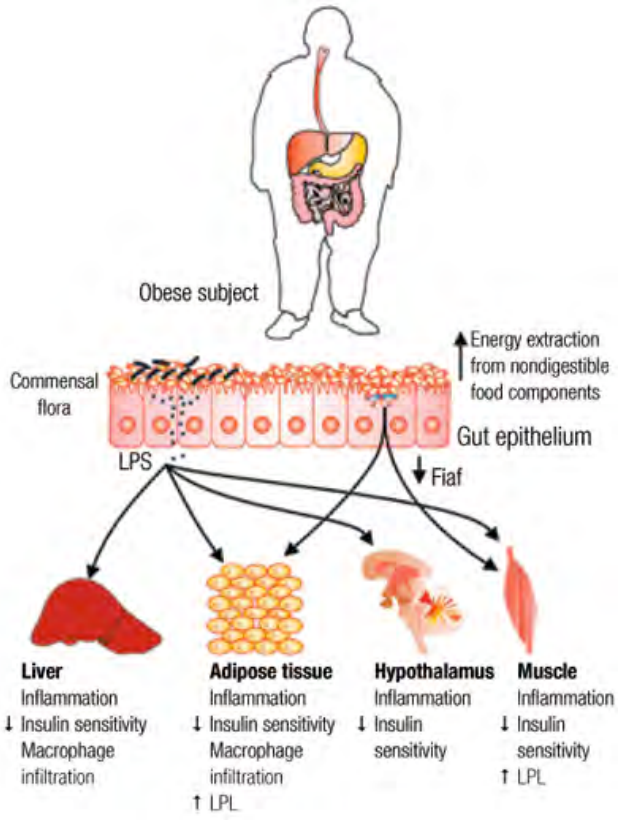


Figure 10: Mechanisms by which microbiota and increased intestinal permeability lead to metabolic impairment. In obese animals that are normally fed HF-diets, bacterial LPS production is increased, and when entering the circulation, triggers an inflammatory response in peripheral organs, such as the liver, adipose tissue, and the hypothalamus. Ensuing peripheral low-grade inflammation leads to impaired insulin sensitivity and metabolic dysregulation [503].

1.3.5 Gut microbiota and obesity

The role of the gut microbiota in obesity is becoming increasingly clear with accumulating evidence. The first study to establish the effect of the microbiota on daily

energy regulation and adiposity established that the lack of microbiota in a mouse model drastically reduces adiposity during standard rodent diet feeding [21]. When given microbiota from a donor mouse, a process referred to as “conventionalization,” GF mice increase adiposity by 60% within two weeks. Similarly, GF mice are protected from DIO maintaining significantly less adiposity relative to conventional (CV) mice. This effect is independent of increases in daily energy intake as conventionalized animals display lower daily food intake relative to GF animals in both low-fat and HF diet feeding. Decreased adiposity in the GF animal also is associated with inhibition of hepatic lipogenesis as conventionalization induces increases in nuclear response elements carbohydrate regulatory element binding protein (ChREBP) and sterol regulatory element binding protein-1 (SREBP-1), both of which regulate lipogenesis. The mechanism of decreased hepatic lipogenesis in the GF animal is most likely due to decreased absorption of glucose, as conventionalization also increases glucose uptake in the small intestine. Thus, by decreasing the amount of glucose shunted to the liver via the hepatic portal vein, hepatocytes increase inhibition of lipogenesis in GF animals. Furthermore, the liver of GF mice displays increases in phosphorylated AMPK and ACC-1, which are potent inhibitors of lipogenesis.

While these factors clearly demonstrate a shift towards peripheral catabolism, the proposed mechanism by which GF animals display decreased adiposity relative to CV animals is through increased expression of FIAF, a regionally expressed inhibitor of LPL [504, 505], which is involved in the rate limiting step of increasing circulating fatty acid uptake in adipocytes. For example, GF FIAF KO animals display similar levels of adiposity as CV animals during both low-fat and HF feeding [21]. These data

demonstrate that the gut microbiota regulates adiposity and the absence of the gut microbiota leads to severely decreased adiposity. While the majority of studies have demonstrated that GF mice indeed display severely diminished adipose deposition, one study in a GF mouse model different from that of previous GF studies demonstrated no effect of the absence of the gut microbiota on adiposity levels [506]. The authors of this study used the C3H mouse model in both the GF and CV state and conducted a series of experiments using low- and HF diets as well as a HF, high sucrose western diet, similar to that of previous studies in GF mice. Feeding the LF and HF diets, GF mice displayed similar and increased levels of adiposity, respectively, relative to CV animals feeding the same diet. However, similar to previous studies performed in GF mice, adiposity levels were significantly decreased in GF animals relative to CV controls when fed a western diet. While the authors of this study found varying levels of adiposity in GF animals relative to CV controls across three different diets, the GF state was always associated with up-regulation of intestinal FIAF. Furthermore, the authors of this study could not detect circulating FIAF, raising the question of the role of intestinal FIAF in metabolic alterations in GF animals. The authors postulated that the high sucrose concentration of the western diet, but not LF and HF diets contributed to the obesity in CV animals, which was absent in the GF group. Despite this, work from Rabot et al. [507] demonstrate that feeding a HF-diet with low sucrose content results in decreased adiposity in GF mice relative to CV controls in the C57Bl/6J mouse model. Thus, the differing strain of mice in this study cannot be excluded as a possible reason for these conflicting results.

Additional studies have shown the gut microbiota in the obese state is responsible for further increases in adiposity relative lean animals [22, 508]. Data from Ley et al. [457] demonstrate that the obese state is associated with alterations in gut microbiota populations. Specifically, the obese ob/ob mouse displays a 50% reduction in Bacteroidetes population, which is compensated by a 50% increase in Firmicutes population. As Firmicutes are fermenting bacteria [509] that release an abundance of SCFA in response to the breakdown of polysaccharides found in the diet, these bacteria may contribute to increased energy extraction from the diet leading to further perturbed energy balance [21]. The direct evidence for the gut microbiota of ob/ob mice playing at least a marginal role in obesity came from a follow-up experiment in which the same group implanted microbiota from lean mice or ob/ob mice into GF animals [508]. In doing this, it was discovered that GF animals conventionalized with ob/ob microbiota resulted in increased adiposity levels relative to conventionalized animals receiving lean donor microbiota. Similar to the ob/ob mouse, DIO mice display phylogenic shifts of the gut microbiota with Bacteroides decreasing and proportional increases in Firmicutes [510]. Additionally, conventionalization of GF animals with the DIO microbiota leads to further increases in adiposity than conventionalization with lean donor microbiota. Surprisingly, placing DIO donors on a carbohydrate or fat restricted diet results in attenuation of this effect demonstrating that adiposity, rather than macronutrient content is responsible for changes in gut microbiota populations in DIO animals. In addition to mouse models, obese humans display reductions in Bacteroidetes populations with proportional increases in Firmicutes, independent of diet composition [511, 512]. Thus, collectively, these studies show that the presence of gut microbiota leads to adipose

deposition through increases in energy harvest via bacterial population changes as well as altering peripheral hepatic and skeletal muscle metabolism. Together, these findings demonstrate a clear role for the microbiota in adiposity and the possible distinct population of an “obese microbiota”; however further experiments have challenged the hypothesis of a distinct microbiota population during obesity [460, 513].

When feeding an obesigenic diet, the growth curves of animals vary greatly and in some instances, subsets of populations can be selected as resistant to DIO (DR) when animals display similar adiposity to that of chow-fed controls [514]. To assess whether diet is a factor in altering gut microbiota populations independent of obesity, Hlidebrandt et al. [513] used a genetically engineered KO model that is resistant to DIO during HF-feeding. Surprisingly, gut microbiota populations were similar in the wild-type DIO animals and the lean KO model. Similarly, in an outbred DIO and DR rats fed a HF-diet, gut microbiota populations are similar; however, HF-feeding reduces the total bacteria number, with increases of Bacteroidiales and Clostridiales, the dominant orders of the Bacteroidetes and Frimicutes phyla, respectively [460]. Interestingly, despite sharing similar bacterial populations, the response to these populations in DIO and DR animals differ. Specifically, DIO animals display increased Toll-like receptor-4 (TLR4) activity, which is a co-receptor to lipopolysaccharide (LPS), and decreased alkaline phosphatase activity, which serves to neutralize LPS. Ultimately, increases in TLR4 activation was associated with increased intestinal permeability in DIO animals, with subsequent increases in circulating inflammatory markers. Given the interaction of the inflammatory pathways and metabolic impairments, the response of DIO animals to the altered gut microbiota population due to HF-feeding clearly plays a role in their obesity.

Together, these findings demonstrate that diet may be a more important factor in determining gut microbiota populations in rat models, and have yielded a recent hesitation to accept the term "obese microbiota." Despite this, whether due to population changes during HF-feeding or the obese state, or the hosts' response to bacterial-produced agents, the microbiota plays an important role in regulating energy balance.

1.4. Overall significance and experimental outline

The gastrointestinal (GI) tract is a specialized sensory system that detects and responds to incoming luminal factors including nutrient-derived signals and micro-organisms. Significant progress has been made in understanding the events underlying chemosensory functions of the gut, but the exact mechanisms involved in nutrient detection are not well understood. In addition to nutrients as previously discussed, the intestinal epithelium comes in direct contact with trillions of micro-organisms that affect gut absorptive, morphological, physiological, and endocrine functions and liver metabolism. Recently, there has been emerging evidence implicating the gut microbiota in energy metabolism and obesity [21, 22, 460, 495, 508]. Sweet substances and fats are known to stimulate intake both at the level of the oral cavity [30], as well as the reinforcement through post-oral influences [348]. Furthermore, the effects of nutritive components of a meal, such as sugars and fats are potent inhibitors of food intake [238, 242, 243]. Despite these evidences that the gut microbiota and nutrients influence peripheral factors controlling food intake, data on how the gut microbiota regulates energy intake and furthermore, intake of specific macronutrients,

such as sweet substances and fat, is scant. Thus, the significance of the proposal of this thesis is three-fold. First, it establishes the importance of the gut microbiota in regulating oral factors regulating food intake, specifically sweet and fat taste. Secondly, it determines the effect of the gut microbiota on post-oral factors regulating nutritive sweet and fat intake. Thirdly, it demonstrates the role of the absence of gut microbiota in hepatic and adipocyte metabolism, which may control food intake and are ultimately associated with body adiposity. Together, the significance of this proposal are to demonstrate the effects of the absence of the gut microbiota on taste and intestinal nutrient detection, intestinal satiety peptides regulating food intake, peripheral metabolism, and how these alterations in GF animals are associated with adiposity.

To establish the role of the gut microbiota in oral and post-oral factors controlling food intake, a series of studies have been conducted to address the following three specific aims:

1.4.1 Specific Aim 1: How the absence of gut microbiota affects oral and post-oral detection of sweet nutrients as well as intestinal sugar transporters

In mice, the absence of gut microbiota results in decreased absorption of a radiolabeled glucose gavage when measured from the distal intestine [22]. However, in GF rats, the expression of the predominant transporter regulating glucose absorption, SGLT1, is increased in the distal intestine [251]. As well, GF mice exhibited marginal increases in consumption of a palatable western diet, which is comprised of a high sucrose content [22]. The effects of the microbiota on oral nutrient detection and proximal intestinal nutrient detection and physiology are not clear, despite data demonstrating increased

dissaccharidases in the proximal intestine [477] and decreases in proximal intestine surface area in GF animals [470]. Whether these alterations in intestinal enzymes and nutrient transporters lead to alterations in sweet solution intake remains unexamined. *The results of Chapter 2 demonstrate the association of intestinal carbohydrate detection and transport, with increased nutritive sweet solution intake in GF mice.*

1.4.2 Specific Aim 2: How gut microbiota affects oral and post-oral detection of lipids and associated changes in nutrient-responsive GPRs and intestinal satiety peptides.

The GF state is characterized as that of mimicking fasting, with depleted adipose depots [21, 22]. As well, fatty acid receptors in the distal intestine of GF rats are significantly up-regulated, denoting a possible role of the microbiota in intestinal fat sensing [312]. Fat is a calorically dense nutrient that when exposed only to the oral cavity is highly preferred during both the fed and fasting condition [77]. Increased fat metabolism, which is observed during the fasting state and HF feeding, is also associated with increased intake of fats [73]. The mechanisms responsible for intestinal fat detection and fat-induced satiation, however, are less understood, but are mediated by CD36 [303], intestinal GPRs [91-93], and satiety peptide release [238, 419]. *Results of experiments examining the hypothesis that GF mice display increases in oral factors promoting fat intake and decreases in intestinal fatty acid receptors and satiety peptides are presented in Chapter 3*.

1.4.3 Specific Aim 3: How gut microbiota affects host liver, intestinal, and adipose metabolic parameters in a GF rat model.

Hepatic and adipose metabolic function has a significant impact on adiposity and the regulation of energy balance. Previous studies using the C57Bl/6J GF mouse model have found that the GF state is characterized by decreased body adiposity, increased lipolysis [21], and protection from HF diet-induced obesity [22] . Despite these findings in the C57Bl/6J model, another strain of GF mice was found to exhibit similar adipose deposition as CV controls during LF or HF-feeding [506], denoting a possible effect of strain and/or animal model on the absence of gut microbiota influencing body adiposity. *To examine whether absence of a gut microbiota in the rat is associated with behavioral, phenotypic, and metabolic changes similar to those reported in the mouse model, Chapter 3 contains results of adiposity, hepatic and adipose metabolism, and adipocyte morphology in the Fisher 344 (F344) GF rat model.*

2 Specific Aim 1: How the absence of gut microbiota affects oral and post-oral detection of sweet nutrients as well as intestinal sugar transporters

2.1 Introduction

A potential role of gut microbiota in influencing food intake, energy homeostasis and weight gain in mice has been suggested. As such, GF mice consume more standard chow while maintaining significantly lower levels of body fat [21]. Additionally, GF mice consume more of a palatable western diet, in which a large portion of energy comes from carbohydrates, despite being resistant to DIO [22]. In the colon of GF mice, expression of fatty acid responsive receptors is increased [312], and expression of colonic SGLT1 is increased in GF rats [251] while GF mice display decreases in distal measurements of glucose absorption [21]. Whether the gut microbiota modulates expression of sweet nutrient-responsive receptors and transporters in the proximal intestine, where nutrients are largely sensed and absorbed, is unknown. Thus, in this first study, we examined preference and intake of nutritive (sucrose) and non-nutritive (saccharin) sweet solutions in germ-free (GF, C57BL/6J) mice compared with normal (NORM, C57BL/6J) control mice using a two-bottle preference test and associated changes in proximal intestine expression of the sweet receptor, T1R3, and the luminal glucose transporter, SGLT-1.

2.3 Summary of results and conclusions

In the current study, we found that GF mice display increased intake of a highly concentrated sucrose solution, which leads to increased daily energy consumption from sucrose. This is most likely dependent upon intestinal rather than oral factors as we found no differences in the preference or consumption of the non-nutritive sweetener saccharin, similar expression of lingual sweet receptors in GF and NORM mice, but increased intestinal T1R3 and SGLT-1. The increased consumption of highly concentrated sucrose solutions is most likely due to the energy of this sweet solution as GF mice display significantly decreased energy stores, which leads to increased energy intake. Furthermore, this effect may be mediated by glucose sensing via T1R3 and/or SGLT-1. As such although T1R3 deficient mice display normal preferences of sucrose after intestinal exposure [42], and intestinal sucrose infusions can condition flavor preferences in these animals [340], they still exhibit decreased consumption of sucrose solutions [42]. The exact mechanism of increased T1R3 is not apparent from this study alone; however, because GF animals display a marked reduction in intestinal surface area [470], which normally would lead to decreases in nutrient absorption. Increases in T1R3 and SGLT-1 may be a means of compensation to these observed morphological differences. We did not measure glucose absorption in these animals, but despite this, we observed no signs of glucose malabsorption, which could occur with the consumption of such large volumes of sucrose. The increased T1R3 may be due to the fact that GF animals display increases in brush border disaccharidases [477], which would lead to higher levels of luminal glucose that stimulate T1R3 expression in GF mice [274] . In turn, as previously demonstrated, increased stimulation of T1R3 would

lead to increased SGLT-1, allowing for greater absorption of luminal glucose [265]. Collectively our data demonstrate that GF animals consume more of a highly concentrated nutritive sweet solution than NORM animals and this is associated with increased intestinal T1R3 and SGLT1 expression in the proximal intestine.

3 *Specific Aim 2: How gut microbiota affects oral and post-oral detection of lipids and associated changes in nutrient-responsive g-protein coupled receptors (GPRs) and intestinal satiety peptides.*

3.1 Introduction

The results from Chapter 2 demonstrate that mice lacking gut microbiota over consume increasing amount of calories from nutritive sweet solutions, a phenomenon associated with increased expression of intestinal “sweet” sensing receptors [515]. The western diet in which GF animals consume more of than CV mice is highly comprised of dietary fat as well. While expression of colonic fatty acid receptors that contribute to intestinal satiety peptide release in GF mice are increased [312], the relative expression in the proximal intestine, which predominantly contributes to intestinal nutrient satiation, is relatively unknown. Therefore, to examine whether our results in Chapter 3 extends to other nutrients, and may be another reason for GF mice’s increased consumption of a western diet, we tested intake and preference of intralipid emulsions (IL, 0.156, 0.313, 0.626, 1.25) in germ free (GF) and control (NORM) mice. Furthermore, we measured the associated changes in expression of lingual CD36 from the posterior lingual epithelium, intestinal fatty acid receptors and satiety peptide expression, and circulating levels of satiety hormones in GF and NORM mice.

3.2 Increased oral detection, but decreased intestinal signaling for fats in mice lacking gut microbiota

Introduction

By the year 2030, half of the American adult population is predicted to be obese, which is attributed primarily to increased caloric intake [516]. As such, the large contribution of calories from dietary fats may play a major role in the development of obesity. Despite the strong link between dietary fat intake and obesity, the factors leading to the over consumption of, and preference for, fats are less clear, but may be due to oral, intestinal, and metabolic influences. For example, rats rapidly consume oils during sham feeding, a process that limits post-oral feedback [77], while post-oral infusion of fat conditions flavor preferences in rats and mice [33, 517]. Furthermore, animals efficient in fat digestion or metabolism consume more fat than inefficient fat digesting and metabolizing counterparts [73]. Intestinal and metabolic factors are profoundly influenced and modulated by the presence of trillions of microbes residing in the intestinal tract, collectively referred to as the gut microbiota, which contribute to altered energy intake and increased adiposity. Recent studies have linked the gut microbiota to obesity and associated alterations in metabolism. For example, germ-free (GF) animals, lacking gut microbiota, are significantly leaner on a standard rodent chow diet than normal (NORM) animals with an intact microbiota despite consuming more energy [21]. Furthermore, most studies show that GF-mice are resistant to diet-induced obesity from a HF or western diet [22, 507], although in one recent study; albeit in a different strain, GF mice gained more weight and body fat than NORM mice on a similar calorie HF diet but differing ingredient composition [506]. The resistance to fat

deposition in GF mice appears to be due to several mechanisms, including decreased hepatic *de novo* lipogenesis. As well, increased systemic lipolysis through increased expression of fasting induced adipocyte factor (FIAF), an intestinal lipoprotein lipase (LPL) inhibitor, which results predominantly from decreased extraction of energy from the diet[22] may play a role in the protection from obesity in GF mice, although the role of FIAF in the relationship between gut colonization and adiposity has been recently disputed (see [506]). In addition to influencing host metabolism, the absence of gut microbiota leads to alterations in intestinal morphology and physiology. We have recently demonstrated that GF mice exhibit increased "sweet" nutrient receptors and, sodium glucose-like transporter 1 (SGLT1) expression in the proximal intestine which was associated with increased sucrose intake [518]. The contribution of nutrient receptors to increased caloric intake in GF animals is not known, however, activation of nutrient responsive receptors leads to release of intestinal satiety peptides, such as CCK, GLP-1 and PYY [92, 93, 312]. Further evidence linking the gut microbiota to intestinal satiety peptides is the demonstration that GF mice conventionalized with donor microbiota display an increase in plasma PYY [312], while prebiotic treatment increases circulating GLP-1 and PYY with concomitant decreases in plasma ghrelin [486]. Together, these results suggest that alterations in nutrient sensing and peptide hormones influencing fat ingestion due to lack of microbiota may result in altered fat intake in GF animals.

In addition to the influence of intestinal nutrient sensing on long-term consumption of dietary fats, oral factors also play an important role in the detection of, and preference for, fats. As such, mice lacking CD36, a putative fatty-acid translocase

located on the posterior lingual epithelium, are unable to develop preferences for low concentrations of oil [101]. Interestingly, expression of CD36 is determined by a variety of factors, including diet and energy status. For example, obese and non-obese animals consuming a HF-diet display decreased expression of CD36 compared to LF-fed or non-obese controls [519]. Conversely, during fasting, mice exhibit increased expression of CD36, an energy state associated with increased detection of fats [519, 520]. Because GF mice display marked reduction in adiposity, reflecting a state of energy deprivation, they may also display increased CD36, leading to increased detection or consumption of fats. Therefore, to examine the impact of the absence of the microbiota on fat intake and preference we first employed two-bottle 48-h access to increasing concentrations of intralipid emulsions in GF and NORM C57Bl/6J mice. Secondly, to assess changes in fat detection components and possible mechanisms involved in increased caloric intake, we measured expression of fatty acid sensors and receptors in the lingual and proximal intestine epithelium as well as peptide content and circulating satiety peptide levels in GF and NORM mice. Finally, we measured plasma lipid metabolites and quantified the enteroendocrine cells in the proximal (duodenum, jejunum) and distal (ileum, colon) intestine of both groups.

Methods

Animals

Throughout all experiments, male C57BL/6J GF mice (n=10) from our germ-free colonies, originally derived from Charles River colonies (ANAXEM, Jouy-en-Josas, France), and normal (NORM) mice (n=10) (Charles River, France) were housed

individually in polycarbonate cages with cedar bedding. Each group (GF or NORM) was housed separately in two Trexler-type isolators (Igenia, France). Throughout the studies, sterility of the germ-free isolator was verified through weekly analysis of mouse fecal samples. Both groups of mice received similar autoclaved, deionized water and irradiated standard rodent chow (Safe Diets, Belgium) *ad libitum,* unless noted otherwise. They were allowed a minimum of one-week acclimation before experimental manipulations began. Procedures were carried out in accordance with the European Guidelines for the Care and Use of Laboratory Animals.

48-h two bottle preference tests

Ten-wk old GF and 9-wk old NORM mice weighing 27.1 ± 0.3g and 24.5 ± 0.5g respectively, were given access to intralipid emulsions that were prepared on the basis of percentage of soybean oil (0.156, 0.313, 0.626, 1.25 % oil (v/v)) in ascending concentrations, and water during 48-h two-bottle testing. Due to technical logistics, only eight out of the 10 rats for each group were used for testing. At the beginning of each test, mice were weighed, the water removed and replaced with 2 similar 250-ml plastic bottles with the spouts penetrating from the top floor of the cage at 2-4 cm distance from the floor and 5-6 cm apart. The positions of the two bottles were alternated every 24-h to control for side preference. Bottles were weighed at the beginning and end of each 24-h test. Between each test, mice received one-bottle access to water. In previous experiments using the same bottles, we found that spillage from water bottles was negligible, therefore we did not account for spillage. Emulsions were presented once every 3–5 days, giving mice access to emulsions at least once a week. At no time did

the mice receive more than two intralipid emulsion concentrations per week. To account for the fact that mice may have altered caloric intake from chow during intralipid presentations, we also measured 48hr chow intake during the final two intralipid tests. A pre-weighed amount of chow was presented before testing, and total intake, accounting for spillage, was measured at the completion of the 48-h tests.

Lingual epithelium and plasma collection

Approximately 3 weeks after completion of two-bottle preference tests for oil emulsions, GF and NORM mice (n = 10 each) were sacrificed for collection of lingual epithelium and plasma after either a fast or re-feeding with intralipid. After an overnight-food deprivation (1700 – 0900-h), half of GF and NORM mice (n = 5 each) received a burette filled with 1-ml of 20% intralipid, while the other received a burette filled with water. Mice were sacrificed via decapitation 30-min after drinking the total volume of intralipid. Trunk blood was collected in EDTA-coated tubes (Becton Dickinson) containing 35 µl aprotonin (Sigma), 20 µl pefabloc (Sigma), and 20 µl DPP-4 inhibitor (Millipore), centrifuged at 3,500 x *g* at 4^{o}C, plasma aliquoted, and stored at -80° for further analysis. The posterior lingual epithelium was collected from fasted mice by excising the tongue, and subdermally injecting 0.5 ml of 1 mg/ml dispase and elastase dissolved in mammalian physiological saline containing 1,2-Bis(2-Aminophenoxy)ethane-N,N,N′,N′-tetraacetic acid (Sigma, France). After 20-min incubation at room temperature, the posterior lingual epithelium containing the circumvallate papillae was dissected under a Stereoscope (Zeiss) and placed into a 1.5-ml microfuge tube containing AllProtect Tissue Reagent (Qiagen, France) and stored at 2^{o}C.

Intestinal Epithelial Cell Collection

For quantification of intestinal epithelial proteins, a separate group of GF and NORM mice (n = 5 per group) were used. Under deep isofluorane anesthesia, the proximal portion of the small intestine, containing the duodenum and jejunum was removed and placed into sterile physiological saline. Intestinal epithelial cells were collected using the everted sac method. Briefly, after excision, proximal intestines were flushed using 10 ml of ambient physiological saline followed by 10 ml oxygenated (95:5 O_2:CO_2) Ca^{+2} and Mg^{+2}-free Krebs-Heinslet buffer. After rinsing, intestines were everted, divided into three segments, and placed into flasks with oxygenated Ca+2, Mg+2-free Krebs-Heinslet buffer with EDTA and DTT. Flasks were placed in a 37^{o}C water bath and shaken for 20 min to dissociate epithelial cells from the connective tissue. The subsequent suspension was collected, centrifuged and washed with sterile Dulbecco's Phosphate Buffered-Saline without Ca^{+2} or Mg^{+2}. This process was repeated three times. Aliquots of isolated intestinal cells were snap frozen and stored at -80^{o}C.

Quantitative Real-Time PCR (qRT-PCR)

Posterior lingual epithelium was lysed and homogenized using a TissueLyser (Qiagen, France) and RNA extracted using a RNEasy Fibrous Tissue Mini-kit (Qiagen, France) according to the manufacturer's instructions. For cDNA synthesis, 2 µg of RNA was reverse transcribed in a reaction volume of 60 µl, using a high-capacity cDNA kit (Applied Biosystems, Courtaboeuf, France). Subsequent cDNA was diluted 5-fold and qPCR performed in a reaction volume of 20µl using an ABI Prism 7700 (Applied

Biosystems, Courtaboeuf, France) thermal cycler. Samples were run in triplicate and transcription levels of CD36 was quantified using Taqman® Gene Expression Assays and Gene Expression Master Mix (Applied Biosystems, Courtaboeuf, France). Relative mRNA expression was quantified using the $2^{-\Delta\Delta CT}$ method with β-actin as internal control.

Western blotting

Isolated intestinal epithelial cell aliquots were thawed on ice and suspended in 1-ml of radioimmunoprecipitation assay (RIPA) buffer containing protease inhibitors (Sigma, France). Cells were lysed and homogenized and the resulting homogenate was centrifuged for 20-min at 14,000 x g at 4^{o}C. The protein concentration in the supernatant was determined with NanoDrop system (GE Healthcare). Soluble protein (100 µg) was then run on SDS-PAGE gels containing 10 - 12% acrylamide, transferred to nitrocellulose membranes, and probed with anti-CD36, GPR40, GPR120, GPR41, FIAF, PYY, GLP-1, and CCK (Santa Cruz Biotechnology) antibodies. Immune complexes were detected by chemiluminescence (GE Healthcare). Quantification was performed by scanning densitometry using ImageJ (NIH, Bethesda, MD, USA) against β-actin (Santa Cruz Biotechnology) as internal control.

Plasma Analysis

Plasma was analyzed for glucose, triglycerides, total cholesterol and total high-density lipoprotein (HDL) using an AU 400 automated biochemical analyzer (Olympus). Additionally, circulating levels of leptin, PYY, and acyl-ghrelin were determined using

Enzyme-linked Immunosorbent Assays (Millipore, France) according to manufacturer's instructions.

Immunohistochemistry (IHC) of enteroendocrine cells

A separate group of overnight food deprived 10-wk old GF and NORM mice (n = 4 per group), were sacrificed, and 3 cm sections of the duodenum, jejunum, ileum, and colon were quickly removed, opened, pinned mucosal side up in agarose coated petri-dishes, and fixed with 4% formaldehyde overnight. Intestinal segments were stored in 75% ethanol, embedded in paraffin, and 4-µm-thick microtome cut sections mounted on glass slides were processed using standard procedures. After deparaffinizing and rehydrating, slides were placed in 6% hydrogen peroxide for 30 minutes, then blocked with PBS/3% BSA / 2% goat serum for one hour. Sections were incubated overnight at 4°C with rabbit polyclonal antibody raised against chromogranin A (1:200, Abcam, ab15160), washed, followed by 1-h incubation at room temperature with biotinylated donkey anti rabbit antibody (1:400, Santa Cruz), incubated with a hematoxylin solution for nuclear staining, and processed using DAB (Dako) for 10-20 seconds. Sections were then dehydrated and mounted with DPX (Sigma), and examined under 100x microscope (Nikon) for enteroendocrine cell counts. Counting was performed manually by two individuals blinded to the treatment by observing five, non-overlapping microscopic areas from similar locations of each intestinal segment between GF and NORM mice.

Statistical Analyses

Differences in bodyweight gain between groups from the start to the end of the experiment were analyzed with student's t-test. Preference for intralipid were determined by the following formula: (48-h intake of intralipid)/(48-h intake of total fluid)*100 and subjected to one-way (group) repeated measures (rm) ANOVA. Additionally, 48-h acceptance (raw intake solution) as well as total calories consumed from intralipid were subjected to two-way (group x concentration) rmANOVA. To determine taste sensitivity to intralipid (concentration at which the animal first prefers tastant over water), we performed paired student's t-test for each concentration within each group. The resulting values from Western blotting and qPCR, were analyzed using student's t-test. Levels of plasma biochemical markers and satiety peptides were analyzed by two-way (group x treatment) ANOVA with Bonferroni post-hoc tests, where appropriate. Enteroendocrine cell counts were calculated for each intestinal segment of GF or NORM group as the total of all five microscopic fields, and analyzed by student's t-test. For all statistical tests, differences were considered significant at $\alpha < 0.05$.

Results

Body weight

There were no significant differences in weight gain between GF (0.8 ± 0.5 g) and NORM (0.9 ± 0.3) mice during the duration of the experiment.

48-h two bottle oil preference and acceptance

There were significant main effects of concentration [$F(3, 42) = 6.4$, $P=0.01$], group [$F(1, 14) = 12.56$, $P<0.01$], and group x concentration interaction [$F(3, 42) = 3.8$, $P<0.05$] on intralipid preference in GF and NORM mice. At the lowest concentration tested (0.156% oil), GF mice preferred intralipid to water more than NORM mice (GF: 87.72 ± 3.2% vs. NORM: 68.09 ± 3.3%; $P < 0.001$) (Fig 1A). When acceptance of 48-h intralipid intake was evaluated, there were significant main effects of concentration [$F(3, 42) = 32.98$, $P<0.0001$] and group [$F(1, 14) = 5.66$, $P < 0.05$], but not group x concentration [$F(3, 42) = 2.02$, $P = 0.13$]. Thus, we found no difference in total intake between GF and NORM mice at any of the concentrations tested (Fig 1B). However, when intake was converted into kilocalories, there was a significant fixed effect of concentration [$F(3, 42) = 78.94$, $P<0.0001$], and group x concentration interaction [$F(3, 42) = 2.90$, $P<0.05$], but not group [$F(1, 14) = 4.43$, $P=0.05$]. Post-hoc analysis revealed a significant difference in caloric intake between GF (1.73 ± 0.2 kcal) and NORM (1.19 ± 0.2 kcal) mice at the highest concentration tested (1.25% oil) ($P < 0.05$) (Fig 1C). Additionally, we found no difference in 48-h solid chow energy intake between GF and NORM mice during exposure to 0.626% (GF: 10.14 ± 0.3kcal; NORM: 10.2 ± 0.3g) or 1.25% (GF: 10.04 ± 0.5g; NORM: 8.77 ± 0.8kcal) intralipid.

Lingual and intestinal CD36 expression

In GF mice, expression of CD36 transcript in the posterior lingual epithelium of fasted mice was up-regulated 3-fold relative to NORM mice ($P<0.05$) (Fig 2A). However, intestinal protein expression of CD36 was down-regulated in GF compared to NORM

mice ($P < 0.05$) (Fig 2B). Additionally, intestinal FIAF expression in GF mice was up-regulated relative to NORM mice ($P < 0.001$) (Fig 2C).

Intestinal nutrient receptor, gut peptide, and lipid-related protein levels

Protein expression of fatty-acid receptors GPR40 ($P< 0.0001$), GPR41 ($P<0.0001$), GPR43 ($P<0.05$), and GPR120 ($P<0.0001$) in the proximal intestine was significantly decreased in GF mice relative to NORM controls (Fig 3). Similarly, protein expression of CCK ($P<0.0001$), GLP-1 ($P<0.001$), and PYY ($P<0.001$) were also significantly decreased in GF compared to NORM mice (Fig 4).

Enteroendocrine cell counts

Total enteroendocrine cells, represented by chromogranin-A stained cells, were increased in the colon ($P < 0.05$), but decreased in the ileum ($P < 0.05$) of GF compared to NORM mice (Fig 5A-B). At the level of the duodenum and jejunum, there were no significant differences between groups.

Plasma Analysis

Plasma gastrointestinal hormone levels were consistently decreased in GF mice compared to NORM controls. Specifically, GF mice had significantly lower levels of leptin in both fasted ($P<0.001$) and re-fed state ($P<0.0001$) compared to NORM mice, and re-feeding increased plasma leptin in both GF ($P<0.001$) and NORM ($P<0.0001$) mice (Fig 6A). In both conditions, GF mice displayed decreased circulating PYY compared to NORM mice ($P<0.0001$ for both conditions) while re-feeding resulted in

increased plasma PYY in both GF and NORM mice ($P<0.0001$ for both) (Fig 6B). Ghrelin levels were also significantly lower in GF mice compared to NORM mice ($P<0.0001$ for both conditions); however, re-feeding decreased plasma ghrelin in NORM ($P<0.001$), but not GF mice (Fig 6C).

During both fasted ($P<0.0001$) and re-fed ($P<0.001$) conditions, GF mice had significantly lowers levels of glucose compared to NORM mice, however, re-feeding increased glucose levels in GF ($P<0.0001$), but not NORM mice (Fig 7A). Triglyceride levels were similar between GF and NORM mice in both conditions (Figure 7B). Total cholesterol was increased in GF compared to NORM mice in both fasted ($P<0.001$) and re-fed ($P<0.001$) conditions. Additionally, total cholesterol levels were elevated after re-feeding in both GF ($P<0.05$) and NORM ($P<0.05$) mice compared to fasting (Fig 7C). Consistent with this, HDL levels were significantly higher in GF mice in both conditions (fasted: $P<0.0001$; re-fed: $P<0.0001$) compared to NORM mice. Additionally, plasma HDL was elevated after re-feeding in both groups of mice ($P<0.0001$ for both) (Fig 7D).

Discussion

Our present studies demonstrate that GF mice display an increased preference for a low concentration of intralipid and consume slightly more intralipid than NORM mice, resulting in increased caloric intake. This increased preference for, and intake of, intralipid in GF mice is associated with increased expression of lingual CD36 and down-regulation of intestinal fatty-acid receptors. Furthermore, GF mice have decreased expression of intestinal satiety peptides CCK, GLP-1, and PYY and lower levels of circulating leptin, PYY and ghrelin. They also have fewer enteroendocrine cells in the

ileum, and more in the colon, but an equal number in the proximal (duodenum, jejunum) intestine, compared to NORM mice. Finally, GF mice display alterations in plasma biochemical markers that mimic a fasting state, with increased fat metabolism and decreased circulating glucose. Together, these results suggest that GF mice have increased oral but decreased post-oral nutrient detection and satiation signaling, contributing to increased energy intake, which most likely occurs as a compensatory mechanism for their decreased energy stores.

Oral and post-oral factors are strong determinants of meal size. For example, consumption of a HF diet leading to increased fat metabolism in rodents is associated with increased acceptance of fat [73]. On the other hand, during the fasting state, lingual sensors for fat detection in the oral cavity are markedly increased [519]. Absence of the gut microbiota in mice results in a dramatic metabolic shift that closely resembles the fasting state of a normal animal [21]. For example, while body weight is similar between GF and normal mice, adiposity in GF mice is severely decreased, which is attributed to significant decreases in liver *de novo* lipogenesis[22]. Furthermore, plasma leptin and glucose are also lower in GF mice, an observation similar to that of a fasted state. These physiological and metabolic changes present in the GF condition may drive increased fat preference and/or intake observed in the current studies. Indeed, we found that GF mice prefer a low concentration of intralipid more than NORM mice while consuming more calories from intralipid at the highest concentration tested. These findings may be explained by the decreased energy state exhibited by GF mice, leading to adaptive changes in the lingual epithelium, such as increased CD36. For example, fasting animals exhibit increased preference of low concentrations of fats and

increased caloric intake from fats [163] Furthermore, our result of increased preference for the low intralipid concentration in GF mice was associated with increased expression of the fatty-acid translocase, CD36, in the posterior lingual epithelium. Expressed on the apical portion of sensory taste cells in the circumvallate papillae, CD36 plays a significant role in detection of LCFAs and acts as a lipid sensor. For example, CD36 KO mice exhibit marked reduction in detection and preference for fats [101]. Additionally, expression of CD36 is elevated during fasting, a physiological state associated with increased oral sensitivity to fats [162, 519]. Conversely, HF-feeding and obesity is associated with decreases in lingual CD36 expression [519]. Thus, increased expression of CD36, leading to increased oral sensitivity to intralipid may be a secondary effect observed and attributed to chronically depleted energy stores observed in GF mice. Additionally, while it is unknown if microbiota in the oral cavity play a role in taste signaling, increased expression of CD36 is most likely independent of changes in taste cell number. Specifically, we previously found no difference in expression of α-gustducin, a marker of bitter and sweet taste receptor cells, T1R2, or T1R3 in the posterior lingual epithelium of GF and control mice [518]. However, despite the fact that GF mice are more sensitive to the low concentration of intralipid, intralipid is a nutritive fat source, and GF mice consume more calories from the high concentration of intralipid, denoting possible alterations in post-oral feedback.

While oral factors influence short-term preference and detection of stimuli, long-term acceptance and preference is predominantly driven by post-oral nutrient feedback, in addition to taste associations, which ultimately stimulate further consumption[161]. For example, intestinal infusions of nutrients paired with a flavored non-nutritive solution

increases intake of that flavored solution [521]. As well, at higher concentrations of intralipid, CD36 KO mice display similar intralipid intake as wild type mice, and preference for nutritive fats is similar to wild type mice after repeated exposures with no impairments in post-oral conditioning. Thus, because GF mice have decreased energy stores and consume more of a nutritive solution than NORM counterparts, the composition and nutritive value of the intralipid, rather than oral factors, may be the main contributing factors for increased energy intake [101]. Although GF mice remain in a chronically fasting state, they also display a host of alterations in intestinal morphology and physiology. Specifically, GF animals have decreased intestinal villus length and crypth depth, lower rates of intestinal cell differentiation, all of which could contribute to impaired nutrient absorption [472]. Indeed, this is true of monosaccharide absorption, which is decreased in GF mice [21], and may be reflective of lower plasma glucose observed in our study. However, absorption of saturated fatty acids in GF mice is increased relative to controls [522, 523], which may be partially due to prolonged intestinal nutrient contact time as intestinal transit time is decreased in GF animals[312]. Finally, GF mice have decreased expression of intestinal CD36, which is predominantly located on the brush border; however, CD36 KO mice display no alterations in fat absorption[524]. Therefore, based on these data, it is unlikely that increased caloric intake in GF mice is due to decreased absorption of fats.

Enteroendocrine cells represent a candidate site of interaction between regulation of energy homeostasis and microbiota as they are exposed to the intestinal luminal environment, act as primary chemoreceptors, and respond to GI nutrients by releasing satiety peptides [525]. Emerging evidence has demonstrated that fatty-acid

responsive GPRs located on enteroendocrine cells are responsible for secretion of gut peptides that control energy intake [91]. Furthermore, metabolic byproducts from the gut microbiota are thought to interact with some of these GPRs. Interestingly, GF animals display altered expression of intestinal nutrient receptors and associated changes in plasma intestinal satiety peptides [312, 482]. In the present study, we found decreased expression of fatty-acid receptors GPR40, 41, 43, and 120 in the proximal intestine of GF mice with parallel decreases in intestinal satiety peptide CCK, PYY, and GLP-1 expression that together may be responsible for increased energy intake in GF mice. While the majority of CCK is released from the proximal intestine, PYY and GLP-1 are predominately secreted from the L-cells located in the distal intestine. However, it has been shown that the duodenum contains enough L-cells that are capable of eliciting satiation through GLP-1 and PYY release [407]. Furthermore, unlike changes in the expression of CD36, which are most likely secondary adaptive responses from lack of intestinal microbial stimulation, down-regulation of intestinal fatty-acid receptors seems to be a consequence of the lack of microbial stimulation. For example GPRs located on the luminal portion of enteroendocrine cells come into direct contact with the microbiota, which secrete nutritive byproducts of fermentation, and may alter nutrient receptor expression [312]. This is of relevance to our study, since secretion of GLP-1 and PYY from the proximal intestine is most likely a function of direct luminal nutrient stimulation, while distally secreted GLP-1 and PYY is thought to be primarily mediated by neural pathways [407]. It is known that consumption of fat or stimulation of intestinal cell lines with fatty acids results in release of satiety peptides such as CCK, PYY and GLP-1 through binding to GPR40, 41, 43, and 120. Specifically, short-chain fatty-acids

(SCFA)-induced release of PYY is mediated by GPR41 and 43 [312], while GPR40 and 120 mediate CCK and GLP-1 secretion stimulated by medium and long-chain fatty acids, respectively [91, 93]. Very few studies have examined the relative influence of the gut microbiota on intestinal satiety peptides and nutrient receptors in the intestine, and no studies have linked these changes to appetitive responses. For example, Samuel et. al. found increased GPR41 in the colon of GF mice, which was associated with decreases in circulating PYY [312]. In our study, we only examined receptor expression in the proximal intestine and the relative distribution of fatty-acid responsive receptors throughout the GI tract is unclear. Our immunohistochemical data show no difference in the EEC number in the proximal intestine between GF and NORM mice. Thus, based on the broad decreases in the small intestinal GPRs and satiety peptide expression it appears that absence of microbiota affects intestinal peptide content rather than enteroendocrine cell numbers.

We also found that circulating levels of leptin, PYY, and ghrelin were all decreased in GF animals relative to controls. Although we have not assessed whole body fat composition in this study, carcasses of GF mice were virtually void of fat pads and we were unable to dissect any quantifiable fat depots from the GF mice. Because the majority of circulating leptin originates from white adipose tissue and GF mice are mostly fat depleted [21], decreased circulating leptin in GF mice is reflective of decreased adiposity. In addition, in a separate study we found that GF mice displayed drastically reduced fat mass (significantly less epididymal fat pad mass: GF: 0.04 g vs. NORM: 0.14 g; unpublished) which was very similar to what we qualitatively observed in the mice from this current study. Our results are consistent with previous published

work using GF C57Bl/6J mice. However, as mentioned in the introduction, decreased adiposity was not observed in GF male adult C3H mice fed a high fat diet [506] which may be attributed to strain difference and the type of diet used. Thus with a 30% reduction in circulating leptin observed in our GF mice, a chronic energy deficit may be the main driving factor for increased caloric intake from intralipid. Similarly, PYY, which is released mainly from the distal intestine, where the majority of microbiota resides, is also decreased in GF mice. SCFA are potent stimulators of PYY release [526], thus it is not surprising that, decreased delivery of SCFA in the distal intestine, due to lack of the microbiota, results in decreased circulating PYY, similar to that proposed previously. GF mice also had lower plasma levels of ghrelin compared to controls. As the only known orexigenic hormone released mainly from the stomach and duodenum, ghrelin is elevated during fasting and increases food intake and adiposity in rodent models when administered exogenously [218]. Based on this and given the constant energy deficits of the GF mice, one would expect increased circulating ghrelin in fasted GF mice. The reason for this effect is not immediately clear but changes in GI tract morphology, such as differences in X/A-cell number may be responsible. As expected, intralipid feeding increased leptin and PYY levels in both GF and NORM mice; however, re-feeding decreased ghrelin in NORM, but not GF mice, which may be reflective of the chronic fasting state in these animals.

In addition to changes in satiety hormone levels, we found slight alterations in circulating biochemical parameters. For example, plasma glucose was decreased in GF mice relative to NORM controls, an effect predictive of the energy deficits in the GF model and consistent with previous reports [22, 507]. Equally, we found that the

intestinal glycoprotein FIAF, a lipoprotein lipase inhibitor, was significantly upregulated in GF mice. This is not unexpected, since intestinal microbiota promotes fat storage by suppressing intestinal expression of FIAF[21] and fasting increases FIAF expression[504]. However, the role of intestinal FIAF as an inhibitor of lipoprotein lipase in peripheral tissues of GF mice has been recently disputed [506]. FIAF stimulates lipolysis, resulting in elevated plasma triglycerides and lipoproteins with subsequent reduction in fat stores [527]. While we found no differences in total plasma TG levels, we found increases in plasma cholesterol and HDL in GF mice, consistent with the physical associations of FIAF with plasma lipoproteins [527]. Recent evidence suggests that serum TG levels are not altered in GF animals, but decreased LPL activity in this model has an effect on circulating TG levels [21, 528]. The reasons for the discrepancy in these findings regarding increased FIAF, yet unaltered plasma TG levels is not completely clear. While FIAF is indeed an important factor altering LPL activity in adipose tissue, recently, it has been suggested that intestinal FIAF levels do not influence circulating FIAF, as GF mice displayed increased intestinal FIAF but no difference in plasma FIAF compared to CV mice [506]. Furthermore, FIAF is a potent inhibitor of angiogenesis [529], and the gut microbiota has a profound ability to influence intestinal angiogenesis [530]. Thus, intestinal FIAF may serve as local contributor to angiogenesis rather than circulating metabolism. Additionally, cholesterol levels are typically unaltered or increased in GF rodents relative to controls during standard chow feeding [22, 528, 531], and decreased during HF-feeding [507]. Interestingly, increased circulating markers of fat metabolism are associated with increased acceptance of fat[73], supporting our behavioral findings. Together, these data confirms previous

reports that markers of lipid metabolism are dramatically altered in GF animals and are influenced by energy status and feeding conditions.

In summary, we have shown that GF mice prefer a low concentration of intralipid more than NORM mice and consume more calories from a high concentration of intralipid. Increased preference for, and caloric intake of, intralipid was associated with an increase in lingual CD36 expression and concomitant decrease in intestinal fatty-acid responsive receptors and satiety peptide content. As well, circulating biochemical markers indicated a shift toward increased fat metabolism in GF mice, while circulating satiety hormones signified decreased energy stores. Collectively, these results demonstrate, for the first time, that in addition to profound effects on energy status of the animal resulting in a significant loss of adipose stores and subsequent metabolic changes, the absence of gut microbiota profoundly alters the physiological mechanisms and molecular substrates responsible for nutrient detection and signaling pathways that ultimately affect feeding behavior.

Figure legends

Fig 1. Preference (A), raw intake (B), and calorie intake from intralipid emulsions (C) in GF and NORM C57B6/J mice during 48-h two-bottle sucrose vs. water tests. (A) GF mice preferred the lowest concentration (0.156% oil) of intralipid emulsion tested more than NORM mice. (B) Intake of intralipid emulsions was similar across all concentrations tested. (C) GF mice consumed more energy from the highest concentration (1.25% oil) of intralipid emulsion tested. Data are expressed as means ± SEM. *P<0.05 compared to NORM

Fig 2. Gene expression of (A) lingual CD36, and protein expression of (B) intestinal CD36, and (C) intestinal FIAF. (A) GF mice exhibited 3-fold up-regulation of lingual CD36 mRNA in the posterior lingual epithelium relative to NORM mice. (B) Expression of intestinal CD36 was down-regulated in GF mice compared to NORM controls. (C) Intestinal FIAF expression was increased over 3-fold in GF mice relative to NORM mice Data are expressed as means ± SEM. *P<0.05 compared to NORM, **P<0.01, ***P<0.001

Fig 3. Intestinal epithelial protein expression of fatty-acid responsive receptors in GF and NORM mice. GF mice displayed down-regulation of proximal intestinal GPR40, 41, 43, and 120 relative to NORM mice. Data are expressed as means ± SEM. *P<0.05 compared to NORM mice, ***P<0.001.

Fig 4. Intestinal epithelial protein expression of satiety peptide in GF and NORM mice. Data are expressed as means ± SEM. GF mice exhibited down-regulation of proximal intestinal CCK, GLP-1 and PYY relative to NORM mice.**P<0.01 compared to NORM mice, ***P<0.001.

Fig 5. (A) Cell counts of enteroendocrine cells expressing chromogrannin-A and (B) representative microphotographs of ileum and colon sections at 100X magnification. GF mice had less enteroendocrine cells in the ileum, but more in the colon compared to NORM controls. Data are expressed as means ± SEM. *P<0.05 compared to NORM mice.

Fig 6. Plasma levels of (A) leptin, (B) ghrelin, and (C) PYY in GF and NORM mice following an overnight fast or refeeding with 1-ml of 20% intralipid. (A) Plasma leptin was lower in GF mice relative to NORM controls. Refeeding elevated plasma leptin in both groups. (B) GF mice displayed lower levels of plasma PYY compared to NORM mice. Refeeding increased plasma PYY in both groups. (C) GF mice exhibited lower circulating ghrelin in both conditions. Refeeding increased plasma ghrelin in NORM, but not GF mice. Data are expressed as means ± SEM. **P<0.01 compared to NORM mice, ***P<0.001. ††P<0.01 compared to fasting condition within group, ††† P<0.001.

Fig 7. Plasma levels of (A) glucose, (B) cholesterol, (C) total triglycerides, and (D) HDL in GF and NORM mice following an overnight fast or refeeding with 1-ml of 20% intralipid. (A) Plasma glucose levels of GF mice were lower than NORM controls and

refeeding increased glucose levels in GF, but not NORM mice. (B) Plasma triglycerides were similar between both groups and both feeding conditions. (C) Plasma total cholesterol was increased in GF mice relative to NORM controls and refeeding increased cholesterol levels in both NORM and GF mice. Total plasma HDL was increased in GF mice relative to NORM mice with refeeding increasing total HDL in both groups. Data are expressed as means ± SEM. **P<0.01 compared to NORM mice, ***P<0.001. †P<0.05 compared to fasting condition within group, †† P<0.01.

References

1. Wang YC, McPherson K, Marsh T, Gortmaker SL, Brown M (2011) Health and economic burden of the projected obesity trends in the USA and the UK. Lancet 378: 815-825.
2. Mindell S, Smith GP, Greenberg D (1990) Corn oil and mineral oil stimulate sham feeding in rats. Physiol Behav 48: 283-287.
3. Lucas F, Sclafani A (1996) The composition of the maintenance diet alters flavor-preference conditioning by intragastric fat infusions in rats. Physiol Behav 60: 1151-1157.
4. Sclafani A, Glendinning JI (2005) Sugar and fat conditioned flavor preferences in C57BL/6J and 129 mice: oral and postoral interactions. Am J Physiol Regul Integr Comp Physiol 289: R712-720.
5. Reed DR, Tordoff MG, Friedman MI (1991) Enhanced acceptance and metabolism of fats by rats fed a HF diet. Am J Physiol 261: R1084-1088.
6. Backhed F, Ding H, Wang T, Hooper LV, Koh GY, et al. (2004) The gut microbiota as an environmental factor that regulates fat storage. Proc Natl Acad Sci U S A 101: 15718-15723.
7. Backhed F, Manchester JK, Semenkovich CF, Gordon JI (2007) Mechanisms underlying the resistance to diet-induced obesity in germ-free mice. Proc Natl Acad Sci U S A 104: 979-984.
8. Rabot S, Membrez M, Bruneau A, Gerard P, Harach T, et al. (2010) Germ-free C57BL/6J mice are resistant to HF-diet-induced insulin resistance and have altered cholesterol metabolism. FASEB J.
9. Fleissner CK, Huebel N, Abd El-Bary MM, Loh G, Klaus S, et al. (2010) Absence of intestinal microbiota does not protect mice from diet-induced obesity. Br J Nutr 104: 919-929.
10. Swartz TD, Duca FA, de Wouters T, Sakar Y, Covasa M (2011) Up-regulation of intestinal type 1 taste receptor 3 and sodium glucose luminal transporter-1 expression and increased sucrose intake in mice lacking gut microbiota. Br J Nutr: 1-10.

11. Samuel BS, Shaito A, Motoike T, Rey FE, Backhed F, et al. (2008) Effects of the gut microbiota on host adiposity are modulated by the short-chain fatty-acid binding G protein-coupled receptor, Gpr41. Proc Natl Acad Sci U S A 105: 16767-16772.
12. Tanaka T, Katsuma S, Adachi T, Koshimizu TA, Hirasawa A, et al. (2008) Free fatty acids induce cholecystokinin secretion through GPR120. Naunyn Schmiedebergs Arch Pharmacol 377: 523-527.
13. Hirasawa A, Tsumaya K, Awaji T, Katsuma S, Adachi T, et al. (2005) Free fatty acids regulate gut incretin glucagon-like peptide-1 secretion through GPR120. Nat Med 11: 90-94.
14. Delzenne NM, Cani PD, Daubioul C, Neyrinck AM (2005) Impact of inulin and oligofructose on gastrointestinal peptides. Br J Nutr 93 Suppl 1: S157-161.
15. Sclafani A, Ackroff K, Abumrad NA (2007) CD36 gene deletion reduces fat preference and intake but not post-oral fat conditioning in mice. Am J Physiol Regul Integr Comp Physiol 293: R1823-1832.
16. Martin C, Passilly-Degrace P, Gaillard D, Merlin JF, Chevrot M, et al. The lipid-sensor candidates CD36 and GPR120 are differentially regulated by dietary lipids in mouse taste buds: impact on spontaneous fat preference. PLoS One 6: e24014.
17. Zhang XJ, Zhou LH, Ban X, Liu DX, Jiang W, et al. (2011) Decreased expression of CD36 in circumvallate taste buds of HF diet induced obese rats. Acta Histochem 113: 663-667.
18. Martin C, Passilly-Degrace P, Gaillard D, Merlin JF, Chevrot M, et al. (2011) The lipid-sensor candidates CD36 and GPR120 are differentially regulated by dietary lipids in mouse taste buds: impact on spontaneous fat preference. PLoS One 6: e24014.
19. Swartz TD, Hajnal A, Covasa M (2010) Altered orosensory sensitivity to oils in CCK-1 receptor deficient rats. Physiol Behav 99: 109-117.
20. Glendinning JI, Beltran F, Benton L, Cheng S, Gieseke J, et al. (2010) Taste does not determine daily intake of dilute sugar solutions in mice. Am J Physiol Regul Integr Comp Physiol 299: R1333-1341.
21. Ackroff K, Sclafani A (1991) Flavor preferences conditioned by sugars: rats learn to prefer glucose over fructose. Physiol Behav 50: 815-824.

22. Cherbuy C, Honvo-Houeto E, Bruneau A, Bridonneau C, Mayeur C, et al. (2010) Microbiota matures colonic epithelium through a coordinated induction of cell cycle-related proteins in gnotobiotic rat. Am J Physiol Gastrointest Liver Physiol 299: G348-357.
23. Demarne Y, Corring T, Pihet A, Sacquet E (1982) Fat absorption in germ-free and conventional rats artificially deprived of bile secretion. Gut 23: 49-57.
24. Demarne Y, Sacquet E, Garnier H (1972) [Gastrointestinal flora and fat digestion in monogastrics]. Ann Biol Anim Biochim Biophys 12: 509-524.
25. Goudriaan JR, Dahlmans VE, Febbraio M, Teusink B, Romijn JA, et al. (2002) Intestinal lipid absorption is not affected in CD36 deficient mice. Mol Cell Biochem 239: 199-202.
26. Engelstoft MS, Egerod KL, Holst B, Schwartz TW (2008) A gut feeling for obesity: 7TM sensors on enteroendocrine cells. Cell Metab 8: 447-449.
27. Liou AP, Lu X, Sei Y, Zhao X, Pechhold S, et al. (2011) The G-protein-coupled receptor GPR40 directly mediates long-chain fatty acid-induced secretion of cholecystokinin. Gastroenterology 140: 903-912.
28. Goodlad RA, Ratcliffe B, Fordham JP, Ghatei MA, Domin J, et al. (1989) Plasma enteroglucagon, gastrin and peptide YY in conventional and germ-free rats refed with a fibre-free or fibre-supplemented diet. Q J Exp Physiol 74: 437-442.
29. Theodorakis MJ, Carlson O, Michopoulos S, Doyle ME, Juhaszova M, et al. (2006) Human duodenal enteroendocrine cells: source of both incretin peptides, GLP-1 and GIP. Am J Physiol Endocrinol Metab 290: E550-559.
30. Cherbut C, Ferrier L, Roze C, Anini Y, Blottiere H, et al. (1998) Short-chain fatty acids modify colonic motility through nerves and polypeptide YY release in the rat. Am J Physiol 275: G1415-1422.
31. Tschop M, Smiley DL, Heiman ML (2000) Ghrelin induces adiposity in rodents. Nature 407: 908-913.
32. Kersten S, Mandard S, Tan NS, Escher P, Metzger D, et al. (2000) Characterization of the fasting-induced adipose factor FIAF, a novel peroxisome proliferator-activated receptor target gene. J Biol Chem 275: 28488-28493.

33. Mandard S, Zandbergen F, van Straten E, Wahli W, Kuipers F, et al. (2006) The fasting-induced adipose factor/angiopoietin-like protein 4 is physically associated with lipoproteins and governs plasma lipid levels and adiposity. J Biol Chem 281: 934-944.

34. Velagapudi VR, Hezaveh R, Reigstad CS, Gopalacharyulu P, Yetukuri L, et al. (2011) The gut microbiota modulates host energy and lipid metabolism in mice. J Lipid Res 51: 1101-1112.

35. Le Jan S, Amy C, Cazes A, Monnot C, Lamande N, et al. (2003) Angiopoietin-like 4 is a proangiogenic factor produced during ischemia and in conventional renal cell carcinoma. Am J Pathol 162: 1521-1528.

36. Stappenbeck TS, Hooper LV, Gordon JI (2002) Developmental regulation of intestinal angiogenesis by indigenous microbes via Paneth cells. Proc Natl Acad Sci U S A 99: 15451-15455.

37. Wostmann BS, Kan DF (1964) The Cholesterol-Lowering Effect of Commercial Diet Fed to Germfree and Conventional Rats. J Nutr 84: 277-282.

Figure 1

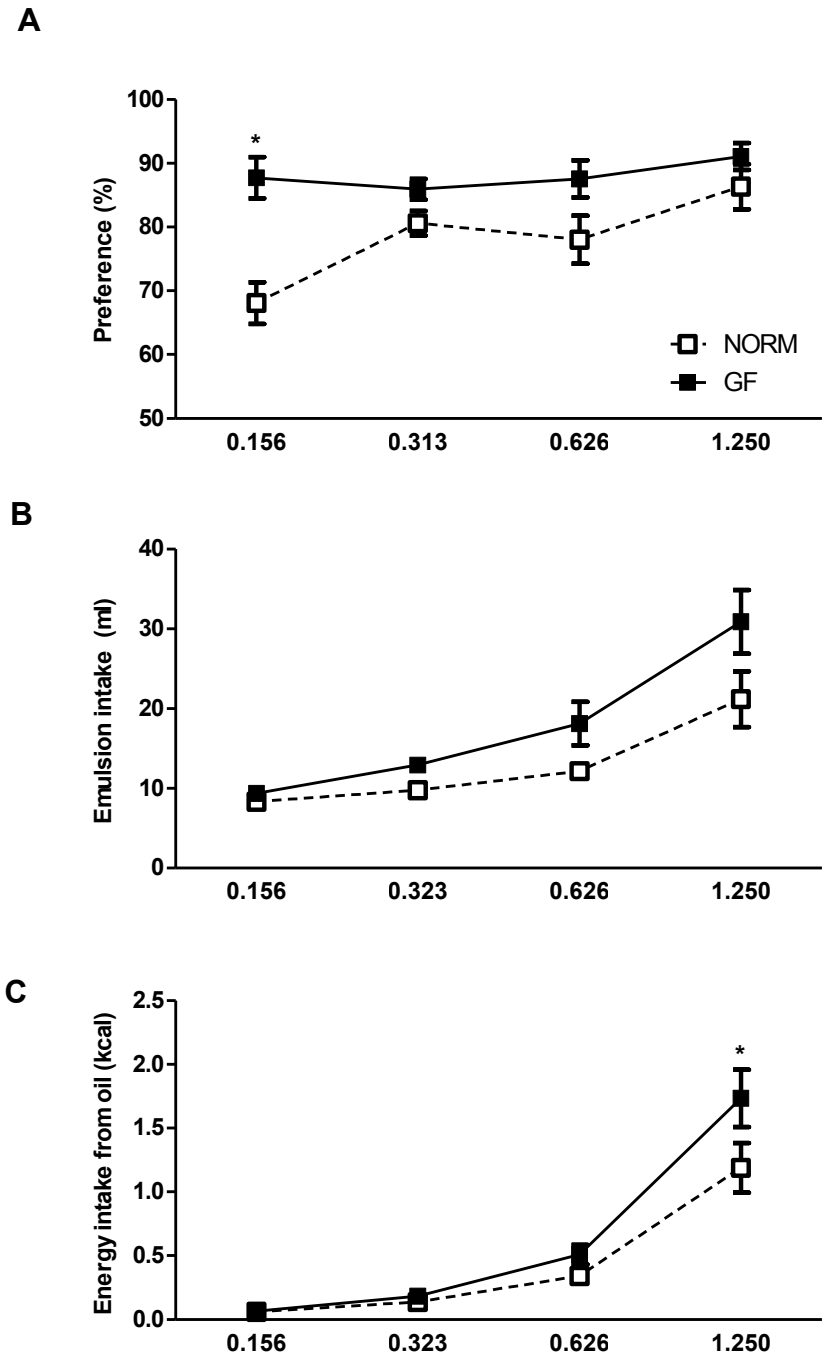
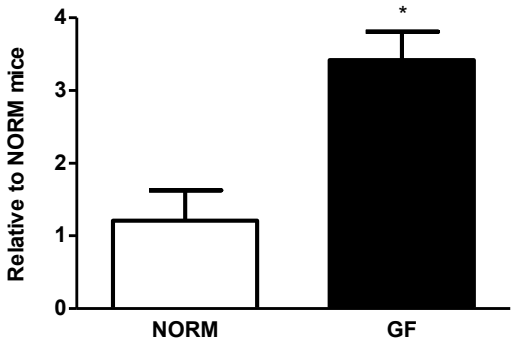
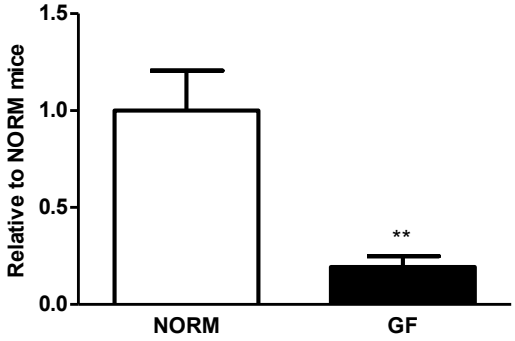

A
Preference (%)
100
90
80
70
60
50
*
NORM
GF
0.156
0.313
0.626
1.250
B
Emulsion intake (ml)
40
30
20
10
0
0.156
0.323
0.626
1.250
C
Energy intake from oil (kcal)
2.5
2.0
1.5
1.0
0.5
0.0
*
0.156
0.323
0.626
1.250

Figure 2

A


Relative to NORM mice
4
3
2
1
0
*
NORM
GF

B


Relative to NORM mice
1.5
1.0
0.5
0.0
**
NORM
GF

C

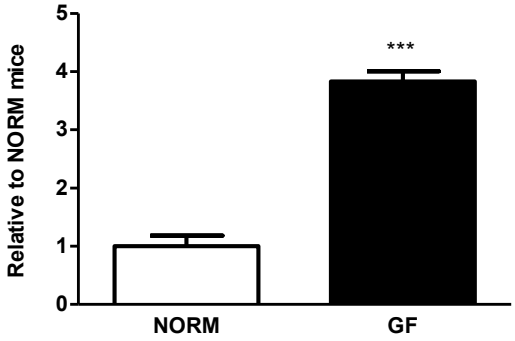

Relative to NORM mice
5
4
3
2
1
0
***
NORM
GF

Figure 3

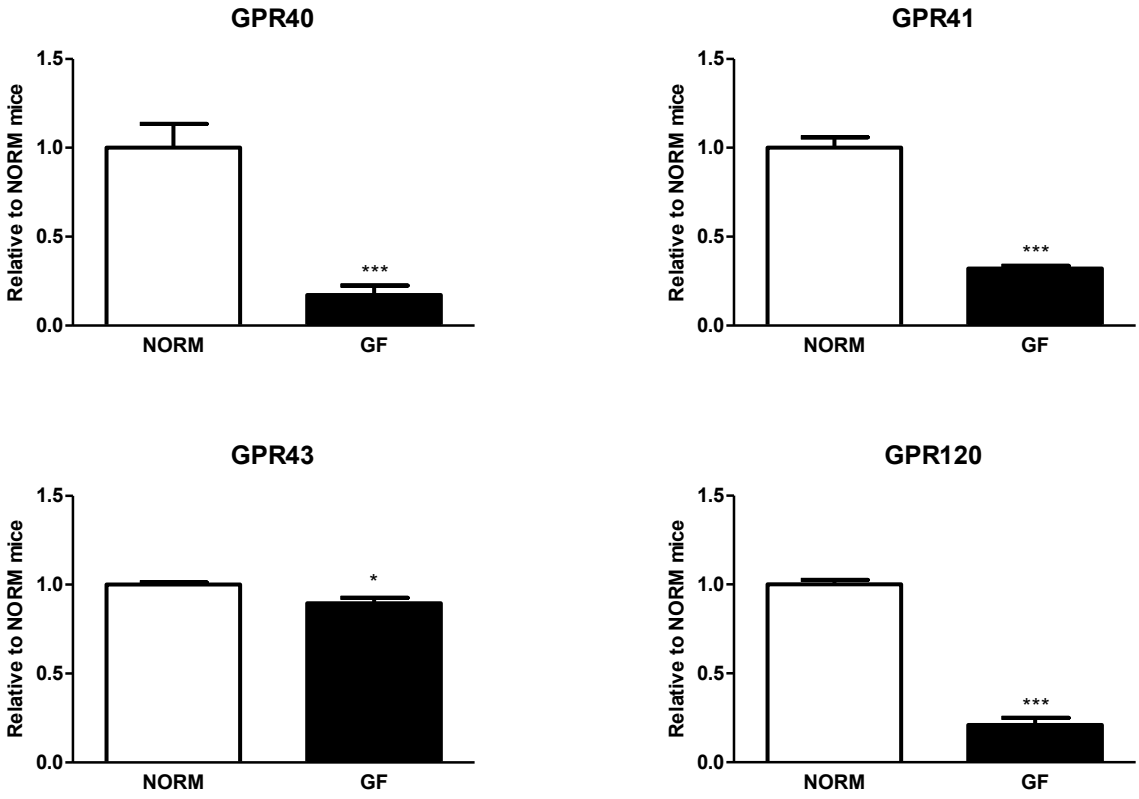

GPR40
Relative to NORM mice
1.5
1.0
0.5
0.0
***
NORM
GF
GPR41
Relative to NORM mice
1.5
1.0
0.5
0.0
***
NORM
GF
GPR43
Relative to NORM mice
1.5
1.0
0.5
0.0
*
NORM
GF
GPR120
Relative to NORM mice
1.5
1.0
0.5
0.0
***
NORM
GF

Figure 4

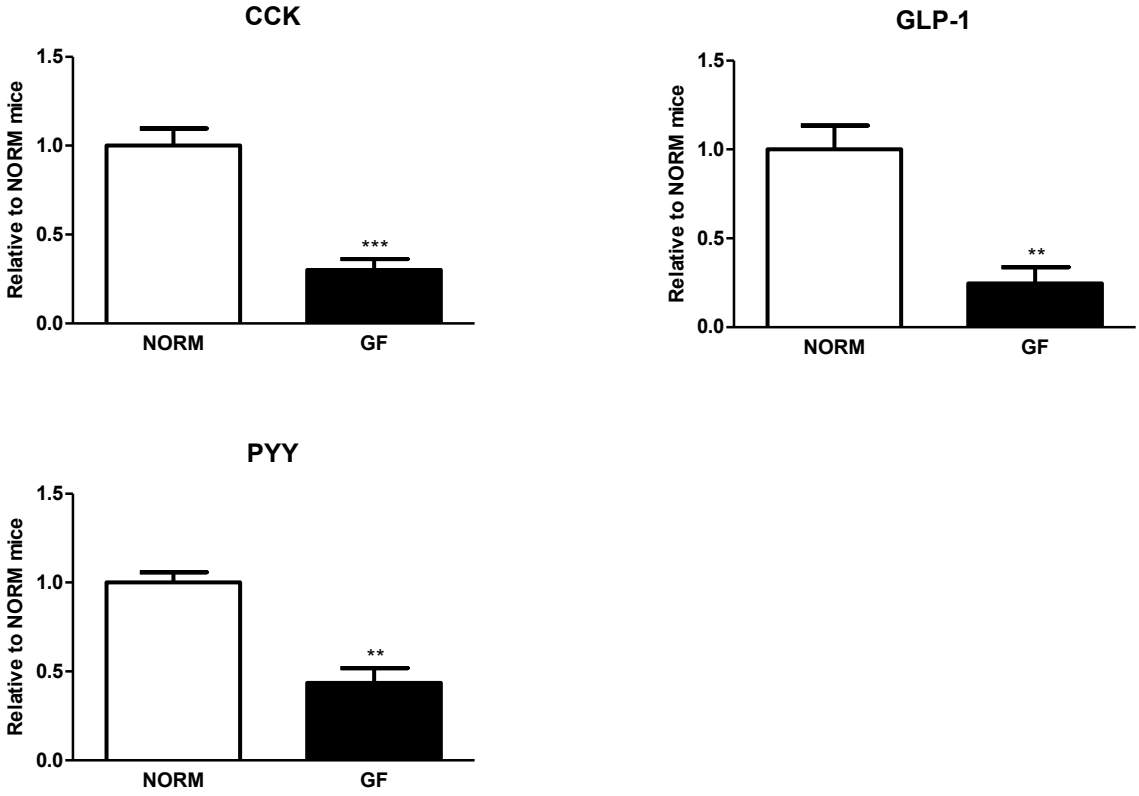

CCK
Relative to NORM mice
1.5
1.0
0.5
0.0
***
NORM
GF
GLP-1
Relative to NORM mice
1.5
1.0
0.5
0.0
**
NORM
GF
PYY
Relative to NORM mice
1.5
1.0
0.5
0.0
**
NORM
GF

Figure 5

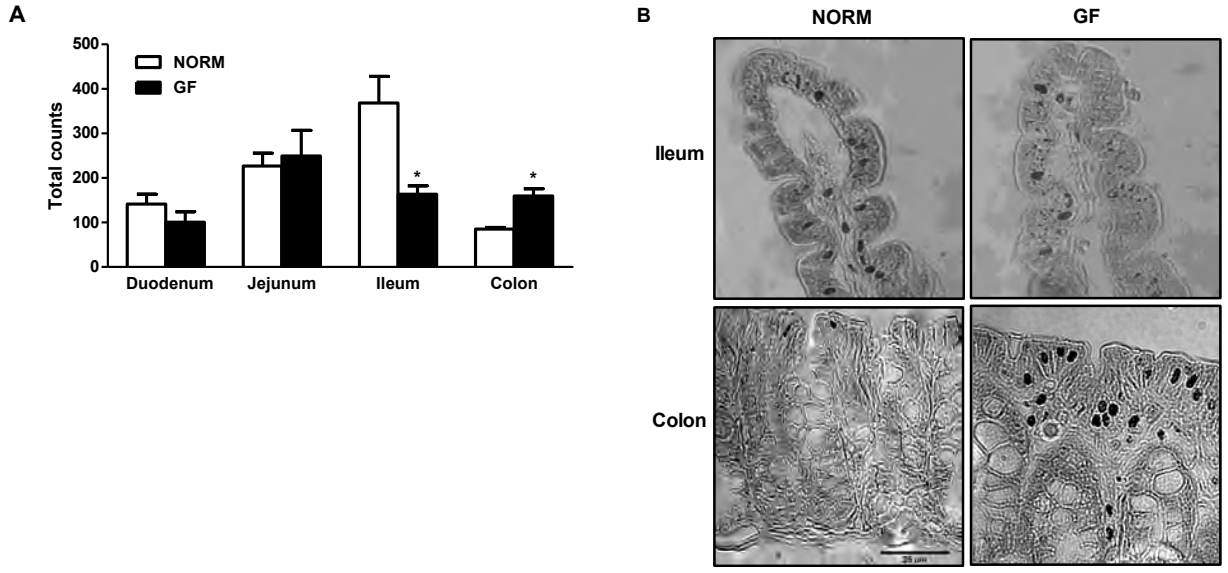

A
500
400
300
200
100
0
Total counts
NORM
GF
*
*
Duodenum
Jejunum
Ileum
Colon
B
NORM
GF
Ileum
Colon

Figure 6

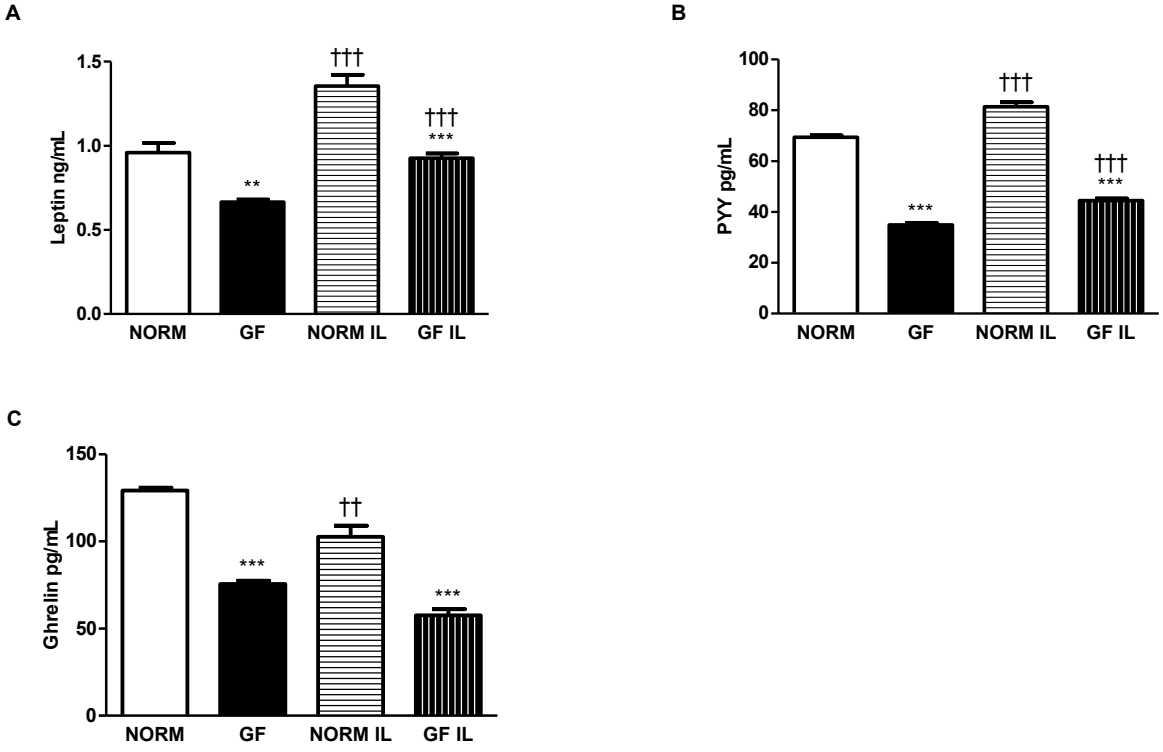
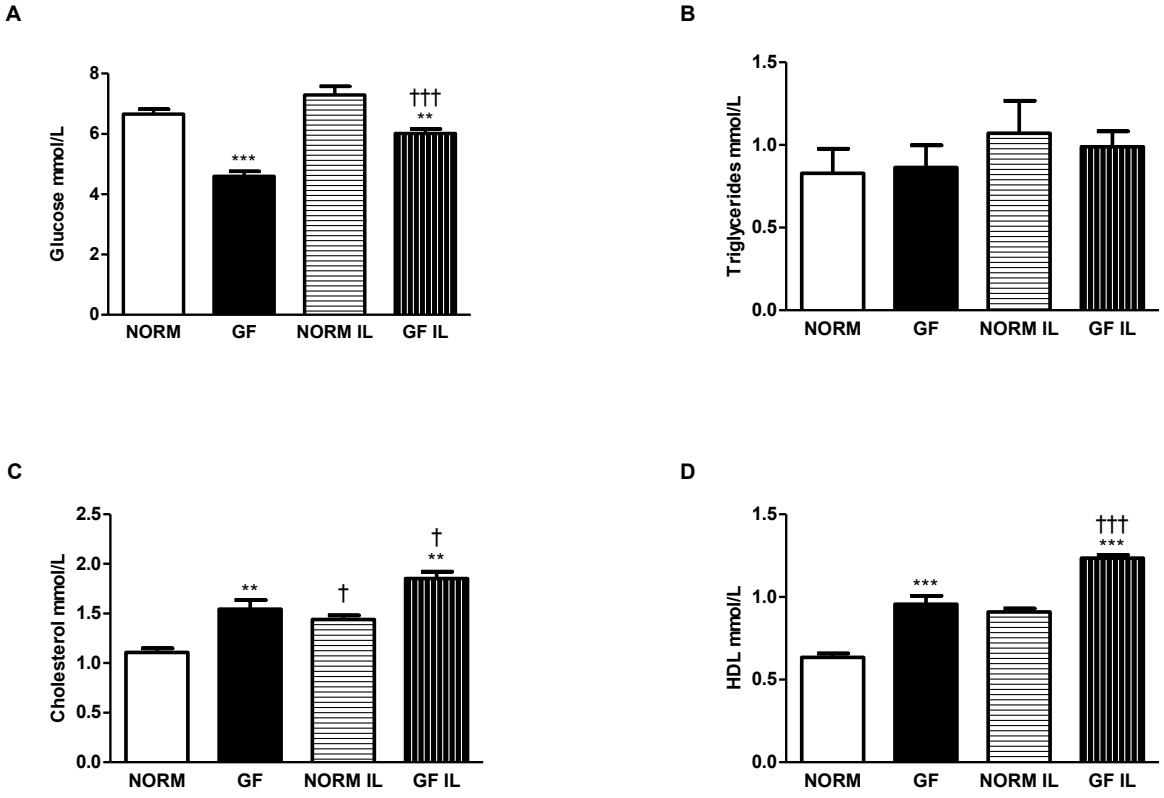

A
Leptin ng/mL
1.5
1.0
0.5
0.0
**
†††
†††
***
NORM
GF
NORM IL
GF IL
B
PYY pg/mL
100
80
60
40
20
0
***
†††
†††
***
NORM
GF
NORM IL
GF IL
C
Ghrelin pg/mL
150
100
50
0
***
††
***
NORM
GF
NORM IL
GF IL

Figure 7


A
Glucose mmol/L
8
6
4
2
0
***
**
†††
NORM
GF
NORM IL
GF IL
B
Triglycerides mmol/L
1.5
1.0
0.5
0.0
NORM
GF
NORM IL
GF IL
C
Cholesterol mmol/L
2.5
2.0
1.5
1.0
0.5
0.0
**
†
**
†
NORM
GF
NORM IL
GF IL
D
HDL mmol/L
1.5
1.0
0.5
0.0
***
***
†††
NORM
GF
NORM IL
GF IL

3.3 Specific Aim 2: Summary of results and conclusions

The present study demonstrated that GF mice display increased preference of a lower concentration of intralipid while consuming more of a higher concentration. As well, we also found increases in intestinal FIAF, a marker of fasting, in GF mice, with decreases in circulating levels of the adipocyte hormone leptin, which is highly correlated with adiposity [532]. The finding of increased preference of a low intralipid concentration in these animals is thought to be due to increased lingual CD36, which allows for better detection of oral fats. The increased intake of more energy dense intralipid emulsions; however, is thought to be due to decreased intestinal expression of fatty acid receptors and intestinal satiety peptides. Increased lingual CD36 in our current study is not surprising as GF mice display significantly decreased energy stores [21, 22], and expression of lingual CD36 is highly correlated with feeding states [163]. For example, fasting leads to increases in CD36 expression [163] while obesity is associated with decreased lingual CD36 [520]. The decrease in intestinal fatty acid receptors is most likely due to the exhibited fasting state of these animals. As such, mice lacking CD36 are less sensitive to intestinal infusions of fat [303], and by expressing less intestinal CD36, GF mice are most likely less sensitive to intestinal fats. This is not associated with decreased intestinal fat absorption; however, as CD36 KO mice do not display steatorrhea [297, 533]. Furthermore, decreases in intestinal CD36 lead to decreased chylomicron formation [533], which is a critical process in intestinal fat satiation [295]. Finally, the decreased intestinal satiety peptides and secretion, which may be due to CD36 via chylomicron formation, may also be due to decreases in intestinal GPRs, which are largely responsible for their secretion. As intestinal satiety peptides often

serve to control food intake, decreased levels of these peptides would allow for GF mice to consume more fat. Thus, collectively, these data demonstrate that GF mice display an energy state mimicking fasting, which leads to increased oral CD36 that is associated with increased fat preference and decreased intestinal fat receptors that could contribute to decreased intestinal satiety peptide levels and increased energy intake from fats.

4 Specific Aim 3: How gut microbiota affects host liver, intestinal, and adipose metabolic parameters in a GF rat model

4.1 Introduction

The results from Chapter 2 and 3 collectively demonstrated that the absence of gut microbiota in C57Bl/6J mice results in alterations in feeding behavior, impaired intestinal nutrient detection, and is associated with changed in expression and secretion of gut peptides relative to NORM controls. Previous studies using a GF mouse model have found that the GF state is characterized by decreased body adiposity, increased hepatic lipolysis, and protection from HF diet-induced obesity [22]. These results were predominantly attributed to increases in intestinal FIAF as GF-derived FIAF KO animals exhibit similar adiposity levels as CV controls [21, 22]. Despite this landmark finding, a more recent finding has demonstrated that in a C3N mouse model, GF mice exhibit increases in intestinal FIAF yet similar or decreased adiposity levels [506]. Therefore, the effect of the GF status on adiposity may be specific to the mouse strain used. Furthermore, it is unclear whether the lack of gut microbiota produces similar effects in the GF rat model as in the C57Bl/6J mouse. Therefore, the focus of this study was to examine whether the absence of a gut microbiota in the Fisher 344 (F344) rat is associated with behavioral, phenotypic and metabolic changes similar to those reported in the mouse model.

4.2 Absence of gut microbiota is not protective of fat deposition in the GF F344 rat model

Introduction

Studies comparing germ-free (GF) animals, devoid of gut microbiota, and conventional (NORM) animals, which have an intact microbiota, have revealed a wealth of information on the microbiota and how it affects physiology, endocrinology, and metabolism of the host it inhabits. Recently, the gut microbiota of the distal gastrointestinal tract has been identified as a possible factor influencing energy intake and obesity. For example, when GF mice receive microbiota from a lean donor, a term referred to as "conventionalization," the animals rapidly increase body adiposity, with little change in body weight while maintained on chow. Interestingly, while mice increase adipose deposition after conventionalization, they reduce caloric intake, which can be partially attributed to the increased absorption of energy from byproducts of microbial fermentation in the distal GI tract [21]. These results are further extended to a western diet, when GF mice are protected from HF diet induced obesity relative to NORM controls [22]. Additionally, while receiving microbiota from a lean donor results in increased adiposity, further increases are noted in animals conventionalized from genetically obese or dietary-induced obese animals [22, 508, 510].

Currently, there are multiple alterations including morphological, physiological, endocrine, and metabolic factors that are thought to contribute to the increased adiposity and altered feeding behavior seen in GF animals compared to NORM animals. For example, the GF status is characterized by marked shortening of the intestinal villus and decreases in crypt depth relative to controls [472]. Furthermore, expression of

nutrient receptors and transporters is markedly altered throughout the gastrointestinal tract [312], which may be a primary effect due to lack of microbial stimulation or secondary to alterations in intestinal morphology or an energy state similar to that of fasting. As well, proximal intestine glucose absorption is decreased in GF mice [21], which may attribute to the observed increases in liver lipolysis [22]. Intestinal transit time has also been observed to be decreased in GF mice, due primarily to decreases in gastric emptying, resulting from decreased secretion of the intestinal satiety peptide PYY [312] as well as decreased proximal intestine CCK, GLP-1, and PYY. However, more recently, decreased adiposity in the GF mouse has been primarily attributed to decreased hepatic lipogenesis.

The GF state is characterized by increased fatty acid oxidation and inhibition of lipogenesis due to the chronically low levels of adiposity observed in these animals [21, 22, 507, 508]. The main mechanism of decreased lipogenesis, is thought to be through fasting-induced adipocyte factor (fiaf), an inhibitor of lipoprotein lipase (LPL) that is produced in the intestinal epithelium [21, 22]. As such, GF mice express increased fiaf in the intestine, but not liver or adipose, and fiaf knock-out (KO) mice express significantly lower adipose LPL, and decreased liver *de novo* lipogenesis [21, 22]. Furthermore, while GF mice are resistant to obesity, GF fiaf KO mice are not resistant to ensuing obesity, demonstrating a critical role for fiaf in energy balance [21]. Despite this wealth of data, more recently, Fleissner et al found that GF mice are not resistant to diet-induced obesity, and exhibit increased adipose deposition, yet up-regulated intestinal fiaf, an effect attributed to diet [506]. Whether the altered metabolism observed in the GF mouse model extends to other species, commonly used to examine

feeding behavior, is relatively unclear. Similar to mice, GF rats maintain similar body weights as control animals, and exhibit increased food and water intake, which is thought to occur due to decreased energy extraction from the diet in GF rats [467]. Therefore, it is possible that due to decreased energy extraction from the gut microbiota, and associated morphological changes, GF rats maintain lower levels of adiposity relative to NORM controls. Thus, to better determine the contribution of gut microbiota on influencing peripheral metabolism and adiposity in rats, we examined adiposity in the GF Fisher F344 rat during standard rodent chow feeding with associated alterations in fiaf expression as well as markers of hepatic and adipose lipogenesis and adipogenesis.

Methods

Subjects

Throughout all experiments, adult male GF F344 rats from our local germ-free facility (ANAXEM, Jouy-en-Josas, FRANCE) and NORM F344 rats (Charles River, L'Arboseilles, FRANCE) were used. All rats were housed in polycarbonate cages with cedar bedding, except where noted otherwise and placed in Trexler-type isolators (Igenia, FRANCE). Rats were given *ad libitum* access to sterlized standard rodent chow (SAFE diets, Belgium) and sterile deionized water throughout experiments.

Body weight and food intake

Adult male rats (n = 5 NORM, 5 GF) were placed in polycarbonate cages with stainless steel metal bottoms to allow for accurate food spillage collection, one week prior to food intake measurements. After acclimation, 24-h food intake was measured for 5

consecutive days, accounting for spillage. Body weights of rats were also recorded every day for five days after which rats were switched back to cedar bedding.

Terminal tissue collection

Three separate groups of naïve rats (Group 1: n = 10 NORM, 10 GF; Group 2: n = 15 NORM, 15 GF; Group 3: n = 6 NORM, 6 GF) were used to measure relative epididymal fat pad mass in NORM and GF rats. Rats from Group 3 in the adiposity measurements were subsequently used to examine expression of intestinal glucose transporters and markers of peripheral metabolism. Rats were decapitated and fresh trunk blood collected in EDTA-coated tubes and the proximal intestine comprising both the duodenum and jejunum was excised and placed into physiological saline for the isolation of intestinal epithelial cells as described previously. Briefly, after excision, proximal intestines were flushed using 10 ml of ambient physiological saline, everted and divided into three segments, and placed into oxygenated Ca^{+2}, Mg^{+2}-free Krebs-Heinslet buffer with EDTA and DTT. Flasks containing sections were placed in a 37^{o}C water bath and shaken for 20 minutes to dissociate epithelial cells from the connective tissue. The suspension was collected, centrifuged and washed with Dulbecco's Phosphate Buffered-Saline (DPBS, Lonza). Afterwards, cell extract aliquots were snap frozen and stored at -80^{o}C until further analysis. Samples from liver were taken and snap frozen until further analysis. Epididymal, retroperitoneal, and visceral adipose tissue were excised and weighed, and samples of epididymal fat pads were snap frozen for analysis. The remainder of the epididymal fat pad was post-fixed in paraformaldehyde, embedded in paraffin, and 4-µm sections were stained with

hematoxylin and eosin. Adipocyte size was measured in at least 150 rat and analyzed using NIS Elements (Nikon).

qRT-PCR

Intestinal epithelial cells, adipose tissue, and liver tissues were lysed and homogenized with a rotor homogenizer and RNA extracted using TRiZol (Invitrogen). Resulting RNA was quantified with a Nanodrop (Thermo Scientific) and for all tissues, 10 µg of RNA was reverse transcribed into 100 µl cDNA using the high capacity cDNA kit (Applied Biosystem, Courtabeouf, FRANCE). Subsequent cDNA for all samples was diluted 5-fold and used for qRT-PCR using taqman gene expression master mix and inventoried taqman gene expression assays (Table 1) in an ABI Prism.

Western Blotting

Liver samples used for western blotting were homogenized in Radio Immuno Precipitation Assay (RIPA) buffer containing protease inhibitors (Sigma) using a rotor homogenizer. The resulting homogenate was centrifuged for 20 min at 13,000 RPM at 4^{o}C, the supernatant was transferred, and protein quantified using a nanodrop system (Thermo Scientific). Solubilized liver proteins (50 - 100 µg) were run on 8% acrylamide SDS-PAGE gels and transferred to nitrocellulose membranes, and probed with anti-bodies for ACC, ACC-P, AMPK, and AMPK-P (Santa Cruz Biotechnologies). Immune complexes were detected by chemiluminescence (GE Healthcare). Quantification was performed by densitometric analysis using ImageJ (NIH, Bethesda, MD, USA) and

ACC-P and AMPK-P were normalized to ACC and AMPK (Santa Cruz Biotechnology) as internal controls, respectively.

Adipocyte morphology

Epididymal fat pads were post-fixed in 4% paraformaldehyde overnight and then transferred to 75% ethanol. Subsequently, fat pads were impeded in paraffin and sectioned at 4 μm, and hematoxylin and eosin stained. Microphotographs of adipocytes were captured at 10X magnification, converted to binary using photoshop, and adipocyte surface area was measured in at least 150 adipocytes for each animal (n = 6 animals for each group) using NSI Elements (Nikon).

Statistical analysis

All values are expressed as mean ± SEM and statistical analyses performed using GraphPad™ Prism (version 5.0, San Diego, CA). Average 5-d body weight and food intake of GF and NORM rats was analyzed by one-way (type) analysis of variance (ANOVA). Body weights, adiposity, plasma biochemical analyses, and gene and protein expression of intestinal, liver and adipose tissues were analyzed using unpaired student's t-tests with Welch's correction for unequal variance where appropriate. Adipocyte surface area measurements were analyzed by one-way (type) repeated measures ANOVA.

Results

Food intake, body weight, adiposity, fiaf expression and biochemical analyses

During measurement of food intake, germ-free and NORM rats were of similar body weights (GF: 320 ± 7 g vs NORM: 319 ± 8 g; P = 0.91). GF animals consumed more food than NORM controls daily on average, but this did not reach significance (GF: 19.8 ± 1.1 g vs NORM: 18.7 ± 0.6 g; P = 0.42). In animals where epididymal fat pads were quantified, we found a significant increase of body weight in GF rats from Group 3 relative to all other groups. Similarly, relative epididymal adiposity was unchanged between GF and CV groups, with the exception of GF rats in Group 3 that displayed increased adiposity (Table 1). Additionally, in this GF group, relative retroperitoneal (P < 0.01), and total fat pad (P < 0.01) adiposity was increased (Fig 1A). Despite the observed increased adiposity in GF rats, fiaf expression was up-regulated over two-fold in the proximal small intestine (P < 0.05) and up-regulated in the liver (P < 0.01), but down-regulated in adipose tissue (P < 0.01) relative to NORM controls (Fig 1B). Plasma analyses revealed that GF rats had significantly higher circulating HDL (P < 0.05) and total cholesterol (P < 0.01), and an increase in plasma TG, which did not reach significance (P = 0.05) (Table 2).

Expression of intestinal glucose transporters and liver and adipocyte metabolic markers

In GF rats, intestinal expression of SGLT1 (P < 0.01) and GLUT2 (P < 0.01) transcripts were significantly down-regulated compared to intestinal expression of NORM rats (Fig 2A). Down-regulation of intestinal sugar transport coincided with decreased gene expression of liver lipogenesis markers, ACC (P < 0.01), SREBP (P < 0.01), and FAS (P

< 0.0001) relative to NORM rats as well (Fig 2B). Although GF mice expressing less total AMPK in the liver relative to NORM controls ($P < 0.05$), protein expression of phosphorylated AMPK ($P < 0.05$) was increased in GF rats compared to NORM controls, with an increase in phosphorylated ACC as well, which did not reach significance ($P = 0.17$) (Fig 2C). Analysis of adipocyte expression of adipogenesis markers revealed decreases in LPL ($P < 0.01$), PPAR-γ ($P < 0.01$), and aBP2 ($P < 0.01$) (Fig 3A); however, there were increases in expression of lipogenic enzymes ACC ($P < 0.05$) and FAS ($P < 0.01$) in GF rats' epididymal fat pads.

Adipose morphology

Increases in lipogenic enzyme expression was associated with observed increases in adipocyte surface area in GF rats relative to NORM controls, but this did not reach statistical significance ($P = 0.05$).

Discussion

In our current study, we have shown that in the F344 rat, the absence of the gut microbiota is not a major factor preventing adipose deposition. While food intake was similar in GF and NORM rats, relative adiposity of GF rats was relatively unchanged, and in one group, adiposity was increased compared to NORM rats. Despite increased adiposity in this group, these animals displayed increased intestinal fiaf. Furthermore, while expression of proximal intestine glucose transporters and markers of lipogenesis in the liver was decreased, adipocyte lipogenesis markers were increased. Finally, the increased lipogenesis in adipocytes of GF rats was associated with modest increases in

adipocyte size. Together, these changes suggest that lack of the microbiota, which leads to a decreased energy state in the intestine and liver, and decreased hepatic *de novo* lipogenesis in the rat, is not a factor in the protection from adiposity deposition in this model. Furthermore, lack of microbiota results in increases in anabolic pathways in adipose tissue of F344 rats, which may account for the relative difference of adiposity in this model compared to GF mice models.

The GF status generally is associated with extremely low levels of adiposity and decreased extraction of energy from the diet, due to lack of microbial fermentation in the distal intestine. For example, Backhed et al. demonstrated that despite similar body weights, GF mice are significantly leaner than NORM mice and consume more food over 24-h, with increased energy in the feces [21]. Additionally, when placed on a HF diet, GF mice increase adiposity relative to chow feeding, but remain significantly leaner than NORM animals fed either a chow or HF diet [22]. In our current findings, we have demonstrated that GF and NORM rats have no differences in 24-h food intake when animals were of similar body weights and similar adiposity. However, in a second group of animals, GF rats weighed more than NORM controls, and exhibited increased adiposity relative to body weight. While GF rats in this group displayed general increases in adiposity, they still expressed increased intestinal and liver fiaf, which has been a suggested factor in preventing obesity in GF mice [21, 22]. Similar to our current findings, Fleissman et al reported that while GF mice exhibited increased intestinal fiaf, the maintained increased levels of adiposity and were not resistant to HF diet induced obesity [506]. Furthermore, GF mice in their study did not exhibit increases in plasma fiaf, suggesting that intestinal fiaf may not have a role in circulating

metabolism, but rather intestinal angiogenesis. As such, intestinal fiaf may be elevated in GF animals due to the lack of intestinal cell differentiation observed in this model [472]. While we did not measure plasma fiaf, thus being unable to exclude whether intestinal fiaf in our study had a role in circulating metabolism, we observed increases in liver fiaf, yet decreases in adipocyte fiaf. Previous reports demonstrate liver and adipocyte fiaf are largely unchanged, or slightly increased in GF animals [21]. Nevertheless, circulating biochemical markers show that increases in fiaf expression as well as the GF state during standard chow feeding are highly associated with increases in circulating triglycerides and cholesterol [21, 22, 534], which we found in our study. Reflective of the fasting state typically observed in GF mice, blood glucose is also decreased, which is similar to our current findings. This could also be due to a decrease in glucose absorption, as found previously [21]. Thus, while intestinal fiaf may be important in metabolism regulation of GF mice, delivery of intestinal sugars to the liver may be the primary factor influencing this metabolic state. For example, decreased glucose delivery to the liver via intestinal absorption can shift liver metabolism towards catabolic pathways [535]. Additionally, the finding that the absence of glucose in an obesigenic diet results in normalized adiposity of GF animals suggests that the inability to absorb dietary sugars is important in influencing GF mice adiposity levels.

Nutrients absorbed from the intestine are sent through the portal vein for metabolism in the liver. Thus, decreases in extraction of energy from the diet, predominantly due to decreased absorption of SCFAs and sugars in the intestine may be one cause of the decreased liver lipogenesis in GF animals. To examine if the increased liver fiaf, which may be reflective of a negative energy state in the liver was

associated with changes in proximal intestine nutrient absorption, we measured transcript expression of SGLT1 and GLUT2, the two major transporters responsible for glucose absorption. We found that both proximal intestine SGLT1 and GLUT2 were decreased in GF rats, which was associated with slight decreases in plasma glucose levels, all of which would contribute to decreasing liver lipogenesis. This is in accordance with previous data demonstrating decreased intestinal absorption of glucose in GF mice. Together with increased intestinal fiaf expression, decreased expression of proximal intestine glucose transporters leading to decreased glucose absorption may be another reason for the decreases in liver lipogenesis observed in the GF model.

When we examined makers of liver lipogenesis, we found, expression of two important regulators of *de novo* fatty acid synthesis, ACC and FAS, were down-regulated in the liver of GF rats signifying that the GF status in rats is associated with this previously observed phenomenon in mice. Furthermore, expression of SREBP, a key transcription factor of glucose-induced hepatocyte lipogenesis and activator of ACC and FAS, was decreased in GF rats. Thus, from our study, it appears that the observed down-regulation of ACC and FAS expression in the liver may be through a SREBP-dependent mechanism. To further assess the energy status in the liver of GF animals, we examined protein expression of hepatic phosphorylated AMPK and ACC. AMP-activated protein kinase reflects cellular energy stores and the activation of AMPK through phosphorylation is dependent upon factors that signal decreased energy status [535]. In a fasting state, P-AMPK increases the generation of ATP through catabolic pathways, which is predominantly through inhibition of lipogenesis and stimulation of β-

oxidation. The mechanism responsible for inhibition of lipogenesis, and thus increased β-oxidation is the phosphorylation of the lipogenic enzyme, ACC, by P-AMPK which leads to its inactivation. We found that protein expression of P-AMPK and P-ACC was up-regulated in liver of GF rats, which may denote increased β-oxidation of the liver, and thus, decreased lipogenesis. Together, these data suggest that the GF status is indeed associated with decreased makers *de novo* lipogenesis, which are, surprisingly, independent of adiposity in this group of animals. Despite these findings, it is plausible that increased expression of local factors in adipocytes, and downstream of the decreased adipocyte fiaf observed, which are responsible for adipogenesis and lipogenesis may be one reason for the observed increase in adiposity observed in the GF rat.

Changes in adiposity are reflective of adipocyte hyperplasia or hypertrophy, which are due to increased adipogenesis and lipogenesis, respectively. For this reason, we examined the transcript expression of adipogenic factors PPAR-γ and ABP2 as well as key enzymes of fatty acid synthesis, ACC, FAS, and LPL, in the epididymal fat pads of GF and NORM rats. Peroxisome proliferator activated receptor is a nuclear transcription receptor that is a key regulator of adipogenesis via proteins such as ABP2 [536]. In general, we found that GF rats displayed increases in adipocyte lipogenesis markers yet decreased markers of adipogenesis. For example GF rats displayed decreased expression of PPAR and ABP2. Conversely, expression of ACC and FAS, two important enzymes responsible for fatty acid synthesis were increased. Interestingly, intestinal fiaf-induced LPL inhibition had been thought to be responsible for the observed decrease in adiposity in GF animals. As such, fiaf-deficient GF mice

are obese and fiaf-deficient mice display increased LPL activity [21]. However, these current data suggest that despite increased intestinal fiaf, adiposity is not decreased in GF rats and this is independent of LPL expression as well. Unlike LPL, the increase of ACC and FAS may be an underlying mechanism responsible for increased adiposity observed in GF rats. Being the committed step to fatty acid biosynthesis, ACC may induce the accumulation of fatty acids into adipocytes, and lead to increased adipocyte hypertrophy. Indeed, we did find modest increases in adipocyte surface area in the epididymal fat of GF rats relative to NORM controls. Therefore, from these results, we demonstrate that increased adiposity in GF rats from this group may be due to increased fatty acid synthesis and adipocyte size. Interestingly, adipocyte hypertrophy is associated with metabolic abnormalities, such as diabetes [537, 538]; however, GF rats in our study exhibited similar or lower levels for fasting plasma glucose. The discrepancy between these two findings may be explained by the observed increased in adipocyte size occurring in the epididymal fat pad. As such, we found no difference in the visceral fat mass and did not examine visceral adipocyte morphology, which is an independent predictor of diabetes and metabolic dysregulation [538]. While we found modest adipocyte hypertrophy in the GF rat, the exact mechanism may involve increased ACC and FAS as well as possible differences in fatty acid uptake.

In summary, our current results indicate that the absence of gut microbiota do not result in reduced adiposity in the GF F344 rat model, contrary to previous results using C57Bl/6J mice. Furthermore, while decreased adiposity in GF mice has been attributed to increased intestinal fiaf, a possible circulating metabolic regulator, and decreases in liver lipogenesis, we found that in one group of GF rats that displayed increased

adiposity, intestinal fiaf was still up-regulated, similar to that of a previous study[506]. Finally, the increase in adiposity in GF rat was associated with increased fatty acid synthesis markers in epididymal fat pads of GF rats, leading to adipocyte hypertrophy, and overall was independent of hepatic lipogenic markers. Collectively, these results demonstrate that the absence of gut microbiota in rats may not be important in preventing adiposity deposition in this rat model while maintaining importance in intestinal nutrient transporter expression and liver metabolism.

Table 1: Body weight and relative epididymal adiposity in GF and NORM rats

	Group 1		Group 2		Group 3	
	NORM	**GF**	**NORM**	**GF**	**NORM**	**GF**
n	(10)	(10)	(15)	(15)	(6)	(6)
Body weight	267 ± 5[a]	278 ± 15[a]	267 ± 12[a]	271 ± 17[a]	284 ± 7[a]	324 ± 22[b]
Epi. fat (%)	1.84 ± 0.22[a]	1.95 ± 0.35[a]	1.83 ± 0.20[a]	1.83 ± 0.29[a]	1.65 ± 0.20[a]	2.38 ± 0.30[b]

Columns in a row with differing letters denote significant difference.

Table 2: Plasma biochemical markers in NORM and GF rats

	NORM	GF
Glucose (mg/dl)	176.5 ± 19.0	137.1 ± 6.0
Triglycerides (mg/dl)	167.8 ± 10.7	213.6 ± 16.9
Cholesterol (mg/dl)	64.73 ± 4.9	87.2 ± 2.0*
HDL (mg/dl)	33.6 ± 2.8	44.7 ± 3.0*

*denotes significant difference from NORM rats

Figure legends

Fig 1. Relative fat pad mass (A), and fiaf tissue expression (B) in NORM (open bars) and GF (closed bars) rats from Group 3. (A) Relative epididymal, retroperitoneal, and total fat pad masses were increased in GF rats compared to NORM controls. (B) Intestinal and liver fiaf were up-regulated and down-regulated in adipose tissue of GF rats relative to NORM rats. Data are expressed as mean ± SEM. *P<0.05, **P<0.01 relative NORM controls

Fig 2. Intestinal glucose transporter mRNA expression (A), gene expression of hepatic lipogenesis markers (B), and hepatic phosphorylated AMPK and ACC-1 (C) in NORM and GF rats from Group 3. (A) GF rats exhibited decreased expression of both GLUT and SGLT1 in the proximal intestine. (B) Markers of hepatic lipogenesis were decreased in GF rats. (C) Enzymes responsible for inactivating lipogenesis were increased in GF rats

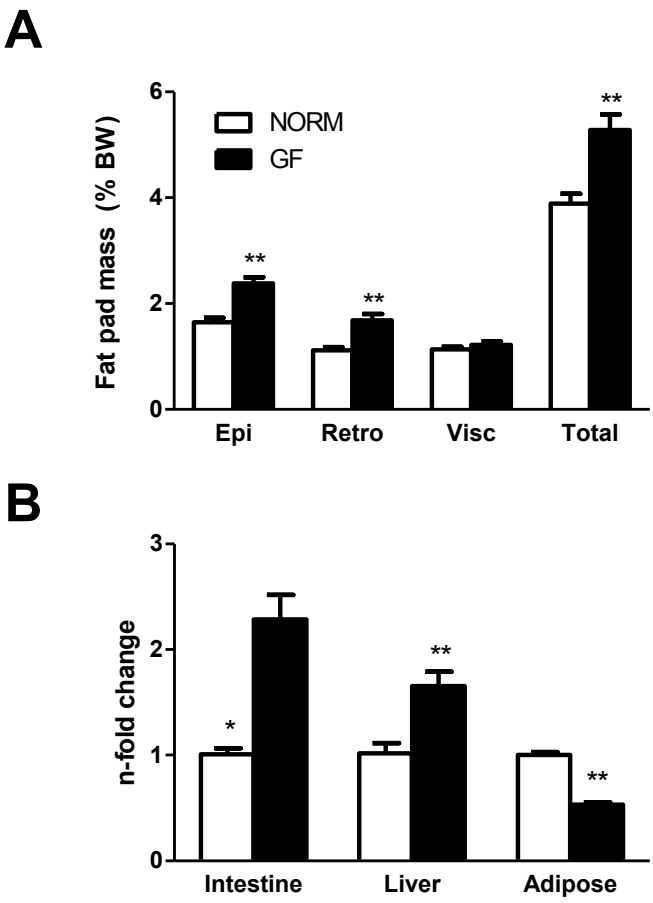
Fig 3. Markers of fatty acid synthesis and adipogenesis (A) and average adipocyte surface area and adipocyte morphology (B and C) in epididymal fat pads of NORM and GF rats from Group 3. GF rats expressed increased ACC-1 and FAS, but decreased markers of adipogenesis. (B) Average surface area measurements revealed modest adipocyte hypertrophy in GF mice. (C) Microphotographs (10X magnification) of adipocytes from NORM and GF rats.

References

1. Backhed F, Ding H, Wang T, Hooper LV, Koh GY, et al. (2004) The gut microbiota as an environmental factor that regulates fat storage. Proc Natl Acad Sci U S A 101: 15718-15723.
2. Backhed F, Manchester JK, Semenkovich CF, Gordon JI (2007) Mechanisms underlying the resistance to diet-induced obesity in germ-free mice. Proc Natl Acad Sci U S A 104: 979-984.
3. Turnbaugh PJ, Backhed F, Fulton L, Gordon JI (2008) Diet-induced obesity is linked to marked but reversible alterations in the mouse distal gut microbiome. Cell Host Microbe 3: 213-223.
4. Turnbaugh PJ, Ley RE, Mahowald MA, Magrini V, Mardis ER, et al. (2006) An obesity-associated gut microbiome with increased capacity for energy harvest. Nature 444: 1027-1031.
5. Cherbuy C, Honvo-Houeto E, Bruneau A, Bridonneau C, Mayeur C, et al. (2010) Microbiota matures colonic epithelium through a coordinated induction of cell cycle-related proteins in gnotobiotic rat. Am J Physiol Gastrointest Liver Physiol 299: G348-357.
6. Samuel BS, Shaito A, Motoike T, Rey FE, Backhed F, et al. (2008) Effects of the gut microbiota on host adiposity are modulated by the short-chain fatty-acid binding G protein-coupled receptor, Gpr41. Proc Natl Acad Sci U S A 105: 16767-16772.
7. Rabot S, Membrez M, Bruneau A, Gerard P, Harach T, et al. (2010) Germ-free C57BL/6J mice are resistant to HF-diet-induced insulin resistance and have altered cholesterol metabolism. FASEB J.
8. Fleissner CK, Huebel N, Abd El-Bary MM, Loh G, Klaus S, et al. (2010) Absence of intestinal microbiota does not protect mice from diet-induced obesity. Br J Nutr 104: 919-929.
9. Wostmann BS, Larkin C, Moriarty A, Bruckner-Kardoss E (1983) Dietary intake, energy metabolism, and excretory losses of adult male germfree Wistar rats. Lab Anim Sci 33: 46-50.

10. Lin ZJ, Zhang B, Liu XQ, Yang HL (2009) Abdominal fat accumulation with hyperuricemia and hypercholesterolemia quail model induced by high fat diet. Chin Med Sci J 24: 191-194.
11. Kahn BB, Alquier T, Carling D, Hardie DG (2005) AMP-activated protein kinase: ancient energy gauge provides clues to modern understanding of metabolism. Cell Metab 1: 15-25.
12. Bastie C, Luquet S, Holst D, Jehl-Pietri C, Grimaldi PA (2000) Alterations of peroxisome proliferator-activated receptor delta activity affect fatty acid-controlled adipose differentiation. J Biol Chem 275: 38768-38773.
13. Jernas M, Palming J, Sjoholm K, Jennische E, Svensson PA, et al. (2006) Separation of human adipocytes by size: hypertrophic fat cells display distinct gene expression. FASEB J 20: 1540-1542.
14. Weyer C, Foley JE, Bogardus C, Tataranni PA, Pratley RE (2000) Enlarged subcutaneous abdominal adipocyte size, but not obesity itself, predicts type II diabetes independent of insulin resistance. Diabetologia 43: 1498-1506.

Figure 1


A
Fat pad mass (% BW)
NORM
GF
0
2
4
6
**
**
**
Epi
Retro
Visc
Total
B
n-fold change
0
1
2
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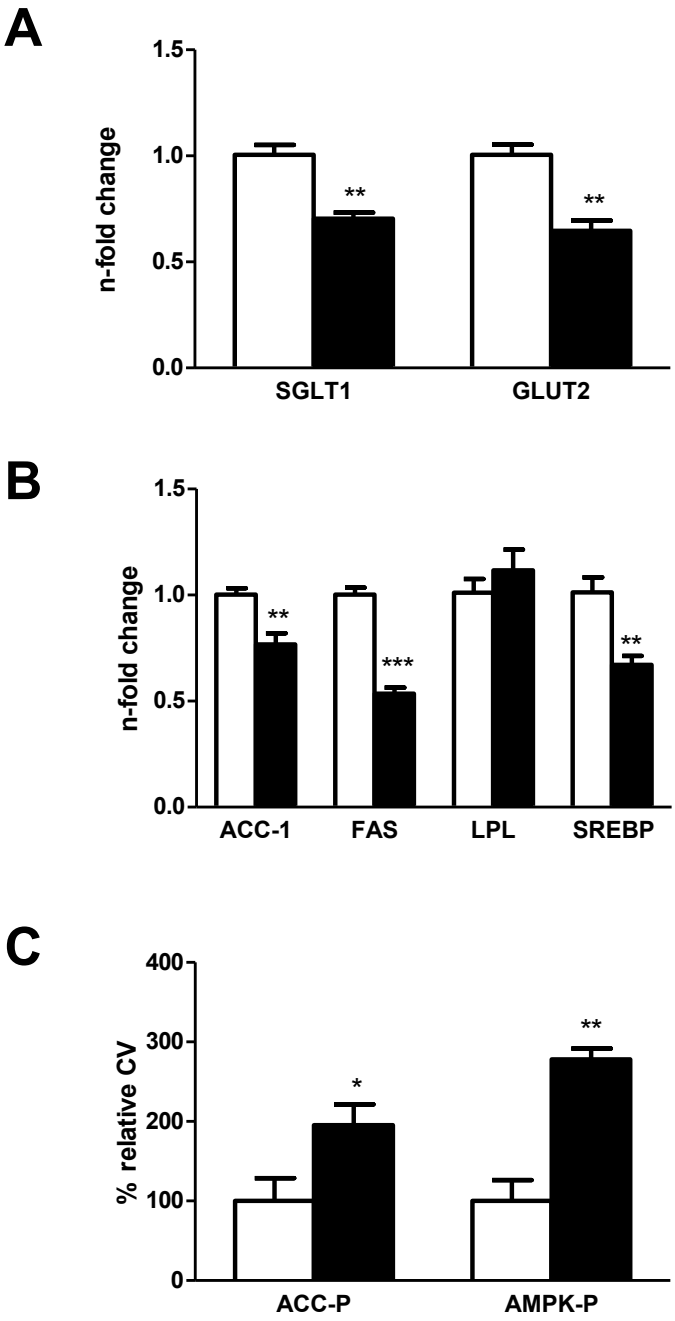
Intestine
Liver
Adipose

Figure 2


A
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1.5
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SGLT1
GLUT2
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ACC-1
FAS
LPL
SREBP
C
% relative CV
400
300
200
100
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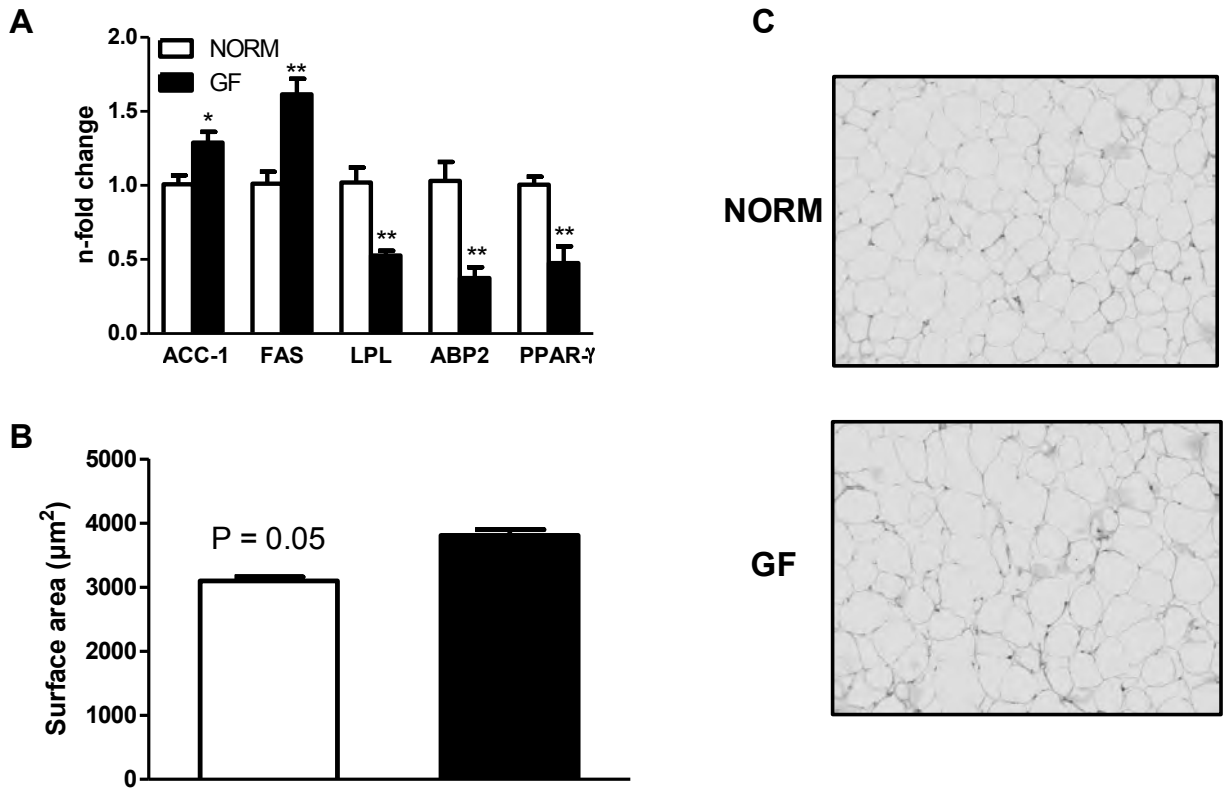
ACC-P
AMPK-P

Figure 3


A
NORM
GF
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2.0
1.5
1.0
0.5
0.0
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ACC-1
FAS
LPL
ABP2
PPAR-γ
B
Surface area (μm²)
5000
4000
3000
2000
1000
0
P = 0.05
C
NORM
GF

4.3 Specific Aim 3: Summary of results and conclusions

The final experiments from these collective sets of studies demonstrate that in the F344 rat model, the GF status is not predictive of decreased adiposity. The findings of absence of decreased adiposity in GF rats occurs despite increases in intestinal FIAF expression, increases in markers of hepatic fat lipolysis, and increased circulating biochemical markers resembling that of GF mice, which are extremely lean. While adiposity was similar between GF and NORM rats the first two groups, in a third group we examined, GF rats displayed increased adiposity that was due primarily to increased epididymal and retroperitoneal fat pad masses. Furthermore, while intestinal FIAF was up-regulated and intestinal glucose transporters were down-regulated, both of which were associated with decreased markers of hepatic lipogenesis. We also directly showed a negative energy balance in the liver of GF rats as they displayed increases in P-AMPK and P-ACC, which inhibit lipogenesis. One mechanism responsible for this hepatic energy status could be the decreased delivery of glucose to the liver as observed by the decreases in intestinal glucose transporters. Despite these findings, we found that adipocytes of GF rats had increases in lipogenesis enzymes with decreases in adipogenesis factors. Histological examination of the epididymal fat pads of GF rats supported these findings as GF rats displayed modest adipocyte hypertrophy relative to control rats. The immediate reason for the lack effects of the GF state on adiposity in this model is not clear from these findings alone as the GF model is typically described as maintaining a fasting state [21, 22], which is associated with general decreases in liver and adipocyte lipogenesis [22]. However, the increases in adipocyte lipogenesis markers in our study may signal that local factors, independent of liver

metabolism, regulate adiposity in this model. As well, fatty acid absorption may be enhanced in the adipocytes of GF rats, leading to compensatory changes in adiposity due to the lack of microbiota.

5. General summary

5.1 General results

Collectively, these experiments demonstrate the influential role of the gut microbiota on feeding behavior of specific nutrients, intestinal nutrient detection, and intestinal and liver metabolism. In *Chapter 2* of this thesis, we presented that the GF C57Bl/6J mouse, which exhibits reduced adiposity, increases consumption of highly concentrated nutritive sweet solutions, while maintaining the same affinity for nonnutritive sweet solutions as control animals. These behavioral effects were associated with similar levels of the lingual sweet taste receptor between groups, but significant up-regulation of intestinal T1R3 and the active glucose transporter, SGLT1 [515]. Together, these data implicate intestinal sugar sensing in the increased consumption of nutritive sweet solutions. Additionally, in *Chapter 3,* we demonstrate that the same GF mouse model also displays increased preference and acceptance for nutritive oil emulsions. This increase in oil preference and acceptance was associated with increased expression of lingual CD36 and decreased expression of intestinal fatty acid sensors and intestinal satiety peptides, respectively. The significance of these studies is that unlike intestinal sugar detection, which is increased in GF mice, intestinal fat detection is decreased, allowing for greater consumption of highly nutritive oil emulsions. Finally, in *Chapter 4,* we reveal that the effect of the gut microbiota on adiposity and energy balance may be species specific as GF F344 rats do not display severely reduced adiposity stores, despite similar findings of increased intestinal FIAF and inhibition of hepatic lipogenesis in this model. However, the relative modest increase in adiposity in one group of GF rats is associated with increases in local adipocyte lipogenesis enzymes and increased

adipocyte size. Together, these data demonstrate the broad effects of the absence of microbiota on oral and post-oral nutrient sensing in GF mice to increase consumption of nutritive stimuli and how the effect of microbiota has differing effects on peripheral metabolism in GF animal models (Figure 1).

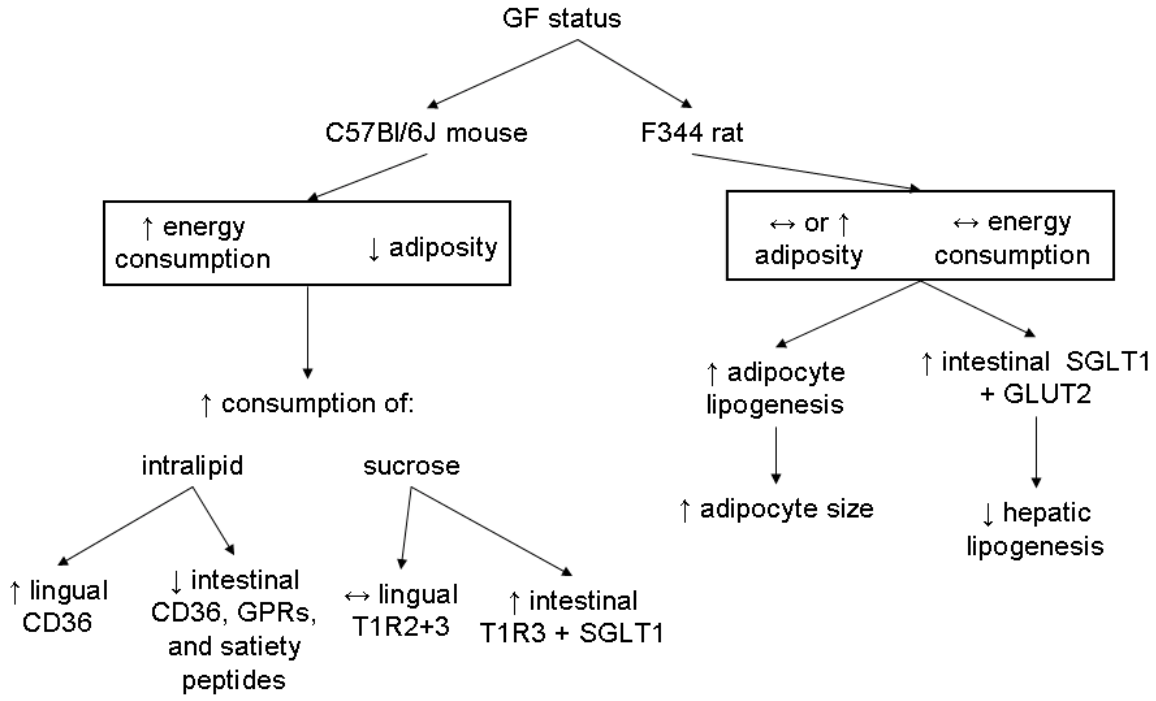

Figure 1: The effect of the GF status on oral and intestinal nutrient receptors, metabolism, and adiposity. The GF status in C57Bl/6J mice is characterized by decreased adiposity, leading to increases in consumption of fat and sugar, which is associated with alterations in intestinal fat and sugar detection. In the F344 rat, the GF status is not associated with decreased adiposity or altered energy consumption. While hepatic lipogenesis is decreased in the GF F344 rat, adipocyte lipogenesis is increased, which is associated with increased adipocyte size in the epididymal fat pad.

5.2 General discussion

Germ-free animals are a model that allow for the examination of the effects of the gut microbiota on various metabolic, physiological, and endocrine parameters. Previously, GF models have been used to examine intestinal digestive [476, 477], morphological [470, 472], and physiological [473] effects of the microbiota; however, more recently a case for the gut microbiota affecting energy balance has been made. Originally, in 2004, using C57Bl/6J mice, Backhed et al reported that the gut microbiota was a key regulator of energy balance as mice lacking microbiota were extremely lean compared to control animals that normally possess a gut microbiota and conventionalized animals [21]. Additionally these same GF mice are resistant to obesity during HF-feeding [22], an effect replicated more recently [507]. Despite the relative lack of adiposity in GF mice, these animals consume more energy, an affect attributed largely to lack of energy extraction from the diet [21]. Furthermore, due to the expression of various glycosylhydrolyases that breakdown dietary fibers and are not inherent to the mammalian genome [461], expression of distal nutrient receptors are altered in GF mice [312]. While decreased energy extraction is most likely the primary reason for the energy deficit in GF mice, the specific mechanisms in this model controlling the consumption of macronutrients that comprise a meal are poorly understood.

The detection and control of nutrient intake occurs primarily at two levels: oral and post-oral [539]. While the first involves almost exclusively the lingual epithelium [25], afferent taste pathways [118], and ultimately reward centers [540], the latter is controlled primarily by intestinal chemosensing [541] with input from mechanic and metabolic signals arising from the stomach [180] and liver [542], respectively. The

lingual contribution to at least short-term food intake is apparent by studies demonstrating that KO of various receptors localized on TRCs in the lingual epithelium, which maintain affinity for a variety of taste stimuli, results in attenuated or abolished preference for that specific taste, and decreased afferent nerve transmission following lingual application of tastants [46, 79]. For example, T1R2+3, a g-protein coupled transmembrane receptor complex is activated by sweet stimuli and KO of this receptor complex results in total abolishment of oral sweet detection and subsequent CT nerve activation in response to sweet stimuli [46]. For fat taste, KO of the fatty acid translocase CD36 [79] or GPR120 [83] also results in abolished preference for a range of nutritive and non-nutritive oil emulsions. As well, the central pathways involving taste for these palatable stimuli are strong determinants for their intake as oral stimulation with saccharin [138], sucrose [141], or oil [142] results in the release of DA from reward centers, such as the NAcc while dopamine deficient mice are hypophagic and normalization of DA levels increases food consumption [543]. Taste modalities are heavily influenced by energy balance as well. For example, genetically obese rodents display decreased oral sensitivity to both sweet [143, 144] and fat [162] stimuli. The finding of decreased lingual sensitivity to sweet tastants is most likely a direct function of impaired leptin signaling that is observed in many obese rodent models [544]. For example, the absence of leptin, the application of sweet stimuli on the lingual epithelium results in decreased CT nerve responses; however, application of leptin normalizes this response [146]. In our findings, we did not find a difference in taste preferences with saccharin or sucrose or associated changes with T1R2+3 expression in the lingual epithelium of GF mice [515]. Thus, from these findings alone, it does not appear that

sweet taste differs in this model. In respect to fat taste; however, we found that GF mice displayed increased oral sensitivity to fats, preferring a low concentration of intralipid more than NORM mice. This finding was associated with increased expression of lingual CD36. For both of sweet solutions and oil emulsions, we found differences in intake of nutritive stimuli, thus, post-oral factors involving the intestine could also influence our behavioral findings. More recently, it has been suggested that while oral factors clearly influence short-term intake, the post-oral consequences of nutrients may play a larger role in establish long-term feeding behavior [161].

Nutrients in the intestine evoke the release of signals inducing satiation, and may serve as satiation signals themselves [246]. For example, intestinal infusion of carbohydrates, fats, or protein results in reductions in short-term food intake [242, 243], which is though to require digestion and nutritive value [291], but not absorption or metabolism of nutrients [245]. As such, while glucose polymers and fatty acids induce satiation [238], inhibition of dissacharidases [249] or lipases [291] results in attenuation of this behavioral response. Furthermore, blockade of the active glucose transporter, SGLT1, does not prevent glucose-induced satiation [245], intravascular glucose infusions are not efficient in reducing food intake [428], and SCFA and MCFA that are readily absorbed do not induce satiation as effectively as LCFA, which require chylomicron formation [242]. This finding may depend upon intestinal fatty acid sensors, such as CD36 or GPRs, as CD36 is integral for proximal, but not distal, intestine fat absorption [299] and chylomicron formation [298], an area where nutrient satiation is strongest [248]. Furthermore, in our studies, we found that increased intralipid intake in GF mice was associated with decreased proximal intestine CD36, GPR40, 41, 43, and

120. Thus, GF mice may overconsume nutritive sources of fat due to decreased intestinal fat sensing. For example, CD36, the apically expressed fatty acid translocase mediates fat-induced satiation as CD36 KO mice display attenuation of intestinal fat satiation. As well, GPRs lining the intestinal tract are at least in part responsible for the secretion of intestinal satiety peptides that mediate nutrient satiation [91, 93, 312].

The mediation intestinal nutrient satiation is hypothesized to be due predominantly to the secretion of intestinal satiety signals acting on vagal afferents or via the circulation in response to nutrients. Direct support of this comes from the evidence that administration of satiety peptide receptor antagonists results in attenuation of abolishment of intestinal nutrient satiation [238]. Additionally, vagotomy via chemical or surgical means abolishes intestinal nutrient and satiety peptide-induced satiation [242, 243]. However, because feeding is still regulated in the absence of vagal synapses in the intestine, it is not the only means to govern intestinal satiation. Intestinal nutrient infusions also stimulate the release of satiety peptides in the lymph [545] and circulation [546] signaling a likely endocrine pathway for these peptides to mediate satiation. For example, vascular infusions of CCK or GLP-1 inhibit feeding in the absence of vagal signaling [200]. The ARC, which is open to the circulation and lacks a permanent blood brain barrier at the median eminence, expresses receptors for various gut peptides [547]. The direct evidence for the mediation of the forebrain in intestinal satiety signaling is that ablation of transduction pathways to this forebrain nucleus results in abolishment of satiety peptide-induced satiation [548]. As satiety peptides are released in response to a meal, both humans [549] and rodent models [550] display decreased fasting levels of intestinal and plasma satiety peptides, which

rise upon feeding. Predictably, we found that in GF mice, which exhibit a fasting state characterized by low levels of adiposity [21], both intestinal and plasma levels of satiety peptides were decreased. Our results further confirm these decreased levels of body adiposity by demonstrating the reduction of circulating leptin in GF mice. Collectively, these data demonstrate that the associated changes with decreased satiety signals and fat sensing may contribute to the increased consumption of fats displayed by GF mice.

While nutrients in the intestine indeed reduce food intake, the post-oral learning and pairing with taste also has a stimulating effect on feeding behavior [551]. Both carbohydrates [552] and fat [339] are potent nutrients in conditioning learned flavor preferences as intestinal infusions of either nutrient condition preferences of a paired non-nutritive flavor; however, carbohydrates are by far a stronger conditioning nutrient [339]. While intestinal glucose can also reinforce behavior, and conditioned flavor preferences clearly involve brain areas implicated in reward [356, 553, 554], the intestinal detection of nutrients clearly is the first step in this process [330]. Similarly to satiation, post-oral nutrient conditioning is hypothesized to require digestive [350] and pre-absorptive intestinal machinery [330] as KO of CD36 [101] does not prevent post-oral conditioning. Additionally, intravascular glucose infusions [330] and non-nutritive sweet stimuli [340] or oil emulsions [555] condition weak or no flavor preferences. The finding that GF mice display increased sucrose intake with associated up-regulation of T1R3 and SGLT1 may implicate T1R3 in establishing increased nutritive sweet acceptance in this model. As well, T1R3 KO mice, display decreased acceptance of sucrose, despite normalization of sucrose preference during multiple exposures to sucrose [42]. Despite these two somewhat convincing pieces of evidence, T1R3 KO

mice display normal glucose conditioned flavor preferences demonstrating this intestinal glucose sensor may not be responsible for the increased sucrose intake in GF mice [340]. However, similar to sweet taste, intestinal "sweet" sensing at high volumes and concentrations of sucrose, such as tested in our study, may play a pivotal role in the establishment of sucrose intake as high carbohydrate diets increase SGLT1 expression via a T1R3-dependent mechanism [265, 266]. To clarify this uncertainty, further testing of glucose conditioned flavor preferences in GF mice is sure to elucidate whether post-oral signaling is responsible for the increased intake of sucrose in this model. Finally, the derivation of T1R3 KO animals in the GF state would allow for the demonstration that T1R3 in this model is at least in part responsible for the increased intake of sucrose.

While the GF C57Bl/6J mouse model is clearly characterized by decreased adiposity [21, 22, 507], the relationship of the gut microbiota and adiposity in other mouse strains and even other rodent models is less clear [460, 506, 513]. In normal C57Bl/6J mice, microbiota populations play a significant role in energy balance as genetically obese ob/ob mice display significantly different microbiota populations from lean controls with proportional increases in firmicutes and decreases in bacteroides [457]. Furthermore, when GF animals are conventionalized with genetically obese or lean donor microbiota, animals receiving the obese donor microbiota exhibit increased adiposity relative to recipients of lean donor microbiota [508]. This finding has also been extended to mice made obese through HF feeding, and is not an effect of the diet as switching obese animals to a LF-diet and conventionalizing GF animals still results in increased adipose deposition relative to recipients of lean donor microbiota during HF-feeding [510]. Despite these multiple evidences that the microbiota clearly plays a role

in energy balance, more recent evidences in a different strain of mice and rats suggest that the absence of gut microbiota is not necessarily a predictor of decrease adiposity [506]. Using GF C3N mice fed either a LF, HF or western diet, Fleissner et al demonstrated that GF mice exhibit equal or increased adiposity levels relative to control mice during LF or HF-feeding [506]. During western diet feeding; however, GF mice maintained lower adiposity levels than control animals. While the effect in their study was attributed to the carbohydrate content of the diet, because both the LF- and HF-diet contain low dietary sugar content while the western diet contains a high level of sucrose, this explanation is in direct conflict with a previous study [507].

The major factor that is hypothesized to contribute to the decreased energy state exhibited by GF C57Bl/6J mice is the increases in intestinal FIAF [21, 22], which we confirm in our current data from *Chapter 3*. As such, KO of FIAF in GF animals results in similar adiposity as conventionalized control animals [21, 22]. Fast-induced adipocyte factor is a peripheral regulator of metabolism through direct inhibition of LPL. As such, transgenic FIAF models display increased fat metabolism and increased markers of circulating biochemical markers, such as total cholesterol, HDL, and triglycerides [505]. Similarly, GF animals from our study displayed increases in these markers as well. The hepatic metabolism of GF animals is thought to be the primary factor in shifting peripheral metabolism to lipolysis. For example, GF mice display decreases in intestinal glucose absorption, leading to a decreased hepatic glucose shunt, decreases in liver glycogen, and as a result, hepatic lipogenesis is inhibited [21]. As such, the hepatocytes of GF mice display increases in P-AMPK, which occurs during periods of fasting, and leads to the inactivation of ACC, a key rate limiting enzyme of

lipogenesis, via phosphorylation [22]. Germ-free mice also display broad decreases in hepatic lipogenic factors and increased hepatic LPL activity, demonstrating the increased lipolysis in this model [21, 22]. In both humans [556] and rodents [557], hepatic lipogenesis is associated with weight gain and obesity as well. However, in the study demonstrating GF C3N mice do not exhibit decreased adiposity, intestinal FIAF was increased in all GF animals, and circulating FIAF was non-existant with no measurements in liver metabolism [506]. This led the authors to hypothesize that intestinal FIAF, which has anti-angiogenic properties, is not a factor regulating peripheral metabolism. In our data, we clearly demonstrate that in a group of GF rats, hepatic lipogenesis markers are decreased and that the liver is in a negative energy state, perhaps due to the decreased intestinal expression of glucose transporters. The apparent increases in local lipogenic factors in adipose tissue most likely can explain the increased adipocyte size and modest increases in epididymal fat pads in one group of GF rats. As such, increased adipocyte lipogenesis and adipogenesis are associated with increases in adiposity in rodent models [558]. However, our results also demonstrate variability in the GF F344 rat as another group of GF animals exhibited similar adiposity relative to normal controls. These results demonstrate the importance of both strain and species in examining the effect of the microbiota. This evidence can also be extrapolated to humans in which unlike rats, and more like mice, obesity, rather than diet, may have a larger effect on the gut microbial populations [511].

5.3 General conclusions and perspectives

Overall, these data lay out the preliminary studies to assess the relative effect of the gut microbiota on oral and post-oral factors controlling energy balance in rodent models. Of course, in a normal environment, humans and animals co-exist with prokaryotic organisms and are not devoid of an intestinal microbiota. Other environmental factors or genetic factors, such as diet or receptor polymorphisms are well documented in affecting the gut microbiota populations and the host organism's response to microbiota secreted products. Furthermore, the effect of specific microbiota populations on these factors controlling energy balance remains elusive. For example, genetically obese rodent models display increases in consumption of palatable foods with decreases in oral sensitivity to these stimuli with decreases in intestinal nutrient satiation [143]. Whether this is an effect of the microbiota is unknown and could provide for fruitful evidence demonstrating an effect of "obese" microbiota shaping food preferences and leading to consumption of palatable foods. As well, the interaction between diet and inherent microbiota populations may provide clues to the prevalence of DIO in rodent models. Using comprehensive conventionalization studies, in which GF mice receive HF or chow-fed DIO or DR donor microbiota, and are subsequently fed chow and HF diets we could determine the effect of the microbiota, diet, and its interaction in promoting changes in nutrient sensing, satiation signaling, and the obese state.

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6.3 Abbreviations

2-DG – 2-deoxy-D-glucose

ACC-1 – acetyl-CoA decarboxylase-1

AMPK – adenosine monophosphate kinase

ARC – arcuate nucleus

ATP – adenosine triphosphate

BMI – body mass index

cAMP – cyclic adenosine monophosphate

CCK – cholecystokinin

ChREBP – carbohydrate regulatory element binding protein

CNS – central nervous system

CT – chorda tympani nerve

CTA – conditioned taste aversion

CV – conventional

DA – dopamine

Diet-induced obesity (DIO)

DR – diet-induced obese resistant

DRK – delayed rectifying potassium channels

ENS – enteric nervous system

ERK – extracellular receptor kinase

Fat mass and obesity-associated protein (FTO)

GC – gustatory cortex

GF – germ-free

GH – growth hormone

GHS-R1a – growth hormone secretagogue receptor-1a

GLP-1 – glucagon-like peptide-1

GLP-2 – glucagon-like peptide-2

GOAT – gastric O-acyl transferase

GP – glossopharyngeal nerve

GPR – g-protein coupled receptor

HF – high-fat

IGLE – intraganglionic laminar endings

IMA – intramuscular array

KO – knock-out

LCFA – long-chain fatty-acid

LepR – leptin receptor

LF – low-fat

LiCl – lithium chloride

LPS – lipopolysaccharide

MCFA – medium-chain fatty-acid

NAcc – nucleus accumbens

NORM – normal

NTS – nucleus of the solitary tract

OEA – oleoylethanolamide

OLETF – Otsuka Long-Evans Tokushima Fatty

OM – Osborne-Mendle rat

PBN – parabrachial nucleus

PLCβ2 – phospholipase C β2

PPAR-α – peroxisome proliferative factor-α

PYY – peptide YY

SCFA – short-chain fatty-acid

SGLT1 – sodium-glucose transporter 1

SREBP-1 – sterol regulatory element binding protein-1

T1R2 – taste type 1 receptor 2

T1R3 – taste type 1 receptor 3

Taste receptor cells (TRCs)

TLR4 – Toll-like receptor-4

TRPM5 – transient receptor potential melastatin-5 channel

7 References

1. Flegal, K.M., et al., *Prevalence and trends in obesity among US adults, 1999-2008.* JAMA, 2010. **303**(3): p. 235-41.
2. *Controlling the global obesity epidemic*. 2008 2012 [cited 2012 4/26/2012].
3. Finkelstein, E.A., et al., *Annual medical spending attributable to obesity: payer-and service-specific estimates.* Health Aff (Millwood), 2009. **28**(5): p. w822-31.
4. Levy, E., et al., *The economic cost of obesity: the French situation.* Int J Obes Relat Metab Disord, 1995. **19**(11): p. 788-92.
5. *WHO Global database country profiles: France*. 2007 [cited 2012 05/03/2012].
6. Halaas, J.L., et al., *Weight-reducing effects of the plasma protein encoded by the obese gene.* Science, 1995. **269**(5223): p. 543-6.
7. Farooqi, I.S., *FTO and obesity: the missing link.* Cell Metab, 2011. **13**(1): p. 7-8.
8. Meyre, D., et al., *Prevalence of loss-of-function FTO mutations in lean and obese individuals.* Diabetes, 2010. **59**(1): p. 311-8.
9. Wardle, J., et al., *Obesity associated genetic variation in FTO is associated with diminished satiety.* J Clin Endocrinol Metab, 2008. **93**(9): p. 3640-3.
10. Duca, F.A., et al., *Decreased intestinal nutrient response in diet-induced obese rats: role of gut peptides and nutrient receptors.* Int J Obes (Lond), 2012.
11. Farley, C., et al., *Meal pattern analysis of diet-induced obesity in susceptible and resistant rats.* Obes Res, 2003. **11**(7): p. 845-51.
12. Levin, B.E. and A.A. Dunn-Meynell, *Defense of body weight against chronic caloric restriction in obesity-prone and -resistant rats.* Am J Physiol Regul Integr Comp Physiol, 2000. **278**(1): p. R231-7.

13. Levin, B.E. and A.A. Dunn-Meynell, *Defense of body weight depends on dietary composition and palatability in rats with diet-induced obesity.* Am J Physiol Regul Integr Comp Physiol, 2002. **282**(1): p. R46-54.

14. Levin, B.E., A.A. Dunn-Meynell, and W.A. Banks, *Obesity-prone rats have normal blood-brain barrier transport but defective central leptin signaling before obesity onset.* Am J Physiol Regul Integr Comp Physiol, 2004. **286**(1): p. R143-50.

15. Levin, B.E., et al., *Abnormalities of leptin and ghrelin regulation in obesity-prone juvenile rats.* Am J Physiol Endocrinol Metab, 2003. **285**(5): p. E949-57.

16. Ji, H. and M.I. Friedman, *Reduced capacity for fatty acid oxidation in rats with inherited susceptibility to diet-induced obesity.* Metabolism, 2007. **56**(8): p. 1124-30.

17. Weigle, D.S. and B.E. Levin, *Defective dietary induction of uncoupling protein 3 in skeletal muscle of obesity-prone rats.* Obes Res, 2000. **8**(5): p. 385-91.

18. Nefti, W., et al., *A high-fat diet attenuates the central response to within-meal satiation signals and modifies the receptor expression of vagal afferents in mice.* Am J Physiol Regul Integr Comp Physiol, 2009. **296**(6): p. R1681-6.

19. Reidelberger, R.D., et al., *Effects of exendin-4 alone and with peptide YY(3-36) on food intake and body weight in diet-induced obese rats.* Obesity (Silver Spring), 2011. **19**(1): p. 121-7.

20. Trevaskis, J.L., et al., *Interaction of leptin and amylin in the long-term maintenance of weight loss in diet-induced obese rats.* Obesity (Silver Spring), 2010. **18**(1): p. 21-6.

21. Backhed, F., et al., *The gut microbiota as an environmental factor that regulates fat storage.* Proc Natl Acad Sci U S A, 2004. **101**(44): p. 15718-23.

22. Backhed, F., et al., *Mechanisms underlying the resistance to diet-induced obesity in germ-free mice.* Proc Natl Acad Sci U S A, 2007. **104**(3): p. 979-84.

23. Zuniga, J.R., N. Chen, and I.J. Miller, Jr., *Effects of chorda-lingual nerve injury and repair on human taste.* Chem Senses, 1994. **19**(6): p. 657-65.

24. Bailey, E.H. and E.L. Nichols, *On the Sense of Taste.* Science, 1888. **11**(268): p. 145-6.

25. Chandrashekar, J., et al., *The receptors and cells for mammalian taste.* Nature, 2006. **444**(7117): p. 288-94.

26. Chandrashekar, J., et al., *The cells and peripheral representation of sodium taste in mice.* Nature, 2010. **464**(7286): p. 297-301.

27. Huang, Y.A., et al., *Presynaptic (Type III) cells in mouse taste buds sense sour (acid) taste.* J Physiol, 2008. **586**(Pt 12): p. 2903-12.

28. Smith, D.V. and S.J. St John, *Neural coding of gustatory information.* Curr Opin Neurobiol, 1999. **9**(4): p. 427-35.

29. Sclafani, A. and M. Abrams, *Rats show only a weak preference for the artificial sweetener aspartame.* Physiol Behav, 1986. **37**(2): p. 253-6.

30. Weingarten, H.P. and S.D. Watson, *Sham feeding as a procedure for assessing the influence of diet palatability on food intake.* Physiol Behav, 1982. **28**(3): p. 401-7.

31. Geary, N. and G.P. Smith, *Pimozide decreases the positive reinforcing effect of sham fed sucrose in the rat.* Pharmacol Biochem Behav, 1985. **22**(5): p. 787-90.

32. Davis, J.D. and G.P. Smith, *The conditioned satiating effect of orosensory stimuli.* Physiol Behav, 2009. **97**(3-4): p. 293-303.

33. Sclafani, A. and J.I. Glendinning, *Sugar and fat conditioned flavor preferences in C57BL/6J and 129 mice: oral and postoral interactions.* Am J Physiol Regul Integr Comp Physiol, 2005. **289**(3): p. R712-20.

34. Sclafani, A., *Enhanced sucrose and Polycose preference in sweet "sensitive" (C57BL/6J) and "subsensitive" (129P3/J) mice after experience with these saccharides.* Physiol Behav, 2006. **87**(4): p. 745-56.

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

36. Hoon, M.A., et al., *Putative mammalian taste receptors: a class of taste-specific GPCRs with distinct topographic selectivity.* Cell, 1999. **96**(4): p. 541-51.

37. Kitagawa, M., et al., *Molecular genetic identification of a candidate receptor gene for sweet taste.* Biochem Biophys Res Commun, 2001. **283**(1): p. 236-42.

38. Li, X., *T1R receptors mediate mammalian sweet and umami taste.* Am J Clin Nutr, 2009. **90**(3): p. 733S-737S.

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

40. Kim, M.R., et al., *Regional expression patterns of taste receptors and gustducin in the mouse tongue.* Biochem Biophys Res Commun, 2003. **312**(2): p. 500-6.

41. Inoue, M., et al., *Allelic variation of the Tas1r3 taste receptor gene selectively affects taste responses to sweeteners: evidence from 129.B6-Tas1r3 congenic mice.* Physiol Genomics, 2007. **32**(1): p. 82-94.

42. Zukerman, S., et al., *T1R3 taste receptor is critical for sucrose but not Polycose taste.* Am J Physiol Regul Integr Comp Physiol, 2009. **296**(4): p. R866-76.

43. Montmayeur, J.P., et al., *A candidate taste receptor gene near a sweet taste locus.* Nat Neurosci, 2001. **4**(5): p. 492-8.

44. Pin, J.P. and F. Acher, *The metabotropic glutamate receptors: structure, activation mechanism and pharmacology.* Curr Drug Targets CNS Neurol Disord, 2002. **1**(3): p. 297-317.

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

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

47. Glendinning, J.I., et al., *Differential effects of sucrose and fructose on dietary obesity in four mouse strains.* Physiol Behav, 2010. **101**(3): p. 331-43.

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

49. Li, X., et al., *Pseudogenization of a sweet-receptor gene accounts for cats' indifference toward sugar.* PLoS Genet, 2005. **1**(1): p. 27-35.

50. Jiang, P., et al., *Molecular mechanisms of sweet receptor function.* Chem Senses, 2005. **30 Suppl 1**: p. i17-8.

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

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

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

54. Avenet, P., F. Hofmann, and B. Lindemann, *Transduction in taste receptor cells requires cAMP-dependent protein kinase.* Nature, 1988. **331**(6154): p. 351-4.

55. Avenet, P., F. Hofmann, and B. Lindemann, *Signalling in taste receptor cells: cAMP-dependent protein kinase causes depolarization by closure of 44 pS K-channels.* Comp Biochem Physiol A Comp Physiol, 1988. **90**(4): p. 681-5.

56. Cummings, T.A., C. Daniels, and S.C. Kinnamon, *Sweet taste transduction in hamster: sweeteners and cyclic nucleotides depolarize taste cells by reducing a K+ current.* J Neurophysiol, 1996. **75**(3): p. 1256-63.

57. Cummings, T.A., J. Powell, and S.C. Kinnamon, *Sweet taste transduction in hamster taste cells: evidence for the role of cyclic nucleotides.* J Neurophysiol, 1993. **70**(6): p. 2326-36.

58. Perez, C.A., et al., *A transient receptor potential channel expressed in taste receptor cells.* Nat Neurosci, 2002. **5**(11): p. 1169-76.

59. Bernhardt, S.J., et al., *Changes in IP3 and cytosolic Ca2+ in response to sugars and non-sugar sweeteners in transduction of sweet taste in the rat.* J Physiol, 1996. **490 (Pt 2)**: p. 325-36.

60. Wong, G.T., K.S. Gannon, and R.F. Margolskee, *Transduction of bitter and sweet taste by gustducin.* Nature, 1996. **381**(6585): p. 796-800.

61. Tomonari, H., et al., *Galpha-gustducin is extensively coexpressed with sweet and bitter taste receptors in both the soft palate and fungiform papillae but has a different functional significance.* Chem Senses, 2012. **37**(3): p. 241-51.

62. Ruiz-Avila, L., et al., *Dominant loss of responsiveness to sweet and bitter compounds caused by a single mutation in alpha -gustducin.* Proc Natl Acad Sci U S A, 2001. **98**(15): p. 8868-73.

63. Margolskee, R.F., *The molecular biology of taste transduction.* Bioessays, 1993. **15**(10): p. 645-50.

64. Finger, T.E., et al., *ATP signaling is crucial for communication from taste buds to gustatory nerves.* Science, 2005. **310**(5753): p. 1495-9.

65. Huang, Y.J., et al., *The role of pannexin 1 hemichannels in ATP release and cell-cell communication in mouse taste buds.* Proc Natl Acad Sci U S A, 2007. **104**(15): p. 6436-41.

66. Romanov, R.A., et al., *Voltage dependence of ATP secretion in mammalian taste cells.* J Gen Physiol, 2008. **132**(6): p. 731-44.

67. Chaudhari, N. and S.D. Roper, *The cell biology of taste.* J Cell Biol, 2010. **190**(3): p. 285-96.

68. Chale-Rush, A., J.R. Burgess, and R.D. Mattes, *Evidence for human orosensory (taste?) sensitivity to free fatty acids.* Chem Senses, 2007. **32**(5): p. 423-31.

69. Chale-Rush, A., J.R. Burgess, and R.D. Mattes, *Multiple routes of chemosensitivity to free fatty acids in humans.* Am J Physiol Gastrointest Liver Physiol, 2007. **292**(5): p. G1206-12.

70. Mattes, R.D., *Oral detection of short-, medium-, and long-chain free fatty acids in humans.* Chem Senses, 2009. **34**(2): p. 145-50.

71. Stewart, J.E., et al., *Oral sensitivity to fatty acids, food consumption and BMI in human subjects.* Br J Nutr, 2010. **104**(1): p. 145-52.

72. Hamilton, C.L., *Rat's Preference for High Fat Diets.* J Comp Physiol Psychol, 1964. **58**: p. 459-60.

73. Reed, D.R., M.G. Tordoff, and M.I. Friedman, *Enhanced acceptance and metabolism of fats by rats fed a high-fat diet.* Am J Physiol, 1991. **261**(5 Pt 2): p. R1084-8.

74. Takeda, M., M. Imaizumi, and T. Fushiki, *Preference for vegetable oils in the two-bottle choice test in mice.* Life Sci, 2000. **67**(2): p. 197-204.

75. Tsuruta, M., et al., *The orosensory recognition of long-chain fatty acids in rats.* Physiol Behav, 1999. **66**(2): p. 285-8.

76. Smith, J.C., et al., *Orosensory factors in the ingestion of corn oil/sucrose mixtures by the rat.* Physiol Behav, 2000. **69**(1-2): p. 135-46.

77. Mindell, S., G.P. Smith, and D. Greenberg, *Corn oil and mineral oil stimulate sham feeding in rats.* Physiol Behav, 1990. **48**(2): p. 283-7.

78. Reed, D.R., M.G. Tordoff, and M.I. Friedman, *Sham-feeding of corn oil by rats: sensory and postingestive factors.* Physiol Behav, 1990. **47**(4): p. 779-81.

79. Gaillard, D., et al., *The gustatory pathway is involved in CD36-mediated orosensory perception of long-chain fatty acids in the mouse.* FASEB J, 2008. **22**(5): p. 1458-68.

80. Tordoff, M.G. and D.R. Reed, *Sham-feeding sucrose or corn oil stimulates food intake in rats.* Appetite, 1991. **17**(2): p. 97-103.

81. Fukuwatari, T., et al., *Expression of the putative membrane fatty acid transporter (FAT) in taste buds of the circumvallate papillae in rats.* FEBS Lett, 1997. **414**(2): p. 461-4.

82. Laugerette, F., et al., *CD36 involvement in orosensory detection of dietary lipids, spontaneous fat preference, and digestive secretions.* J Clin Invest, 2005. **115**(11): p. 3177-84.

83. Cartoni, C., et al., *Taste preference for fatty acids is mediated by GPR40 and GPR120.* J Neurosci, 2010. **30**(25): p. 8376-82.

84. Matsumura, S., et al., *Colocalization of GPR120 with phospholipase-Cbeta2 and alpha-gustducin in the taste bud cells in mice.* Neurosci Lett, 2009. **450**(2): p. 186-90.

85. Matsumura, S., et al., *GPR expression in the rat taste bud relating to fatty acid sensing.* Biomed Res, 2007. **28**(1): p. 49-55.

86. Gilbertson, T.A., et al., *Fatty acid responses in taste cells from obesity-prone and -resistant rats.* Physiol Behav, 2005. **86**(5): p. 681-90.

87. Gilbertson, T.A. and J.D. Boughter, Jr., *Taste transduction: appetizing times in gustation.* Neuroreport, 2003. **14**(7): p. 905-11.

88. Gilbertson, T.A., et al., *Fatty acid modulation of K+ channels in taste receptor cells: gustatory cues for dietary fat.* Am J Physiol, 1997. **272**(4 Pt 1): p. C1203-10.

89. Liu, L., et al., *Expression and characterization of delayed rectifying K+ channels in anterior rat taste buds.* Am J Physiol Cell Physiol, 2005. **289**(4): p. C868-80.

90. Honore, E., et al., *External blockade of the major cardiac delayed-rectifier K+ channel (Kv1.5) by polyunsaturated fatty acids.* Proc Natl Acad Sci U S A, 1994. **91**(5): p. 1937-41.

91. Liou, A.P., et al., *The G-protein-coupled receptor GPR40 directly mediates long-chain fatty acid-induced secretion of cholecystokinin.* Gastroenterology, 2011. **140**(3): p. 903-12.

92. Tanaka, T., et al., *Free fatty acids induce cholecystokinin secretion through GPR120.* Naunyn Schmiedebergs Arch Pharmacol, 2008. **377**(4-6): p. 523-7.

93. Hirasawa, A., et al., *Free fatty acids regulate gut incretin glucagon-like peptide-1 secretion through GPR120.* Nat Med, 2005. **11**(1): p. 90-4.

94. Saitoh, O., A. Hirano, and Y. Nishimura, *Intestinal STC-1 cells respond to five basic taste stimuli.* Neuroreport, 2007. **18**(18): p. 1991-5.

95. Harmon, C.M. and N.A. Abumrad, *Binding of sulfosuccinimidyl fatty acids to adipocyte membrane proteins: isolation and amino-terminal sequence of an 88-kD protein implicated in transport of long-chain fatty acids.* J Membr Biol, 1993. **133**(1): p. 43-9.

96. Simons, P.J., et al., *Apical CD36 immunolocalization in human and porcine taste buds from circumvallate and foliate papillae.* Acta Histochem, 2011. **113**(8): p. 839-43.

97. Kawai, T. and T. Fushiki, *Importance of lipolysis in oral cavity for orosensory detection of fat.* Am J Physiol Regul Integr Comp Physiol, 2003. **285**(2): p. R447-54.

98. Rac, M.E., K. Safranow, and W. Poncyljusz, *Molecular basis of human CD36 gene mutations.* Mol Med, 2007. **13**(5-6): p. 288-96.

99. Baillie, A.G., C.T. Coburn, and N.A. Abumrad, *Reversible binding of long-chain fatty acids to purified FAT, the adipose CD36 homolog.* J Membr Biol, 1996. **153**(1): p. 75-81.

100. Ibrahimi, A., et al., *Expression of the CD36 homolog (FAT) in fibroblast cells: effects on fatty acid transport.* Proc Natl Acad Sci U S A, 1996. **93**(7): p. 2646-51.

101. Sclafani, A., K. Ackroff, and N.A. Abumrad, *CD36 gene deletion reduces fat preference and intake but not post-oral fat conditioning in mice.* Am J Physiol Regul Integr Comp Physiol, 2007. **293**(5): p. R1823-32.

102. Tepper, B.J. and K.L. Keller, *Can we taste fats?* The Scientist, 2011. **November/December 2011**.

103. Gousset, K., et al., *Important role of raft aggregation in the signaling events of cold-induced platelet activation.* Biochim Biophys Acta, 2004. **1660**(1-2): p. 7-15.

104. Liu, D. and E.R. Liman, *Intracellular Ca2+ and the phospholipid PIP2 regulate the taste transduction ion channel TRPM5.* Proc Natl Acad Sci U S A, 2003. **100**(25): p. 15160-5.

105. Sclafani, A., et al., *Fat and carbohydrate preferences in mice: the contribution of alpha-gustducin and Trpm5 taste-signaling proteins.* Am J Physiol Regul Integr Comp Physiol, 2007. **293**(4): p. R1504-13.

106. El-Yassimi, A., et al., *Linoleic acid induces calcium signaling, Src kinase phosphorylation, and neurotransmitter release in mouse CD36-positive gustatory cells.* J Biol Chem, 2008. **283**(19): p. 12949-59.

107. Huang, Y.A., Y. Maruyama, and S.D. Roper, *Norepinephrine is coreleased with serotonin in mouse taste buds.* J Neurosci, 2008. **28**(49): p. 13088-93.

108. Huang, Y.J., et al., *Mouse taste buds use serotonin as a neurotransmitter.* J Neurosci, 2005. **25**(4): p. 843-7.

109. Huang, Y.J., et al., *Mouse taste buds release serotonin in response to taste stimuli.* Chem Senses, 2005. **30 Suppl 1**: p. i39-40.

110. Oakley, B., et al., *The morphogenesis of mouse vallate gustatory epithelium and taste buds requires BDNF-dependent taste neurons.* Brain Res Dev Brain Res, 1998. **105**(1): p. 85-96.

111. Takeda, M. and K. Kitao, *Effect of monoamines on the taste buds in the mouse.* Cell Tissue Res, 1980. **210**(1): p. 71-8.

112. Takeda, M., et al., *Biogenic monoamines in developing taste buds of mouse circumvallate papillae.* Arch Histol Jpn, 1981. **44**(5): p. 485-95.

113. Herness, S. and F.L. Zhao, *The neuropeptides CCK and NPY and the changing view of cell-to-cell communication in the taste bud.* Physiol Behav, 2009. **97**(5): p. 581-91.

114. Herness, S., et al., *Expression and physiological actions of cholecystokinin in rat taste receptor cells.* J Neurosci, 2002. **22**(22): p. 10018-29.

115. Lu, S.G., F.L. Zhao, and S. Herness, *Physiological phenotyping of cholecystokinin-responsive rat taste receptor cells.* Neurosci Lett, 2003. **351**(3): p. 157-60.

116. Shen, T., et al., *Co-expression patterns of the neuropeptides vasoactive intestinal peptide and cholecystokinin with the transduction molecules alpha-gustducin and T1R2 in rat taste receptor cells.* Neuroscience, 2005. **130**(1): p. 229-38.

117. Zhao, F.L., et al., *Expression, physiological action, and coexpression patterns of neuropeptide Y in rat taste-bud cells.* Proc Natl Acad Sci U S A, 2005. **102**(31): p. 11100-5.

118. Carleton, A., R. Accolla, and S.A. Simon, *Coding in the mammalian gustatory system.* Trends Neurosci, 2010. **33**(7): p. 326-34.

119. Lemon, C.H. and D.V. Smith, *Neural representation of bitter taste in the nucleus of the solitary tract.* J Neurophysiol, 2005. **94**(6): p. 3719-29.

120. Geran, L.C. and S.P. Travers, *Single neurons in the nucleus of the solitary tract respond selectively to bitter taste stimuli.* J Neurophysiol, 2006. **96**(5): p. 2513-27.

121. Geran, L.C. and S.P. Travers, *Bitter-responsive gustatory neurons in the rat parabrachial nucleus.* J Neurophysiol, 2009. **101**(3): p. 1598-612.

122. Travers, S.P. and L.C. Geran, *Bitter-responsive brainstem neurons: characteristics and functions.* Physiol Behav, 2009. **97**(5): p. 592-603.

123. Roussin, A.T., et al., *Variability in responses and temporal coding of tastants of similar quality in the nucleus of the solitary tract of the rat.* J Neurophysiol, 2008. **99**(2): p. 644-55.

124. Monroe, S. and P.M. Di Lorenzo, *Taste responses in neurons in the nucleus of the solitary tract that do and do not project to the parabrachial pons.* J Neurophysiol, 1995. **74**(1): p. 249-57.

125. Lemon, C.H. and R.F. Margolskee, *Contribution of the T1r3 taste receptor to the response properties of central gustatory neurons.* J Neurophysiol, 2009. **101**(5): p. 2459-71.

126. Jones, L.M., et al., *Natural stimuli evoke dynamic sequences of states in sensory cortical ensembles.* Proc Natl Acad Sci U S A, 2007. **104**(47): p. 18772-7.

127. Stapleton, J.R., et al., *Ensembles of gustatory cortical neurons anticipate and discriminate between tastants in a single lick.* Front Neurosci, 2007. **1**(1): p. 161-74.

128. Stapleton, J.R., et al., *Rapid taste responses in the gustatory cortex during licking.* J Neurosci, 2006. **26**(15): p. 4126-38.

129. Grossman, S.E., et al., *Learning-related plasticity of temporal coding in simultaneously recorded amygdala-cortical ensembles.* J Neurosci, 2008. **28**(11): p. 2864-73.

130. Yasoshima, Y. and T. Yamamoto, *Short-term and long-term excitability changes of the insular cortical neurons after the acquisition of taste aversion learning in behaving rats.* Neuroscience, 1998. **84**(1): p. 1-5.

131. Accolla, R. and A. Carleton, *Internal body state influences topographical plasticity of sensory representations in the rat gustatory cortex.* Proc Natl Acad Sci U S A, 2008. **105**(10): p. 4010-5.

132. Sclafani, A. and K. Ackroff, *Reinforcement value of sucrose measured by progressive ratio operant licking in the rat.* Physiol Behav, 2003. **79**(4-5): p. 663-70.

133. Yoneda, T., et al., *Reinforcing effect for corn oil stimulus was concentration dependent in an operant task in mice.* Life Sci, 2007. **81**(23-24): p. 1585-92.

134. Norgren, R., *Taste pathways to hypothalamus and amygdala.* J Comp Neurol, 1976. **166**(1): p. 17-30.

135. Hajnal, A., R. Norgren, and P. Kovacs, *Parabrachial coding of sapid sucrose: relevance to reward and obesity.* Ann N Y Acad Sci, 2009. **1170**: p. 347-64.

136. Small, D.M., M. Jones-Gotman, and A. Dagher, *Feeding-induced dopamine release in dorsal striatum correlates with meal pleasantness ratings in healthy human volunteers.* Neuroimage, 2003. **19**(4): p. 1709-15.

137. Volkow, N.D., et al., *Brain dopamine is associated with eating behaviors in humans.* Int J Eat Disord, 2003. **33**(2): p. 136-42.

138. Mark, G.P., D.S. Blander, and B.G. Hoebel, *A conditioned stimulus decreases extracellular dopamine in the nucleus accumbens after the development of a learned taste aversion.* Brain Res, 1991. **551**(1-2): p. 308-10.

139. Grigson, P.S. and A. Hajnal, *Once is too much: conditioned changes in accumbens dopamine following a single saccharin-morphine pairing.* Behav Neurosci, 2007. **121**(6): p. 1234-42.

140. Hajnal, A. and R. Norgren, *Accumbens dopamine mechanisms in sucrose intake.* Brain Res, 2001. **904**(1): p. 76-84.

141. Hajnal, A., G.P. Smith, and R. Norgren, *Oral sucrose stimulation increases accumbens dopamine in the rat.* Am J Physiol Regul Integr Comp Physiol, 2004. **286**(1): p. R31-7.

142. Liang, N.C., A. Hajnal, and R. Norgren, *Sham feeding corn oil increases accumbens dopamine in the rat.* Am J Physiol Regul Integr Comp Physiol, 2006. **291**(5): p. R1236-9.

143. De Jonghe, B.C., A. Hajnal, and M. Covasa, *Increased oral and decreased intestinal sensitivity to sucrose in obese, prediabetic CCK-A receptor-deficient OLETF rats.* Am J Physiol Regul Integr Comp Physiol, 2005. **288**(1): p. R292-300.

144. Hajnal, A., M. Covasa, and N.T. Bello, *Altered taste sensitivity in obese, prediabetic OLETF rats lacking CCK-1 receptors.* Am J Physiol Regul Integr Comp Physiol, 2005. **289**(6): p. R1675-86.

145. Geiger, B.M., et al., *Deficits of mesolimbic dopamine neurotransmission in rat dietary obesity.* Neuroscience, 2009. **159**(4): p. 1193-9.

146. Kawai, K., et al., *Leptin as a modulator of sweet taste sensitivities in mice.* Proc Natl Acad Sci U S A, 2000. **97**(20): p. 11044-9.

147. Mook, D.G., J.A. Brane, and J.A. Whitt, *Effects of food deprivation on intake of solid and liquid sugars in the rat.* Appetite, 1983. **4**(4): p. 259-68.

148. Hajnal, A., et al., *Obese OLETF rats exhibit increased operant performance for palatable sucrose solutions and differential sensitivity to D2 receptor antagonism.* Am J Physiol Regul Integr Comp Physiol, 2007. **293**(5): p. R1846-54.

149. Hajnal, A., B.C. De Jonghe, and M. Covasa, *Dopamine D2 receptors contribute to increased avidity for sucrose in obese rats lacking CCK-1 receptors.* Neuroscience, 2007. **148**(2): p. 584-92.

150. Chen, K., et al., *Nutritional status alters saccharin intake and sweet receptor mRNA expression in rat taste buds.* Brain Res, 2010. **1325**: p. 53-62.

151. Eny, K.M., et al., *Genetic variation in TAS1R2 (Ile191Val) is associated with consumption of sugars in overweight and obese individuals in 2 distinct populations.* Am J Clin Nutr, 2010. **92**(6): p. 1501-10.

152. Pangborn, R.M. and M. Simone, *Body size and sweetness preference.* J Am Diet Assoc, 1958. **34**(9): p. 924-8.

153. Thompson, D.A., H.R. Moskowitz, and R.G. Campbell, *Effects of body weight and food intake on pleasantness ratings for a sweet stimulus.* J Appl Physiol, 1976. **41**(1): p. 77-83.

154. Underwood, P.J., E. Belton, and P. Hulme, *Aversion to sucrose in obesity.* Proc Nutr Soc, 1973. **32**(3): p. 93A-94A.

155. Wooley, O.W., S.C. Wooley, and R.B. Dunham, *Calories and sweet taste: effects on sucrose preference in the obese and nonobese.* Physiol Behav, 1972. **9**(5): p. 765-8.

156. Drewnowski, A., et al., *Sweet tooth reconsidered: taste responsiveness in human obesity.* Physiol Behav, 1985. **35**(4): p. 617-22.

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

158. Bartoshuk, L.M., et al., *Psychophysics of sweet and fat perception in obesity: problems, solutions and new perspectives.* Philos Trans R Soc Lond B Biol Sci, 2006. **361**(1471): p. 1137-48.

159. Moskowitz, H.R., *The sweetness and pleasantness of sugars.* Am J Psychol, 1971. **84**(3): p. 387-405.

160. Moskowitz, H.R., et al., *Sugar sweetness and pleasantness: evidence for different psychological laws.* Science, 1974. **184**(4136): p. 583-5.

161. Glendinning, J.I., et al., *Taste does not determine daily intake of dilute sugar solutions in mice.* Am J Physiol Regul Integr Comp Physiol, 2010. **299**(5): p. R1333-41.

162. Swartz, T.D., A. Hajnal, and M. Covasa, *Altered orosensory sensitivity to oils in CCK-1 receptor deficient rats.* Physiol Behav, 2010. **99**(1): p. 109-17.

163. Martin, C., et al., *The lipid-sensor candidates CD36 and GPR120 are differentially regulated by dietary lipids in mouse taste buds: impact on spontaneous fat preference.* PLoS One, 2011. **6**(8): p. e24014.

164. Swartz, T.D., D.M. Savastano, and M. Covasa, *Reduced sensitivity to cholecystokinin in male rats fed a high-fat diet is reversible.* J Nutr, 2010. **140**(9): p. 1698-703.

165. Pittman, D.W., et al., *Orosensory detection of fatty acids by obesity-prone and obesity-resistant rats: strain and sex differences.* Chem Senses, 2008. **33**(5): p. 449-60.

166. Gilbertson, T.A., et al., *Dietary fat preferences are inversely correlated with peripheral gustatory fatty acid sensitivity.* Ann N Y Acad Sci, 1998. **855**: p. 165-8.

167. Mela, D.J. and D.A. Sacchetti, *Sensory preferences for fats: relationships with diet and body composition.* Am J Clin Nutr, 1991. **53**(4): p. 908-15.

168. Pangborn, R.M., K.E. Bos, and J.S. Stern, *Dietary fat intake and taste responses to fat in milk by under-, normal, and overweight women.* Appetite, 1985. **6**(1): p. 25-40.

169. Keller, K.L., *Genetic influences on oral fat perception and preference: Presented at the symposium "The Taste for Fat: New Discoveries on the Role of Fat in*

Sensory Perception, Metabolism, Sensory Pleasure and Beyond" held at the Institute of Food Technologists 2011 Annual Meeting, New Orleans, LA, June 12, 2011. J Food Sci, 2012. **77**(3): p. S143-7.

170. Keller, K.L., et al., *Common Variants in the CD36 Gene Are Associated With Oral Fat Perception, Fat Preferences, and Obesity in African Americans.* Obesity (Silver Spring), 2012. **20**(5): p. 1066-73.

171. Busch, W., *Contribution to the physiology of the digestive organs.* Arch Pathol Anat Physiol Klin Med, 1858. **14**: p. 140 - 186.

172. Mook, D.G., *Oral and postingestional determinants of the intake of various solutions in rats with esophageal fistulas.* J Comp Physiol Psychol, 1963. **56**: p. 645 - 659.

173. Gibbs, J., R.C. Young, and G.P. Smith, *Cholecystokinin elicits satiety in rats with open gastric fistulas.* Nature, 1973. **245**(5424): p. 323-5.

174. Davis, J.D. and G.P. Smith, *Learning to sham feed: behavioral adjustments to loss of physiological postingestional stimuli.* Am J Physiol, 1990. **259**(6 Pt 2): p. R1228-35.

175. Eisen, S., et al., *Gastric negative feedback produced by volume and nutrient during a meal in rats.* Am J Physiol Regul Integr Comp Physiol, 2001. **281**(4): p. R1201-14.

176. Phillips, R.J. and T.L. Powley, *Gastric volume rather than nutrient content inhibits food intake.* Am J Physiol, 1996. **271**(3 Pt 2): p. R766-9.

177. Gibbs, J. and G.P. Smith, *Gut peptides and food in the gut produce similar satiety effects.* Peptides, 1982. **3**(3): p. 553-7.

178. Nonidez, J.F., *Afferent nerve endings in the ganglia of the intermuscular plexus of the dog's oesophagus.* J Comp Neurol, 1946. **85**(2): p. 177-89.

179. Rodrigo, J., et al., *Vegetative innervation of the esophagus. II. Intraganglionic laminar endings.* Acta Anat (Basel), 1975. **92**(1): p. 79-100.

180. Berthoud, H.R. and T.L. Powley, *Vagal afferent innervation of the rat fundic stomach: morphological characterization of the gastric tension receptor.* J Comp Neurol, 1992. **319**(2): p. 261-76.

181. Zagorodnyuk, V.P. and S.J. Brookes, *Transduction sites of vagal mechanoreceptors in the guinea pig esophagus.* J Neurosci, 2000. **20**(16): p. 6249-55.

182. Zagorodnyuk, V.P., B.N. Chen, and S.J. Brookes, *Intraganglionic laminar endings are mechano-transduction sites of vagal tension receptors in the guinea-pig stomach.* J Physiol, 2001. **534**(Pt 1): p. 255-68.

183. Seeley, R.J., J.M. Kaplan, and H.J. Grill, *Effect of occluding the pylorus on intraoral intake: a test of the gastric hypothesis of meal termination.* Physiol Behav, 1995. **58**(2): p. 245-9.

184. Kaplan, J.M., A.C. Spector, and H.J. Grill, *Dynamics of gastric emptying during and after stomach fill.* Am J Physiol, 1992. **263**(4 Pt 2): p. R813-9.

185. Bado, A., et al., *The stomach is a source of leptin.* Nature, 1998. **394**(6695): p. 790-3.

186. Mix, H., et al., *Expression of leptin and its receptor in the human stomach.* Gastroenterology, 1999. **117**(2): p. 509.

187. Mix, H., et al., *Expression of leptin and leptin receptor isoforms in the human stomach.* Gut, 2000. **47**(4): p. 481-6.

188. Sobhani, I., et al., *Leptin secretion and leptin receptor in the human stomach.* Gut, 2000. **47**(2): p. 178-83.

189. Guilmeau, S., et al., *Duodenal leptin stimulates cholecystokinin secretion: evidence of a positive leptin-cholecystokinin feedback loop.* Diabetes, 2003. **52**(7): p. 1664-72.

190. Attoub, S., et al., *Physiological role of cholecystokinin B/gastrin receptor in leptin secretion.* Endocrinology, 1999. **140**(10): p. 4406-10.

191. Sobhani, I., et al., *Vagal stimulation rapidly increases leptin secretion in human stomach.* Gastroenterology, 2002. **122**(2): p. 259-63.

192. Burdyga, G., et al., *Expression of the leptin receptor in rat and human nodose ganglion neurones.* Neuroscience, 2002. **109**(2): p. 339-47.

193. Buyse, M., et al., *Expression and regulation of leptin receptor proteins in afferent and efferent neurons of the vagus nerve.* Eur J Neurosci, 2001. **14**(1): p. 64-72.

194. Wang, Y.H., et al., *Two types of leptin-responsive gastric vagal afferent terminals: an in vitro single-unit study in rats.* Am J Physiol, 1997. **273**(2 Pt 2): p. R833-7.

195. Peters, J.H., et al., *Cooperative activation of cultured vagal afferent neurons by leptin and cholecystokinin.* Endocrinology, 2004. **145**(8): p. 3652-7.

196. Peters, J.H., R.C. Ritter, and S.M. Simasko, *Leptin and CCK selectively activate vagal afferent neurons innervating the stomach and duodenum.* Am J Physiol Regul Integr Comp Physiol, 2006. **290**(6): p. R1544-9.

197. Peters, J.H., R.C. Ritter, and S.M. Simasko, *Leptin and CCK modulate complementary background conductances to depolarize cultured nodose neurons.* Am J Physiol Cell Physiol, 2006. **290**(2): p. C427-32.

198. Matson, C.A., et al., *Cholecystokinin and leptin act synergistically to reduce body weight.* Am J Physiol Regul Integr Comp Physiol, 2000. **278**(4): p. R882-90.

199. Matson, C.A., D.F. Reid, and R.C. Ritter, *Daily CCK injection enhances reduction of body weight by chronic intracerebroventricular leptin infusion.* Am J Physiol Regul Integr Comp Physiol, 2002. **282**(5): p. R1368-73.

200. Zhang, J. and R.C. Ritter, *Circulating GLP-1 and CCK-8 reduce food intake by capsaicin-insensitive, nonvagal mechanisms.* Am J Physiol Regul Integr Comp Physiol, 2012. **302**(2): p. R264-73.

201. Lostao, M.P., et al., *Presence of leptin receptors in rat small intestine and leptin effect on sugar absorption.* FEBS Lett, 1998. **423**(3): p. 302-6.

202. Buyse, M., et al., *PepT1-mediated epithelial transport of dipeptides and cephalexin is enhanced by luminal leptin in the small intestine.* J Clin Invest, 2001. **108**(10): p. 1483-94.

203. Fanjul, C., et al., *Leptin regulates sugar and amino acids transport in the human intestinal cell line Caco-2.* Acta Physiol (Oxf), 2012. **205**(1): p. 82-91.

204. Sakar, Y., et al., *Positive regulatory control loop between gut leptin and intestinal GLUT2/GLUT5 transporters links to hepatic metabolic functions in rodents.* PLoS One, 2009. **4**(11): p. e7935.

205. Morton, N.M., et al., *Leptin action in intestinal cells.* J Biol Chem, 1998. **273**(40): p. 26194-201.

206. Stan, S., et al., *Effect of human recombinant leptin on lipid handling by fully differentiated Caco-2 cells.* FEBS Lett, 2001. **508**(1): p. 80-4.

207. Date, Y., et al., *Ghrelin, a novel growth hormone-releasing acylated peptide, is synthesized in a distinct endocrine cell type in the gastrointestinal tracts of rats and humans.* Endocrinology, 2000. **141**(11): p. 4255-61.

208. Kojima, M., et al., *Ghrelin is a growth-hormone-releasing acylated peptide from stomach.* Nature, 1999. **402**(6762): p. 656-60.

209. Wren, A.M., et al., *The novel hypothalamic peptide ghrelin stimulates food intake and growth hormone secretion.* Endocrinology, 2000. **141**(11): p. 4325-8.

210. Lu, S., et al., *Immunocytochemical observation of ghrelin-containing neurons in the rat arcuate nucleus.* Neurosci Lett, 2002. **321**(3): p. 157-60.

211. Hosoda, H., et al., *Ghrelin and des-acyl ghrelin: two major forms of rat ghrelin peptide in gastrointestinal tissue.* Biochem Biophys Res Commun, 2000. **279**(3): p. 909-13.

212. Jeon, T.Y., et al., *Changes in plasma ghrelin concentration immediately after gastrectomy in patients with early gastric cancer.* J Clin Endocrinol Metab, 2004. **89**(11): p. 5392-6.

213. Yabuki, A., et al., *Characterization and species differences in gastric ghrelin cells from mice, rats and hamsters.* J Anat, 2004. **205**(3): p. 239-46.

214. van der Lely, A.J., et al., *Biological, physiological, pathophysiological, and pharmacological aspects of ghrelin.* Endocr Rev, 2004. **25**(3): p. 426-57.

215. Hosoda, H., et al., *Structural divergence of human ghrelin. Identification of multiple ghrelin-derived molecules produced by post-translational processing.* J Biol Chem, 2003. **278**(1): p. 64-70.

216. Yang, J., et al., *Identification of the acyltransferase that octanoylates ghrelin, an appetite-stimulating peptide hormone.* Cell, 2008. **132**(3): p. 387-96.

217. Yokota, I., et al., *Concentration of the n-octanoylated active form of ghrelin in fetal and neonatal circulation.* Endocr J, 2005. **52**(2): p. 271-6.

218. Tschop, M., D.L. Smiley, and M.L. Heiman, *Ghrelin induces adiposity in rodents.* Nature, 2000. **407**(6806): p. 908-13.

219. Wren, A.M., et al., *Ghrelin enhances appetite and increases food intake in humans.* J Clin Endocrinol Metab, 2001. **86**(12): p. 5992.

220. Wren, A.M., et al., *Ghrelin causes hyperphagia and obesity in rats.* Diabetes, 2001. **50**(11): p. 2540-7.

221. Nakazato, M., et al., *A role for ghrelin in the central regulation of feeding.* Nature, 2001. **409**(6817): p. 194-8.

222. Asakawa, A., et al., *Antagonism of ghrelin receptor reduces food intake and body weight gain in mice.* Gut, 2003. **52**(7): p. 947-52.

223. Druce, M.R., et al., *Ghrelin increases food intake in obese as well as lean subjects.* Int J Obes (Lond), 2005. **29**(9): p. 1130-6.

224. Wolden-Hanson, T., *Mechanisms of the anorexia of aging in the Brown Norway rat.* Physiol Behav, 2006. **88**(3): p. 267-76.

225. Ariyasu, H., et al., *Stomach is a major source of circulating ghrelin, and feeding state determines plasma ghrelin-like immunoreactivity levels in humans.* J Clin Endocrinol Metab, 2001. **86**(10): p. 4753-8.

226. Tups, A., et al., *Circulating ghrelin levels and central ghrelin receptor expression are elevated in response to food deprivation in a seasonal mammal (Phodopus sungorus).* J Neuroendocrinol, 2004. **16**(11): p. 922-8.

227. Drazen, D.L., et al., *Effects of a fixed meal pattern on ghrelin secretion: evidence for a learned response independent of nutrient status.* Endocrinology, 2006. **147**(1): p. 23-30.

228. Cummings, D.E., et al., *A preprandial rise in plasma ghrelin levels suggests a role in meal initiation in humans.* Diabetes, 2001. **50**(8): p. 1714-9.

229. Otto, B., et al., *Weight gain decreases elevated plasma ghrelin concentrations of patients with anorexia nervosa.* Eur J Endocrinol, 2001. **145**(5): p. 669-73.

230. Cummings, D.E., et al., *Plasma ghrelin levels after diet-induced weight loss or gastric bypass surgery.* N Engl J Med, 2002. **346**(21): p. 1623-30.

231. Foster-Schubert, K.E., et al., *Acyl and total ghrelin are suppressed strongly by ingested proteins, weakly by lipids, and biphasically by carbohydrates.* J Clin Endocrinol Metab, 2008. **93**(5): p. 1971-9.

232. Williams, D.L., et al., *Meal-related ghrelin suppression requires postgastric feedback.* Endocrinology, 2003. **144**(7): p. 2765-7.

233. Feinle-Bisset, C., et al., *Fat digestion is required for suppression of ghrelin and stimulation of peptide YY and pancreatic polypeptide secretion by intraduodenal lipid.* Am J Physiol Endocrinol Metab, 2005. **289**(6): p. E948-53.

234. Chapman, I.M., et al., *Effects of small-intestinal fat and carbohydrate infusions on appetite and food intake in obese and nonobese men.* Am J Clin Nutr, 1999. **69**(1): p. 6-12.

235. Gibbs, J., S.P. Maddison, and E.T. Rolls, *Satiety role of the small intestine examined in sham-feeding rhesus monkeys.* J Comp Physiol Psychol, 1981. **95**(6): p. 1003-15.

236. Matzinger, D., et al., *Inhibition of food intake in response to intestinal lipid is mediated by cholecystokinin in humans.* Am J Physiol, 1999. **277**(6 Pt 2): p. R1718-24.

237. Reidelberger, R.D., et al., *Postgastric satiety in the sham-feeding rat.* Am J Physiol, 1983. **244**(6): p. R872-81.

238. Yox, D.P., L. Brenner, and R.C. Ritter, *CCK-receptor antagonists attenuate suppression of sham feeding by intestinal nutrients.* Am J Physiol, 1992. **262**(4 Pt 2): p. R554-61.

239. Garnier, L., N. Mei, and J. Melone, *Further data on the inhibitory enterogastric reflex triggered by intestinal osmotic changes in cats.* J Auton Nerv Syst, 1986. **16**(3): p. 171-80.

240. Falasco, J.D., G.P. Smith, and J. Gibbs, *Cholecystokinin suppresses sham feeding in the rhesus monkey.* Physiol Behav, 1979. **23**(5): p. 887-90.

241. Houpt, T.R., K.A. Houpt, and A.A. Swan, *Duodenal osmoconcentration and food intake in pigs after ingestion of hypertonic nutrients.* Am J Physiol, 1983. **245**(2): p. R181-9.

242. Yox, D.P. and R.C. Ritter, *Capsaicin attenuates suppression of sham feeding induced by intestinal nutrients.* Am J Physiol, 1988. **255**(4 Pt 2): p. R569-74.

243. Yox, D.P., H. Stokesberry, and R.C. Ritter, *Vagotomy attenuates suppression of sham feeding induced by intestinal nutrients.* Am J Physiol, 1991. **260**(3 Pt 2): p. R503-8.

244. Yin, T.H. and C.T. Tsai, *Effects of glucose on feeding in relation to routes of entry in rats.* J Comp Physiol Psychol, 1973. **85**(2): p. 258-64.

245. Ritter, R.C., Brenner, L., Yox, D.P., *Participation of vagal sensory neurons in putative satiety signals from the upper gastrointestinal tract*, in *Neuroanatomy and physiology of abdominal vagal afferents*. 1992, CRC Press: Ann Arbor. p. 221 - 248.

246. Ritter, R.C., *Gastrointestinal mechanisms of satiation for food.* Physiol Behav, 2004. **81**(2): p. 249-73.

247. Walls, E.K., et al., *Suppression of meal size by intestinal nutrients is eliminated by celiac vagal deafferentation.* Am J Physiol, 1995. **269**(6 Pt 2): p. R1410-9.

248. Meyer, J.H., et al., *Length of intestinal contact on nutrient-driven satiety.* Am J Physiol, 1998. **275**(4 Pt 2): p. R1308-19.

249. Ritter, R.C., T. Bui, and M. Covasa, *Digestion and absorption are not required for satiation by intestinal maltotriose*, A.-S.f. Neuroscience, Editor. 2001.

250. Shirazi-Beechey, S.P., *Molecular biology of intestinal glucose transport.* Nutr Res Rev, 1995. **8**(1): p. 27-41.

251. Yoshikawa, T., et al., *Comparative expression of hexose transporters (SGLT1, GLUT1, GLUT2 and GLUT5) throughout the mouse gastrointestinal tract.* Histochem Cell Biol, 2011. **135**(2): p. 183-94.

252. Affleck, J.A., P.A. Helliwell, and G.L. Kellett, *Immunocytochemical detection of GLUT2 at the rat intestinal brush-border membrane.* J Histochem Cytochem, 2003. **51**(11): p. 1567-74.

253. Kellett, G.L. and E. Brot-Laroche, *Apical GLUT2: a major pathway of intestinal sugar absorption.* Diabetes, 2005. **54**(10): p. 3056-62.

254. Kellett, G.L. and P.A. Helliwell, *The diffusive component of intestinal glucose absorption is mediated by the glucose-induced recruitment of GLUT2 to the brush-border membrane.* Biochem J, 2000. **350 Pt 1**: p. 155-62.

255. Stumpel, F., et al., *Normal kinetics of intestinal glucose absorption in the absence of GLUT2: evidence for a transport pathway requiring glucose phosphorylation and transfer into the endoplasmic reticulum.* Proc Natl Acad Sci U S A, 2001. **98**(20): p. 11330-5.

256. Shirazi-Beechey, S.P., et al., *Glucose sensing and signalling; regulation of intestinal glucose transport.* Proc Nutr Soc, 2011. **70**(2): p. 185-93.

257. Dyer, J., K.B. Hosie, and S.P. Shirazi-Beechey, *Nutrient regulation of human intestinal sugar transporter (SGLT1) expression.* Gut, 1997. **41**(1): p. 56-9.

258. Ferraris, R.P. and J.M. Diamond, *Crypt/villus site of substrate-dependent regulation of mouse intestinal glucose transporters.* Proc Natl Acad Sci U S A, 1993. **90**(12): p. 5868-72.

259. Shirazi-Beechey, S.P., et al., *Ontogenic development of lamb intestinal sodium-glucose co-transporter is regulated by diet.* J Physiol, 1991. **437**: p. 699-708.

260. Solberg, D.H. and J.M. Diamond, *Comparison of different dietary sugars as inducers of intestinal sugar transporters.* Am J Physiol, 1987. **252**(4 Pt 1): p. G574-84.

261. Shirazi-Beechey, S.P., et al., *Postnatal development of lamb intestinal digestive enzymes is not regulated by diet.* J Physiol, 1991. **437**: p. 691-8.

262. Stearns, A.T., et al., *Rapid upregulation of sodium-glucose transporter SGLT1 in response to intestinal sweet taste stimulation.* Ann Surg, 2010. **251**(5): p. 865-71.

263. Dyer, J., et al., *Glucose sensing in the intestinal epithelium.* Eur J Biochem, 2003. **270**(16): p. 3377-88.

264. Batchelor, D.J., et al., *Sodium/glucose cotransporter-1, sweet receptor, and disaccharidase expression in the intestine of the domestic dog and cat: two species of different dietary habit.* Am J Physiol Regul Integr Comp Physiol, 2011. **300**(1): p. R67-75.

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

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

267. Hofer, D., E. Asan, and D. Drenckhahn, *Chemosensory Perception in the Gut.* News Physiol Sci, 1999. **14**: p. 18-23.

268. Wu, S.V., et al., *Expression of bitter taste receptors of the T2R family in the gastrointestinal tract and enteroendocrine STC-1 cells.* Proc Natl Acad Sci U S A, 2002. **99**(4): p. 2392-7.

269. Dyer, J., et al., *Expression of sweet taste receptors of the T1R family in the intestinal tract and enteroendocrine cells.* Biochem Soc Trans, 2005. **33**(Pt 1): p. 302-5.

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

271. Shi, P. and J. Zhang, *Contrasting modes of evolution between vertebrate sweet/umami receptor genes and bitter receptor genes.* Mol Biol Evol, 2006. **23**(2): p. 292-300.

272. Barfull, A., et al., *Ontogenetic expression and regulation of Na(+)-D-glucose cotransporter in jejunum of domestic chicken.* Am J Physiol Gastrointest Liver Physiol, 2002. **282**(3): p. G559-64.

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

274. Bezencon, C., J. le Coutre, and S. Damak, *Taste-signaling proteins are coexpressed in solitary intestinal epithelial cells.* Chem Senses, 2007. **32**(1): p. 41-9.

275. Ford, H.E., et al., *Effects of oral ingestion of sucralose on gut hormone response and appetite in healthy normal-weight subjects.* Eur J Clin Nutr, 2011. **65**(4): p. 508-13.

276. Reimann, F., et al., *Glucose sensing in L cells: a primary cell study.* Cell Metab, 2008. **8**(6): p. 532-9.

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

278. Cheeseman, C.I., *Upregulation of SGLT-1 transport activity in rat jejunum induced by GLP-2 infusion in vivo.* Am J Physiol, 1997. **273**(6 Pt 2): p. R1965-71.

279. Cottrell, J.J., et al., *Glucagon-like peptide-2 protects against TPN-induced intestinal hexose malabsorption in enterally refed piglets.* Am J Physiol Gastrointest Liver Physiol, 2006. **290**(2): p. G293-300.

280. Ramsanahie, A., et al., *Effect of GLP-2 on mucosal morphology and SGLT1 expression in tissue-engineered neointestine.* Am J Physiol Gastrointest Liver Physiol, 2003. **285**(6): p. G1345-52.

281. Sangild, P.T., et al., *Glucagon-like peptide 2 stimulates intestinal nutrient absorption in parenterally fed newborn pigs.* J Pediatr Gastroenterol Nutr, 2006. **43**(2): p. 160-7.

282. Baldassano, S., et al., *Glucagon-like peptide-2 modulates neurally evoked mucosal chloride secretion in guinea pig small intestine in vitro.* Am J Physiol Gastrointest Liver Physiol, 2009. **297**(4): p. G800-5.

283. Bjerknes, M. and H. Cheng, *Modulation of specific intestinal epithelial progenitors by enteric neurons.* Proc Natl Acad Sci U S A, 2001. **98**(22): p. 12497-502.

284. Debnam, E.S., *Adaptation of hexose uptake by the rat jejunum induced by the perfusion of sugars into the distal ileum.* Digestion, 1985. **31**(1): p. 25-30.

285. Moreto, M., et al., *Involvement of cellular cyclic AMP in theophylline-induced sugar accumulation in chicken intestinal epithelial cells.* Biochim Biophys Acta, 1984. **771**(1): p. 68-73.

286. Nath, S.K. and J.F. Desjeux, *Human intestinal cell lines as in vitro tools for electrolyte transport studies with relevance to secretory diarrhoea.* J Diarrhoeal Dis Res, 1990. **8**(4): p. 133-42.

287. Sharp, P.A. and E.S. Debnam, *The role of cyclic AMP in the control of sugar transport across the brush-border and basolateral membranes of rat jejunal enterocytes.* Exp Physiol, 1994. **79**(2): p. 203-14.

288. Amsler, K., S. Ghatani, and B.A. Hemmings, *cAMP-dependent protein kinase regulates renal epithelial cell properties.* Am J Physiol, 1991. **260**(6 Pt 1): p. C1290-9.

289. Williams, M. and P. Sharp, *Regulation of jejunal glucose transporter expression by forskolin.* Biochim Biophys Acta, 2002. **1559**(2): p. 179-85.

290. Greenberg, D., G.P. Smith, and J. Gibbs, *Oleic acid inhibits sham feeding when duodenally infused while triolein does not*, A.S.f. Neuroscience, Editor. 1988.

291. Meyer, J.H., et al., *Chemical specificities and intestinal distributions of nutrient-driven satiety.* Am J Physiol, 1998. **275**(4 Pt 2): p. R1293-307.

292. Phan, C.T. and P. Tso, *Intestinal lipid absorption and transport.* Front Biosci, 2001. **6**: p. D299-319.

293. Tso, P. and K. Fujimoto, *The absorption and transport of lipids by the small intestine.* Brain Res Bull, 1991. **27**(3-4): p. 477-82.

294. Tso, P. and S.R. Gollamudi, *Pluronic L-81: a potent inhibitor of the transport of intestinal chylomicrons.* Am J Physiol, 1984. **247**(1 Pt 1): p. G32-6.

295. Sakata, Y., et al., *Postabsorptive factors are important for satiation in rats after a lipid meal.* Am J Physiol, 1996. **271**(3 Pt 1): p. G438-42.

296. Lobo, M.V., et al., *Localization of the lipid receptors CD36 and CLA-1/SR-BI in the human gastrointestinal tract: towards the identification of receptors mediating the intestinal absorption of dietary lipids.* J Histochem Cytochem, 2001. **49**(10): p. 1253-60.

297. Drover, V.A., et al., *CD36 mediates both cellular uptake of very long chain fatty acids and their intestinal absorption in mice.* J Biol Chem, 2008. **283**(19): p. 13108-15.

298. Nauli, A.M., et al., *CD36 is important for chylomicron formation and secretion and may mediate cholesterol uptake in the proximal intestine.* Gastroenterology, 2006. **131**(4): p. 1197-207.

299. Nassir, F., et al., *CD36 is important for fatty acid and cholesterol uptake by the proximal but not distal intestine.* J Biol Chem, 2007. **282**(27): p. 19493-501.

300. Goudriaan, J.R., et al., *CD36 deficiency in mice impairs lipoprotein lipase-mediated triglyceride clearance.* J Lipid Res, 2005. **46**(10): p. 2175-81.

301. Petit, V., et al., *Chronic high-fat diet affects intestinal fat absorption and postprandial triglyceride levels in the mouse.* J Lipid Res, 2007. **48**(2): p. 278-87.

302. Le Foll, C., et al., *Characteristics and mechanisms of hypothalamic neuronal fatty acid sensing.* Am J Physiol Regul Integr Comp Physiol, 2009. **297**(3): p. R655-64.

303. Schwartz, G.J., et al., *The lipid messenger OEA links dietary fat intake to satiety.* Cell Metab, 2008. **8**(4): p. 281-8.

304. Fu, J., et al., *Targeted enhancement of oleoylethanolamide production in proximal small intestine induces across-meal satiety in rats.* Am J Physiol Regul Integr Comp Physiol, 2008. **295**(1): p. R45-50.

305. Schwartz, G.J., *Gut fat sensing in the negative feedback control of energy balance--recent advances.* Physiol Behav, 2011. **104**(4): p. 621-3.

306. Rodriguez de Fonseca, F., et al., *An anorexic lipid mediator regulated by feeding.* Nature, 2001. **414**(6860): p. 209-12.

307. Briscoe, C.P., et al., *The orphan G protein-coupled receptor GPR40 is activated by medium and long chain fatty acids.* J Biol Chem, 2003. **278**(13): p. 11303-11.

308. Lan, H., et al., *Lack of FFAR1/GPR40 does not protect mice from high-fat diet-induced metabolic disease.* Diabetes, 2008. **57**(11): p. 2999-3006.

309. Brown, A.J., et al., *The Orphan G protein-coupled receptors GPR41 and GPR43 are activated by propionate and other short chain carboxylic acids.* J Biol Chem, 2003. **278**(13): p. 11312-9.

310. Tazoe, H., et al., *Expression of short-chain fatty acid receptor GPR41 in the human colon.* Biomed Res, 2009. **30**(3): p. 149-56.

311. Dass, N.B., et al., *The relationship between the effects of short-chain fatty acids on intestinal motility in vitro and GPR43 receptor activation.* Neurogastroenterol Motil, 2007. **19**(1): p. 66-74.

312. Samuel, B.S., et al., *Effects of the gut microbiota on host adiposity are modulated by the short-chain fatty-acid binding G protein-coupled receptor, Gpr41.* Proc Natl Acad Sci U S A, 2008. **105**(43): p. 16767-72.

313. Karaki, S., et al., *Short-chain fatty acid receptor, GPR43, is expressed by enteroendocrine cells and mucosal mast cells in rat intestine.* Cell Tissue Res, 2006. **324**(3): p. 353-60.

314. Ichimura, A., et al., *Dysfunction of lipid sensor GPR120 leads to obesity in both mouse and human.* Nature, 2012. **483**(7389): p. 350-4.

315. Cornall, L.M., et al., *Diet-induced obesity up-regulates the abundance of GPR43 and GPR120 in a tissue specific manner.* Cell Physiol Biochem, 2011. **28**(5): p. 949-58.

316. Rayasam, G.V., et al., *Fatty acid receptors as new therapeutic targets for diabetes.* Expert Opin Ther Targets, 2007. **11**(5): p. 661-71.

317. Oh, D.Y., et al., *GPR120 is an omega-3 fatty acid receptor mediating potent anti-inflammatory and insulin-sensitizing effects.* Cell, 2010. **142**(5): p. 687-98.

318. Hara, T., et al., *Novel selective ligands for free fatty acid receptors GPR120 and GPR40.* Naunyn Schmiedebergs Arch Pharmacol, 2009. **380**(3): p. 247-55.

319. Katsuma, S., et al., *Free fatty acids inhibit serum deprivation-induced apoptosis through GPR120 in a murine enteroendocrine cell line STC-1.* J Biol Chem, 2005. **280**(20): p. 19507-15.

320. Sclafani, A., L.J. Fanizza, and A.V. Azzara, *Conditioned flavor avoidance, preference, and indifference produced by intragastric infusions of galactose, glucose, and fructose in rats.* Physiol Behav, 1999. **67**(2): p. 227-34.

321. Ackroff, K. and A. Sclafani, *Conditioned flavor preferences: evaluating postingestive reinforcement by nutrients.* Curr Protoc Neurosci, 2001. **Chapter 8**: p. Unit 8 6F.

322. Ackroff, K., D.B. Drucker, and A. Sclafani, *The CS-US delay gradient in flavor preference conditioning with intragastric carbohydrate infusions.* Physiol Behav, 2012. **105**(2): p. 168-74.

323. Drucker, D.B., K. Ackroff, and A. Sclafani, *Nutrient-conditioned flavor preference and acceptance in rats: effects of deprivation state and nonreinforcement.* Physiol Behav, 1994. **56**(4): p. 701-7.

324. Elizalde, G. and A. Sclafani, *Flavor preferences conditioned by intragastric polycose infusions: a detailed analysis using an electronic esophagus preparation.* Physiol Behav, 1990. **47**(1): p. 63-77.

325. Myers, K.P., *Robust preference for a flavor paired with intragastric glucose acquired in a single trial.* Appetite, 2007. **48**(1): p. 123-7.

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

327. Ramirez, I., *Stimulus specificity in flavor acceptance learning.* Physiol Behav, 1996. **60**(2): p. 595-610.

328. Ramirez, I., *Intragastric carbohydrate exerts both intake-stimulating and intake-suppressing effects.* Behav Neurosci, 1997. **111**(3): p. 612-22.

329. Drucker, D.B. and A. Sclafani, *The role of gastric and postgastric sites in glucose-conditioned flavor preferences in rats.* Physiol Behav, 1997. **61**(2): p. 351-8.

330. Ackroff, K., Y.M. Yiin, and A. Sclafani, *Post-oral infusion sites that support glucose-conditioned flavor preferences in rats.* Physiol Behav, 2010. **99**(3): p. 402-11.

331. Gowans, S.E. and H.P. Weingarten, *Elevations of plasma glucose do not support taste-to-postingestive consequence learning.* Am J Physiol, 1991. **261**(6 Pt 2): p. R1409-17.

332. Oliveira-Maia, A.J., et al., *Intravascular food reward.* PLoS One, 2011. **6**(9): p. e24992.

333. Ackroff, K., et al., *Rapid acquisition of conditioned flavor preferences in rats.* Physiol Behav, 2009. **97**(3-4): p. 406-13.

334. Ackroff, K. and A. Sclafani, *Fructose-conditioned flavor preferences in male and female rats: effects of sweet taste and sugar concentration.* Appetite, 2004. **42**(3): p. 287-97.

335. Ackroff, K., A. Sclafani, and K.V. Axen, *Diabetic rats prefer glucose-paired flavors over fructose-paired flavors.* Appetite, 1997. **28**(1): p. 73-83.

336. Ackroff, K., et al., *Flavor preferences conditioned by intragastric fructose and glucose: differences in reinforcement potency.* Physiol Behav, 2001. **72**(5): p. 691-703.

337. Sclafani, A. and D.L. Williams, *Galactose consumption induces conditioned flavor avoidance in rats.* J Nutr, 1999. **129**(9): p. 1737-41.

338. Sclafani, A. and K. Ackroff, *Flavor preferences conditioned by intragastric glucose but not fructose or galactose in C57BL/6J mice.* Physiol Behav, 2012.

339. Lucas, F. and A. Sclafani, *Differential reinforcing and satiating effects of intragastric fat and carbohydrate infusions in rats.* Physiol Behav, 1999. **66**(3): p. 381-8.

340. Sclafani, A., et al., *Gut T1R3 sweet taste receptors do not mediate sucrose-conditioned flavor preferences in mice.* Am J Physiol Regul Integr Comp Physiol, 2010. **299**(6): p. R1643-50.

341. Wright, E.M., D.D. Loo, and B.A. Hirayama, *Biology of human sodium glucose transporters.* Physiol Rev, 2011. **91**(2): p. 733-94.

342. Sclafani, A., K. Ackroff, and G.J. Schwartz, *Selective effects of vagal deafferentation and celiac-superior mesenteric ganglionectomy on the reinforcing and satiating action of intestinal nutrients.* Physiol Behav, 2003. **78**(2): p. 285-94.

343. Sclafani, A. and F. Lucas, *Abdominal vagotomy does not block carbohydrate-conditioned flavor preferences in rats.* Physiol Behav, 1996. **60**(2): p. 447-53.

344. Lucas, F. and A. Sclafani, *Capsaicin attenuates feeding suppression but not reinforcement by intestinal nutrients.* Am J Physiol, 1996. **270**(5 Pt 2): p. R1059-64.

345. Perez, C., F. Lucas, and A. Sclafani, *Devazepide, a CCK(A) antagonist, attenuates the satiating but not the preference conditioning effects of intestinal carbohydrate infusions in rats.* Pharmacol Biochem Behav, 1998. **59**(2): p. 451-7.

346. Perez, C. and A. Sclafani, *Cholecystokinin conditions flavor preferences in rats.* Am J Physiol, 1991. **260**(1 Pt 2): p. R179-85.

347. Ackroff, K. and A. Sclafani, *Oral and Postoral Determinants of Dietary Fat Appetite.* 2010.

348. Lucas, F. and A. Sclafani, *Flavor preferences conditioned by intragastric fat infusions in rats.* Physiol Behav, 1989. **46**(3): p. 403-12.

349. Ackroff, K., F. Lucas, and A. Sclafani, *Flavor preference conditioning as a function of fat source.* Physiol Behav, 2005. **85**(4): p. 448-60.

350. Ackroff, K. and A. Sclafani, *Effects of the lipase inhibitor orlistat on intake and preference for dietary fat in rats.* Am J Physiol, 1996. **271**(1 Pt 2): p. R48-54.

351. Lucas, F., K. Ackroff, and A. Sclafani, *High-fat diet preference and overeating mediated by postingestive factors in rats.* Am J Physiol, 1998. **275**(5 Pt 2): p. R1511-22.

352. Suzuki, A., T. Yamane, and T. Fushiki, *Inhibition of fatty acid beta-oxidation attenuates the reinforcing effects and palatability to fat.* Nutrition, 2006. **22**(4): p. 401-7.

353. Ren, X., et al., *Nutrient selection in the absence of taste receptor signaling.* J Neurosci, 2010. **30**(23): p. 8012-23.

354. Otsubo, H., et al., *Induction of Fos expression in the rat forebrain after intragastric administration of monosodium L-glutamate, glucose and NaCl.* Neuroscience, 2011. **196**: p. 97-103.

355. Tsurugizawa, T., et al., *Mechanisms of neural response to gastrointestinal nutritive stimuli: the gut-brain axis.* Gastroenterology, 2009. **137**(1): p. 262-73.

356. Touzani, K., R. Bodnar, and A. Sclafani, *Activation of dopamine D1-like receptors in nucleus accumbens is critical for the acquisition, but not the expression, of*

nutrient-conditioned flavor preferences in rats. Eur J Neurosci, 2008. **27**(6): p. 1525-33.

357. Sclafani, A., et al., *Parabrachial nucleus lesions block taste and attenuate flavor preference and aversion conditioning in rats.* Behav Neurosci, 2001. **115**(4): p. 920-33.

358. Tsurugizawa, T., et al., *Blood oxygenation level-dependent response to intragastric load of corn oil emulsion in conscious rats.* Neuroreport, 2009. **20**(18): p. 1625-9.

359. Dela Cruz, J.A., et al., *Roles of dopamine D1 and D2 receptors in the acquisition and expression of fat-conditioned flavor preferences in rats.* Neurobiol Learn Mem, 2012. **97**(3): p. 332-7.

360. Imaizumi, M., M. Takeda, and T. Fushiki, *Effects of oil intake in the conditioned place preference test in mice.* Brain Res, 2000. **870**(1-2): p. 150-6.

361. Ferreira, J.G., et al., *Regulation of fat intake in the absence of flavour signalling.* J Physiol, 2012. **590**(Pt 4): p. 953-72.

362. Schwartz, G.J., et al., *Decreased responsiveness to dietary fat in Otsuka Long-Evans Tokushima fatty rats lacking CCK-A receptors.* Am J Physiol, 1999. **277**(4 Pt 2): p. R1144-51.

363. Greenberg, D., et al., *Differential satiating effects of fats in the small intestine of obesity-resistant and obesity-prone rats.* Physiol Behav, 1999. **66**(4): p. 621-6.

364. Funakoshi, A., et al., *Little or no expression of the cholecystokinin-A receptor gene in the pancreas of diabetic rats (Otsuka Long-Evans Tokushima Fatty = OLETF rats).* Biochem Biophys Res Commun, 1994. **199**(2): p. 482-8.

365. White, C.L., et al., *Effect of meta-chlorophenylpiperazine and cholecystokinin on food intake of Osborne-Mendel and S5B/P1 rats.* Obesity (Silver Spring), 2007. **15**(3): p. 624-31.

366. Matson, C.A. and R.C. Ritter, *Long-term CCK-leptin synergy suggests a role for CCK in the regulation of body weight.* Am J Physiol, 1999. **276**(4 Pt 2): p. R1038-45.

367. Young, R.L., et al., *Expression of taste molecules in the upper gastrointestinal tract in humans with and without type 2 diabetes.* Gut, 2009. **58**(3): p. 337-46.

368. le Roux, C.W., et al., *Gut hormone profiles following bariatric surgery favor an anorectic state, facilitate weight loss, and improve metabolic parameters.* Ann Surg, 2006. **243**(1): p. 108-14.

369. le Roux, C.W., et al., *Attenuated peptide YY release in obese subjects is associated with reduced satiety.* Endocrinology, 2006. **147**(1): p. 3-8.

370. le Roux, C.W., et al., *Gut hormones as mediators of appetite and weight loss after Roux-en-Y gastric bypass.* Ann Surg, 2007. **246**(5): p. 780-5.

371. Kaplan, J.M., R.J. Seeley, and H.J. Grill, *Daily caloric intake in intact and chronic decerebrate rats.* Behav Neurosci, 1993. **107**(5): p. 876-81.

372. Flynn, F.W., K.C. Berridge, and H.J. Grill, *Pre- and postabsorptive insulin secretion in chronic decerebrate rats.* Am J Physiol, 1986. **250**(4 Pt 2): p. R539-48.

373. Grill, H.J. and R. Norgren, *Chronically decerebrate rats demonstrate satiation but not bait shyness.* Science, 1978. **201**(4352): p. 267-9.

374. Lorenz, D.N. and S.A. Goldman, *Vagal mediation of the cholecystokinin satiety effect in rats.* Physiol Behav, 1982. **29**(4): p. 599-604.

375. Walls, E.K., et al., *Selective vagal rhizotomies: a new dorsal surgical approach used for intestinal deafferentations.* Am J Physiol, 1995. **269**(5 Pt 2): p. R1279-88.

376. Dailey, M.J., et al., *Nutrient specific feeding and endocrine effects of jejunal infusions.* Obesity (Silver Spring), 2010. **18**(5): p. 904-10.

377. Tamura, C.S. and R.C. Ritter, *Intestinal capsaicin transiently attenuates suppression of sham feeding by oleate.* Am J Physiol, 1994. **267**(2 Pt 2): p. R561-8.

378. Berthoud, H.R., et al., *Vagal sensors in the rat duodenal mucosa: distribution and structure as revealed by in vivo DiI-tracing.* Anat Embryol (Berl), 1995. **191**(3): p. 203-12.

379. Berthoud, H.R. and L.M. Patterson, *Anatomical relationship between vagal afferent fibers and CCK-immunoreactive entero-endocrine cells in the rat small intestinal mucosa.* Acta Anat (Basel), 1996. **156**(2): p. 123-31.

380. Lynn, P.A. and L.A. Blackshaw, *In vitro recordings of afferent fibres with receptive fields in the serosa, muscle and mucosa of rat colon.* J Physiol, 1999. **518 (Pt 1)**: p. 271-82.

381. Williams, R.M., H.R. Berthoud, and R.H. Stead, *Vagal afferent nerve fibres contact mast cells in rat small intestinal mucosa.* Neuroimmunomodulation, 1997. **4**(5-6): p. 266-70.

382. Jorpes, E. and V. Mutt, *[Secretin, pancreozymin, and cholecystokinin; their physiology and future clinical use.].* Nord Med, 1956. **56**(42): p. 1511-7.

383. Gibbs, J., R.C. Young, and G.P. Smith, *Cholecystokinin decreases food intake in rats.* J Comp Physiol Psychol, 1973. **84**(3): p. 488-95.

384. Liddle, R.A., *Cholecystokinin cells.* Annu Rev Physiol, 1997. **59**: p. 221-42.

385. Glatzle, J., et al., *Cholecystokinin-58 is more potent in inhibiting food intake than cholecystokinin-8 in rats.* Nutr Neurosci, 2008. **11**(2): p. 69-74.

386. Brenner, L., D.P. Yox, and R.C. Ritter, *Suppression of sham feeding by intraintestinal nutrients is not correlated with plasma cholecystokinin elevation.* Am J Physiol, 1993. **264**(5 Pt 2): p. R972-6.

387. Weller, A., et al., *Trypsin inhibitor and maternal reunion increase plasma cholecystokinin in neonatal rats.* Peptides, 1992. **13**(5): p. 939-41.

388. Liddle, R.A., et al., *Proteins but not amino acids, carbohydrates, or fats stimulate cholecystokinin secretion in the rat.* Am J Physiol, 1986. **251**(2 Pt 1): p. G243-8.

389. Ballinger, A.B. and M.L. Clark, *L-phenylalanine releases cholecystokinin (CCK) and is associated with reduced food intake in humans: evidence for a physiological role of CCK in control of eating.* Metabolism, 1994. **43**(6): p. 735-8.

390. Parker, B.A., et al., *Effects of small intestinal and gastric glucose administration on the suppression of plasma ghrelin concentrations in healthy older men and women.* Clin Endocrinol (Oxf), 2005. **62**(5): p. 539-46.

391. South, E.H. and R.C. Ritter, *Capsaicin application to central or peripheral vagal fibers attenuates CCK satiety.* Peptides, 1988. **9**(3): p. 601-12.

392. Woltman, T., D. Castellanos, and R. Reidelberger, *Role of cholecystokinin in the anorexia produced by duodenal delivery of oleic acid in rats.* Am J Physiol, 1995. **269**(6 Pt 2): p. R1420-33.

393. Brenner, L.A. and R.C. Ritter, *Type A CCK receptors mediate satiety effects of intestinal nutrients.* Pharmacol Biochem Behav, 1996. **54**(3): p. 625-31.

394. Woltman, T. and R. Reidelberger, *Role of cholecystokinin in the anorexia produced by duodenal delivery of peptone in rats.* Am J Physiol, 1999. **276**(6 Pt 2): p. R1701-9.

395. Brenner, L. and R.C. Ritter, *Peptide cholesystokinin receptor antagonist increases food intake in rats.* Appetite, 1995. **24**(1): p. 1-9.

396. Moran, T.H., et al., *Blockade of type A, not type B, CCK receptors attenuates satiety actions of exogenous and endogenous CCK.* Am J Physiol, 1992. **262**(1 Pt 2): p. R46-50.

397. Reeve, J.R., Jr., et al., *CCK-58 is the only detectable endocrine form of cholecystokinin in rat.* Am J Physiol Gastrointest Liver Physiol, 2003. **285**(2): p. G255-65.

398. Canova, A. and N. Geary, *Intraperitoneal injections of nanogram CCK-8 doses inhibit feeding in rats.* Appetite, 1991. **17**(3): p. 221-7.

399. Cox, J.E., G.S. Perdue, and W.J. Tyler, *Suppression of sucrose intake by continuous near-celiac and intravenous cholecystokinin infusions in rats.* Am J Physiol, 1995. **268**(1 Pt 2): p. R150-5.

400. Pandya, P.K., et al., *Biochemical regulation of the three different states of the cholecystokinin (CCK) receptor in pancreatic acini.* Biochim Biophys Acta, 1994. **1224**(1): p. 117-26.

401. Talkad, V.D., et al., *Characterization of the three different states of the cholecystokinin (CCK) receptor in pancreatic acini.* Biochim Biophys Acta, 1994. **1224**(1): p. 103-16.

402. Weatherford, S.C., et al., *CCK satiety is differentially mediated by high- and low-affinity CCK receptors in mice and rats.* Am J Physiol, 1993. **264**(2 Pt 2): p. R244-9.

403. Simasko, S.M., et al., *Cholecystokinin increases cytosolic calcium in a subpopulation of cultured vagal afferent neurons.* Am J Physiol Regul Integr Comp Physiol, 2002. **283**(6): p. R1303-13.

404. Roberge, J.N. and P.L. Brubaker, *Secretion of proglucagon-derived peptides in response to intestinal luminal nutrients.* Endocrinology, 1991. **128**(6): p. 3169-74.

405. Dube, P.E. and P.L. Brubaker, *Nutrient, neural and endocrine control of glucagon-like peptide secretion.* Horm Metab Res, 2004. **36**(11-12): p. 755-60.

406. Holst, J.J., *On the physiology of GIP and GLP-1.* Horm Metab Res, 2004. **36**(11-12): p. 747-54.

407. Theodorakis, M.J., et al., *Human duodenal enteroendocrine cells: source of both incretin peptides, GLP-1 and GIP.* Am J Physiol Endocrinol Metab, 2006. **290**(3): p. E550-9.

408. Rozengurt, E. and C. Sternini, *Taste receptor signaling in the mammalian gut.* Curr Opin Pharmacol, 2007. **7**(6): p. 557-62.

409. Layer, P., et al., *Ileal release of glucagon-like peptide-1 (GLP-1). Association with inhibition of gastric acid secretion in humans.* Dig Dis Sci, 1995. **40**(5): p. 1074-82.

410. Cordier-Bussat, M., et al., *Peptones stimulate both the secretion of the incretin hormone glucagon-like peptide 1 and the transcription of the proglucagon gene.* Diabetes, 1998. **47**(7): p. 1038-45.

411. Chu, Z.L., et al., *A role for intestinal endocrine cell-expressed g protein-coupled receptor 119 in glycemic control by enhancing glucagon-like Peptide-1 and glucose-dependent insulinotropic Peptide release.* Endocrinology, 2008. **149**(5): p. 2038-47.

412. Tolhurst, G., F. Reimann, and F.M. Gribble, *Nutritional regulation of glucagon-like peptide-1 secretion.* J Physiol, 2009. **587**(Pt 1): p. 27-32.

413. Mari, A., et al., *Vildagliptin, a dipeptidyl peptidase-IV inhibitor, improves model-assessed beta-cell function in patients with type 2 diabetes.* J Clin Endocrinol Metab, 2005. **90**(8): p. 4888-94.

414. Turton, M.D., et al., *A role for glucagon-like peptide-1 in the central regulation of feeding.* Nature, 1996. **379**(6560): p. 69-72.

415. Flint, A., et al., *Glucagon-like peptide 1 promotes satiety and suppresses energy intake in humans.* J Clin Invest, 1998. **101**(3): p. 515-20.

416. Gutzwiller, J.P., et al., *Glucagon-like peptide-1: a potent regulator of food intake in humans.* Gut, 1999. **44**(1): p. 81-6.

417. Mack, C.M., et al., *Antiobesity action of peripheral exenatide (exendin-4) in rodents: effects on food intake, body weight, metabolic status and side-effect measures.* Int J Obes (Lond), 2006. **30**(9): p. 1332-40.

418. Larsen, P.J., et al., *Systemic administration of the long-acting GLP-1 derivative NN2211 induces lasting and reversible weight loss in both normal and obese rats.* Diabetes, 2001. **50**(11): p. 2530-9.

419. Williams, D.L., D.G. Baskin, and M.W. Schwartz, *Evidence that intestinal glucagon-like peptide-1 plays a physiological role in satiety.* Endocrinology, 2009. **150**(4): p. 1680-7.

420. Scrocchi, L.A., et al., *Glucose intolerance but normal satiety in mice with a null mutation in the glucagon-like peptide 1 receptor gene.* Nat Med, 1996. **2**(11): p. 1254-8.

421. Medeiros, M.D. and A.J. Turner, *Processing and metabolism of peptide-YY: pivotal roles of dipeptidylpeptidase-IV, aminopeptidase-P, and endopeptidase-24.11.* Endocrinology, 1994. **134**(5): p. 2088-94.

422. Medeiros, M.S. and A.J. Turner, *Post-secretory processing of regulatory peptides: the pancreatic polypeptide family as a model example.* Biochimie, 1994. **76**(3-4): p. 283-7.

423. Allen, J.M., et al., *Effects of peptide YY and neuropeptide Y on gastric emptying in man.* Digestion, 1984. **30**(4): p. 255-62.

424. Lundberg, J.M., et al., *Localization of peptide YY (PYY) in gastrointestinal endocrine cells and effects on intestinal blood flow and motility.* Proc Natl Acad Sci U S A, 1982. **79**(14): p. 4471-5.

425. Baba, H., M. Fujimura, and N. Toda, *Mechanism of inhibitory action of peptide YY on cholecystokinin-induced contractions of isolated dog ileum.* Regul Pept, 1990. **27**(2): p. 227-35.

426. Kim, B.J., et al., *Peptide YY is secreted after oral glucose administration in a gender-specific manner.* J Clin Endocrinol Metab, 2005. **90**(12): p. 6665-71.

427. Zhou, J., et al., *Peptide YY and proglucagon mRNA expression patterns and regulation in the gut.* Obesity (Silver Spring), 2006. **14**(4): p. 683-9.

428. Russek, M., *Participation of hepatic glucoreceptors in the control of intake of food.* Nature, 1963. **197**: p. 79-80.

429. Tordoff, M.G. and M.I. Friedman, *Hepatic portal glucose infusions decrease food intake and increase food preference.* Am J Physiol, 1986. **251**(1 Pt 2): p. R192-6.

430. Novin, D., D.A. VanderWeele, and M. Rezek, *Infusion of 2-deoxy-D-glucose into the hepatic-portal system causes eating: evidence for peripheral glucoreceptors.* Science, 1973. **181**(4102): p. 858-60.

431. Baird, J.P., H.J. Grill, and J.M. Kaplan, *Intake suppression after hepatic portal glucose infusion: all-or-none effect and its temporal threshold.* Am J Physiol, 1997. **272**(5 Pt 2): p. R1454-60.

432. Tordoff, M.G., J.P. Tluczek, and M.I. Friedman, *Effect of hepatic portal glucose concentration on food intake and metabolism.* Am J Physiol, 1989. **257**(6 Pt 2): p. R1474-80.

433. Tordoff, M.G. and M.I. Friedman, *Hepatic control of feeding: effect of glucose, fructose, and mannitol infusion.* Am J Physiol, 1988. **254**(6 Pt 2): p. R969-76.

434. Jambor de Sousa, U.L., et al., *Hepatic-portal oleic acid inhibits feeding more potently than hepatic-portal caprylic acid in rats.* Physiol Behav, 2006. **89**(3): p. 329-34.

435. Anil, M.H. and J.M. Forbes, *Feeding in sheep during intraportal infusions of short-chain fatty acids and the effect of liver denervation.* J Physiol, 1980. **298**: p. 407-14.

436. Friedman, M.I., et al., *Fatty acid oxidation affects food intake by altering hepatic energy status.* Am J Physiol, 1999. **276**(4 Pt 2): p. R1046-53.

437. Lutz, T.A., M. Diener, and E. Scharrer, *Intraportal mercaptoacetate infusion increases afferent activity in the common hepatic vagus branch of the rat.* Am J Physiol, 1997. **273**(1 Pt 2): p. R442-5.

438. Lutz, T.A., A. Niijima, and E. Scharrer, *Intraportal infusion of 2,5-anhydro-D-mannitol increases afferent activity in the common hepatic vagus branch.* J Auton Nerv Syst, 1996. **61**(2): p. 204-8.

439. Langhans, W. and E. Scharrer, *Evidence for a vagally mediated satiety signal derived from hepatic fatty acid oxidation.* J Auton Nerv Syst, 1987. **18**(1): p. 13-8.

440. Ritter, S., T.T. Dinh, and M.I. Friedman, *Induction of Fos-like immunoreactivity (Fos-li) and stimulation of feeding by 2,5-anhydro-D-mannitol (2,5-AM) require the vagus nerve.* Brain Res, 1994. **646**(1): p. 53-64.

441. Friedman, M.I. and P.E. Sawchenko, *Evidence for hepatic involvement in control of ad libitum food intake in rats.* Am J Physiol, 1984. **247**(1 Pt 2): p. R106-13.

442. Ji, H. and M.I. Friedman, *Compensatory hyperphagia after fasting tracks recovery of liver energy status.* Physiol Behav, 1999. **68**(1-2): p. 181-6.

443. Tordoff, M.G., N. Rawson, and M.I. Friedman, *2,5-anhydro-D-mannitol acts in liver to initiate feeding.* Am J Physiol, 1991. **261**(2 Pt 2): p. R283-8.

444. Rawson, N.E., et al., *Hepatic phosphate trapping, decreased ATP, and increased feeding after 2,5-anhydro-D-mannitol.* Am J Physiol, 1994. **266**(1 Pt 2): p. R112-7.

445. Koch, J.E., et al., *Temporal relationships between eating behavior and liver adenine nucleotides in rats treated with 2,5-AM.* Am J Physiol, 1998. **274**(3 Pt 2): p. R610-7.

446. Rawson, N.E. and M.I. Friedman, *Phosphate loading prevents the decrease in ATP and increase in food intake produced by 2,5-anhydro-D-mannitol.* Am J Physiol, 1994. **266**(6 Pt 2): p. R1792-6.

447. Rawson, N.E., P.M. Ulrich, and M.I. Friedman, *L-ethionine, an amino acid analogue, stimulates eating in rats.* Am J Physiol, 1994. **267**(2 Pt 2): p. R612-5.

448. Ji, H. and M.I. Friedman, *Fasting plasma triglyceride levels and fat oxidation predict dietary obesity in rats.* Physiol Behav, 2003. **78**(4-5): p. 767-72.

449. Ji, H., L.V. Outterbridge, and M.I. Friedman, *Phenotype-based treatment of dietary obesity: differential effects of fenofibrate in obesity-prone and obesity-resistant rats.* Metabolism, 2005. **54**(4): p. 421-9.

450. Leonhardt, M. and W. Langhans, *Fatty acid oxidation and control of food intake.* Physiol Behav, 2004. **83**(4): p. 645-51.

451. Scharrer, E. and W. Langhans, *Control of food intake by fatty acid oxidation.* Am J Physiol, 1986. **250**(6 Pt 2): p. R1003-6.

452. Cortez-Pinto, H., et al., *Alterations in liver ATP homeostasis in human nonalcoholic steatohepatitis: a pilot study.* JAMA, 1999. **282**(17): p. 1659-64.

453. Nair, S., et al., *Hepatic ATP reserve and efficiency of replenishing: comparison between obese and nonobese normal individuals.* Am J Gastroenterol, 2003. **98**(2): p. 466-70.

454. Rosebury, T., *Microorganisms indigenous to man*. 1962, New York, New York: McGraw-Hill.

455. Savage, D.C., *Microbial ecology of the gastrointestinal tract.* Annu Rev Microbiol, 1977. **31**: p. 107-33.

456. Xu, J., et al., *Evolution of symbiotic bacteria in the distal human intestine.* PLoS Biol, 2007. **5**(7): p. e156.

457. Ley, R.E., et al., *Obesity alters gut microbial ecology.* Proc Natl Acad Sci U S A, 2005. **102**(31): p. 11070-5.

458. Palmer, C., et al., *Development of the human infant intestinal microbiota.* PLoS Biol, 2007. **5**(7): p. e177.

459. Spor, A., O. Koren, and R. Ley, *Unravelling the effects of the environment and host genotype on the gut microbiome.* Nat Rev Microbiol, 2011. **9**(4): p. 279-90.

460. de La Serre, C.B., et al., *Propensity to high-fat diet-induced obesity in rats is associated with changes in the gut microbiota and gut inflammation.* Am J Physiol Gastrointest Liver Physiol, 2010. **299**(2): p. G440-8.

461. Sonnenburg, J.L., et al., *Glycan foraging in vivo by an intestine-adapted bacterial symbiont.* Science, 2005. **307**(5717): p. 1955-9.

462. Cummings, J.H., et al., *Short chain fatty acids in human large intestine, portal, hepatic and venous blood.* Gut, 1987. **28**(10): p. 1221-7.

463. Macfarlane, G.T., G.R. Gibson, and J.H. Cummings, *Comparison of fermentation reactions in different regions of the human colon.* J Appl Bacteriol, 1992. **72**(1): p. 57-64.

464. Roediger, W.E., *Role of anaerobic bacteria in the metabolic welfare of the colonic mucosa in man.* Gut, 1980. **21**(9): p. 793-8.

465. Cummings, J.H. and G.T. Macfarlane, *The control and consequences of bacterial fermentation in the human colon.* J Appl Bacteriol, 1991. **70**(6): p. 443-59.

466. Bergman, E.N., *Energy contributions of volatile fatty acids from the gastrointestinal tract in various species.* Physiol Rev, 1990. **70**(2): p. 567-90.

467. Wostmann, B.S., et al., *Dietary intake, energy metabolism, and excretory losses of adult male germfree Wistar rats.* Lab Anim Sci, 1983. **33**(1): p. 46-50.

468. Wostmann, B. and E. Bruckner-Kardoss, *Development of cecal distention in germ-free baby rats.* Am J Physiol, 1959. **197**: p. 1345-6.

469. Gordon, H.A. and E. Bruckner-Kardoss, *Effect of normal microbial flora on intestinal surface area.* Am J Physiol, 1961. **201**: p. 175-8.

470. Abrams, G.D., H. Bauer, and H. Sprinz, *Influence of the normal flora on mucosal morphology and cellular renewal in the ileum. A comparison of germ-free and conventional mice.* Lab Invest, 1963. **12**: p. 355-64.

471. Lesher, S., H.E. Walburg, Jr., and G.A. Sacher, Jr., *Generation Cycle in the Duodenal Crypt Cells of Germ-Free and Conventional Mice.* Nature, 1964. **202**: p. 884-6.

472. Cherbuy, C., et al., *Microbiota matures colonic epithelium through a coordinated induction of cell cycle-related proteins in gnotobiotic rat.* Am J Physiol Gastrointest Liver Physiol, 2010. **299**(2): p. G348-57.

473. Abrams, G.D. and J.E. Bishop, *Effect of the normal microbial flora on gastrointestinal motility.* Proc Soc Exp Biol Med, 1967. **126**(1): p. 301-4.

474. Riottot, M., E. Sacquet, and C. Leprince, *Variations of bile salt pool size and secretion rate in rats according to the modes of sterilization and preparation of a semi-synthetic diet.* Reprod Nutr Dev, 1980. **20**(5A): p. 1481-8.

475. Sacquet, E., H. Garnier, and P. Raibaud, *[Study of rate of the gastrointestinal transit of spores of a strictly thermophilic strain of Bacillus subtilis in the holoxenic rat, the axenic rat and the cecectomized axenic rat].* C R Seances Soc Biol Fil, 1970. **164**(3): p. 532-7.

476. Reddy, B.S., J.R. Pleasants, and B.S. Wostmann, *Effect of dietary carbohydrates on intestinal disaccharidases in germfree and conventional rats.* J Nutr, 1968. **95**(3): p. 413-9.

477. Reddy, B.S. and B.S. Wostmann, *Intestinal disaccharidase activities in the growing germfree and conventional rats.* Arch Biochem Biophys, 1966. **113**(3): p. 609-16.

478. Dahlqvist, A. and B. Borgstrom, *Digestion and absorption of disaccharides in man.* Biochem J, 1961. **81**: p. 411-8.

479. Corring, T., C. Juste, and C. Simoes-Nunes, *Digestive enzymes in the germ-free animal.* Reprod Nutr Dev, 1981. **21**(3): p. 355-70.

480. Pen, J. and G.W. Welling, *Influence of the microbial flora on the amount of CCK8- and secretin21-27-like immunoreactivity in the intestinal tract of mice.* Comp Biochem Physiol B, 1983. **76**(3): p. 585-9.

481. Pen, J. and G.W. Welling, *The concentration of cholecystokinin in the intestinal tract of germ-free and control mice.* Antonie Van Leeuwenhoek, 1981. **47**(1): p. 84-5.

482. Goodlad, R.A., et al., *Plasma enteroglucagon, gastrin and peptide YY in conventional and germ-free rats refed with a fibre-free or fibre-supplemented diet.* Q J Exp Physiol, 1989. **74**(4): p. 437-42.

483. Arantes, R.M. and A.M. Nogueira, *Distribution of enteroglucagon- and peptide YY-immunoreactive cells in the intestinal mucosa of germ-free and conventional mice.* Cell Tissue Res, 1997. **290**(1): p. 61-9.

484. Arantes, R.M. and A.M. Nogueira, *Increased intracellular content of enteroglucagon in proximal colon is related to intestinal adaptation to germ-free status.* Cell Tissue Res, 2001. **303**(3): p. 447-50.

485. Cani, P.D., et al., *Oligofructose promotes satiety in rats fed a high-fat diet: involvement of glucagon-like Peptide-1.* Obes Res, 2005. **13**(6): p. 1000-7.

486. Delzenne, N.M., et al., *Impact of inulin and oligofructose on gastrointestinal peptides.* Br J Nutr, 2005. **93 Suppl 1**: p. S157-61.

487. Zhou, J., et al., *Dietary resistant starch upregulates total GLP-1 and PYY in a sustained day-long manner through fermentation in rodents.* Am J Physiol Endocrinol Metab, 2008. **295**(5): p. E1160-6.

488. Shen, L., et al., *Dietary resistant starch increases hypothalamic POMC expression in rats.* Obesity (Silver Spring), 2009. **17**(1): p. 40-5.

489. Cai, D., et al., *Local and systemic insulin resistance resulting from hepatic activation of IKK-beta and NF-kappaB.* Nat Med, 2005. **11**(2): p. 183-90.

490. Hotamisligil, G.S., et al., *Increased adipose tissue expression of tumor necrosis factor-alpha in human obesity and insulin resistance.* J Clin Invest, 1995. **95**(5): p. 2409-15.

491. Weisberg, S.P., et al., *Obesity is associated with macrophage accumulation in adipose tissue.* J Clin Invest, 2003. **112**(12): p. 1796-808.

492. Xu, H., et al., *Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance.* J Clin Invest, 2003. **112**(12): p. 1821-30.

493. Wood, L.G., M.L. Garg, and P.G. Gibson, *A high-fat challenge increases airway inflammation and impairs bronchodilator recovery in asthma.* J Allergy Clin Immunol, 2011. **127**(5): p. 1133-40.

494. Ricci, M.R. and B.E. Levin, *Ontogeny of diet-induced obesity in selectively bred Sprague-Dawley rats.* Am J Physiol Regul Integr Comp Physiol, 2003. **285**(3): p. R610-8.

495. Cani, P.D., et al., *Role of gut microflora in the development of obesity and insulin resistance following high-fat diet feeding.* Pathol Biol (Paris), 2008. **56**(5): p. 305-9.

496. Abreu, M.T., *Toll-like receptor signalling in the intestinal epithelium: how bacterial recognition shapes intestinal function.* Nat Rev Immunol, 2010. **10**(2): p. 131-44.

497. Cani, P.D., et al., *Metabolic endotoxemia initiates obesity and insulin resistance.* Diabetes, 2007. **56**(7): p. 1761-72.

498. Davis, J.E., et al., *Tlr-4 deficiency selectively protects against obesity induced by diets high in saturated fat.* Obesity (Silver Spring), 2008. **16**(6): p. 1248-55.

499. Fernandez-Real, J.M., et al., *CD14 monocyte receptor, involved in the inflammatory cascade, and insulin sensitivity.* J Clin Endocrinol Metab, 2003. **88**(4): p. 1780-4.

500. Cani, P.D., et al., *Changes in gut microbiota control metabolic endotoxemia-induced inflammation in high-fat diet-induced obesity and diabetes in mice.* Diabetes, 2008. **57**(6): p. 1470-81.

501. de Lartigue, G., et al., *Diet-induced obesity leads to the development of leptin resistance in vagal afferent neurons.* Am J Physiol Endocrinol Metab, 2011. **301**(1): p. E187-95.

502. Hosoi, T., et al., *Novel pathway for LPS-induced afferent vagus nerve activation: possible role of nodose ganglion.* Auton Neurosci, 2005. **120**(1-2): p. 104-7.

503. Tsukumo, D.M., et al., *Translational research into gut microbiota: new horizons in obesity treatment.* Arq Bras Endocrinol Metabol, 2009. **53**(2): p. 139-44.

504. Kersten, S., et al., *Characterization of the fasting-induced adipose factor FIAF, a novel peroxisome proliferator-activated receptor target gene.* J Biol Chem, 2000. **275**(37): p. 28488-93.

505. Lichtenstein, L., et al., *Angptl4 upregulates cholesterol synthesis in liver via inhibition of LPL- and HL-dependent hepatic cholesterol uptake.* Arterioscler Thromb Vasc Biol, 2007. **27**(11): p. 2420-7.

506. Fleissner, C.K., et al., *Absence of intestinal microbiota does not protect mice from diet-induced obesity.* Br J Nutr, 2010. **104**(6): p. 919-29.

507. Rabot, S., et al., *Germ-free C57BL/6J mice are resistant to high-fat-diet-induced insulin resistance and have altered cholesterol metabolism.* FASEB J, 2010.

508. Turnbaugh, P.J., et al., *An obesity-associated gut microbiome with increased capacity for energy harvest.* Nature, 2006. **444**(7122): p. 1027-31.

509. Barcenilla, A., et al., *Phylogenetic relationships of butyrate-producing bacteria from the human gut.* Appl Environ Microbiol, 2000. **66**(4): p. 1654-61.

510. Turnbaugh, P.J., et al., *Diet-induced obesity is linked to marked but reversible alterations in the mouse distal gut microbiome.* Cell Host Microbe, 2008. **3**(4): p. 213-23.

511. Turnbaugh, P.J., et al., *A core gut microbiome in obese and lean twins.* Nature, 2009. **457**(7228): p. 480-4.

512. Ley, R.E., et al., *Microbial ecology: human gut microbes associated with obesity.* Nature, 2006. **444**(7122): p. 1022-3.

513. Hildebrandt, M.A., et al., *High-fat diet determines the composition of the murine gut microbiome independently of obesity.* Gastroenterology, 2009. **137**(5): p. 1716-24 e1-2.

514. Levin, B.E., et al., *Selective breeding for diet-induced obesity and resistance in Sprague-Dawley rats.* Am J Physiol, 1997. **273**(2 Pt 2): p. R725-30.

515. Swartz, T.D., et al., *Up-regulation of intestinal type 1 taste receptor 3 and sodium glucose luminal transporter-1 expression and increased sucrose intake in mice lacking gut microbiota.* Br J Nutr, 2012. **107**(5): p. 621-30.

516. Wang, Y.C., et al., *Health and economic burden of the projected obesity trends in the USA and the UK.* Lancet, 2011. **378**(9793): p. 815-25.

517. Lucas, F. and A. Sclafani, *The composition of the maintenance diet alters flavor-preference conditioning by intragastric fat infusions in rats.* Physiol Behav, 1996. **60**(4): p. 1151-7.

518. Swartz, T.D., et al., *Up-regulation of intestinal type 1 taste receptor 3 and sodium glucose luminal transporter-1 expression and increased sucrose intake in mice lacking gut microbiota.* Br J Nutr, 2011: p. 1-10.

519. Martin, C., et al., *The lipid-sensor candidates CD36 and GPR120 are differentially regulated by dietary lipids in mouse taste buds: impact on spontaneous fat preference.* PLoS One. **6**(8): p. e24014.

520. Zhang, X.J., et al., *Decreased expression of CD36 in circumvallate taste buds of high-fat diet induced obese rats.* Acta Histochem, 2011. **113**(6): p. 663-7.

521. Ackroff, K. and A. Sclafani, *Flavor preferences conditioned by sugars: rats learn to prefer glucose over fructose.* Physiol Behav, 1991. **50**(4): p. 815-24.

522. Demarne, Y., et al., *Fat absorption in germ-free and conventional rats artificially deprived of bile secretion.* Gut, 1982. **23**(1): p. 49-57.

523. Demarne, Y., E. Sacquet, and H. Garnier, *[Gastrointestinal flora and fat digestion in monogastrics].* Ann Biol Anim Biochim Biophys, 1972. **12**(3): p. 509-24.

524. Goudriaan, J.R., et al., *Intestinal lipid absorption is not affected in CD36 deficient mice.* Mol Cell Biochem, 2002. **239**(1-2): p. 199-202.

525. Engelstoft, M.S., et al., *A gut feeling for obesity: 7TM sensors on enteroendocrine cells.* Cell Metab, 2008. **8**(6): p. 447-9.

526. Cherbut, C., et al., *Short-chain fatty acids modify colonic motility through nerves and polypeptide YY release in the rat.* Am J Physiol, 1998. **275**(6 Pt 1): p. G1415-22.

527. Mandard, S., et al., *The fasting-induced adipose factor/angiopoietin-like protein 4 is physically associated with lipoproteins and governs plasma lipid levels and adiposity.* J Biol Chem, 2006. **281**(2): p. 934-44.

528. Velagapudi, V.R., et al., *The gut microbiota modulates host energy and lipid metabolism in mice.* J Lipid Res, 2011. **51**(5): p. 1101-12.

529. Le Jan, S., et al., *Angiopoietin-like 4 is a proangiogenic factor produced during ischemia and in conventional renal cell carcinoma.* Am J Pathol, 2003. **162**(5): p. 1521-8.

530. Stappenbeck, T.S., L.V. Hooper, and J.I. Gordon, *Developmental regulation of intestinal angiogenesis by indigenous microbes via Paneth cells.* Proc Natl Acad Sci U S A, 2002. **99**(24): p. 15451-5.

531. Wostmann, B.S. and D.F. Kan, *The Cholesterol-Lowering Effect of Commercial Diet Fed to Germfree and Conventional Rats.* J Nutr, 1964. **84**: p. 277-82.

532. Klein, S., et al., *Adipose tissue leptin production and plasma leptin kinetics in humans.* Diabetes, 1996. **45**(7): p. 984-7.

533. Drover, V.A., et al., *CD36 deficiency impairs intestinal lipid secretion and clearance of chylomicrons from the blood.* J Clin Invest, 2005. **115**(5): p. 1290-7.

534. Lin, Z.J., et al., *Abdominal fat accumulation with hyperuricemia and hypercholesterolemia quail model induced by high fat diet.* Chin Med Sci J, 2009. **24**(3): p. 191-4.

535. Kahn, B.B., et al., *AMP-activated protein kinase: ancient energy gauge provides clues to modern understanding of metabolism.* Cell Metab, 2005. **1**(1): p. 15-25.

536. Bastie, C., et al., *Alterations of peroxisome proliferator-activated receptor delta activity affect fatty acid-controlled adipose differentiation.* J Biol Chem, 2000. **275**(49): p. 38768-73.

537. Jernas, M., et al., *Separation of human adipocytes by size: hypertrophic fat cells display distinct gene expression.* FASEB J, 2006. **20**(9): p. 1540-2.

538. Weyer, C., et al., *Enlarged subcutaneous abdominal adipocyte size, but not obesity itself, predicts type II diabetes independent of insulin resistance.* Diabetologia, 2000. **43**(12): p. 1498-506.

539. Duca, F.A. and M. Covasa, *Current and emerging concepts on the role of peripheral signals in the control of food intake and development of obesity.* Br J Nutr, 2012: p. 1-16.

540. Hajnal, A. and R. Norgren, *Taste pathways that mediate accumbens dopamine release by sapid sucrose.* Physiol Behav, 2005. **84**(3): p. 363-9.

541. Steinert, R.E. and C. Beglinger, *Nutrient sensing in the gut: interactions between chemosensory cells, visceral afferents and the secretion of satiation peptides.* Physiol Behav, 2011. **105**(1): p. 62-70.

542. Friedman, M.I., *Obesity and the hepatic control of feeding behavior.* Drug News Perspect, 2007. **20**(9): p. 573-8.

543. Szczypka, M.S., et al., *Viral gene delivery selectively restores feeding and prevents lethality of dopamine-deficient mice.* Neuron, 1999. **22**(1): p. 167-78.

544. Friedman, J.M., *The function of leptin in nutrition, weight, and physiology.* Nutr Rev, 2002. **60**(10 Pt 2): p. S1-14; discussion S68-84, 85-7.

545. Arnold, M., et al., *Meal-Contingent Intestinal Lymph Collection from Awake, Unrestrained Rats.* Am J Physiol Regul Integr Comp Physiol, 2012.

546. Byrnes, D.J., et al., *Radioimmunoassay of cholecystokinin in human plasma.* Clin Chim Acta, 1981. **111**(1): p. 81-9.

547. Elias, C.F., et al., *Chemical characterization of leptin-activated neurons in the rat brain.* J Comp Neurol, 2000. **423**(2): p. 261-81.

548. Abbott, C.R., et al., *The inhibitory effects of peripheral administration of peptide YY(3-36) and glucagon-like peptide-1 on food intake are attenuated by ablation of the vagal-brainstem-hypothalamic pathway.* Brain Res, 2005. **1044**(1): p. 127-31.

549. Seimon, R.V., et al., *Effects of varying combinations of intraduodenal lipid and carbohydrate on antropyloroduodenal motility, hormone release, and appetite in healthy males.* Am J Physiol Regul Integr Comp Physiol, 2009. **296**(4): p. R912-20.

550. Shin, A.C., et al., *Meal-induced hormone responses in a rat model of Roux-en-Y gastric bypass surgery.* Endocrinology, 2010. **151**(4): p. 1588-97.

551. Sclafani, A. and K. Ackroff, *Invited Review: The role of gut nutrient sensing in stimulating appetite and conditioning food preferences.* Am J Physiol Regul Integr Comp Physiol, 2012.

552. Elizalde, G. and A. Sclafani, *Starch-based conditioned flavor preferences in rats: influence of taste, calories and CS-US delay.* Appetite, 1988. **11**(3): p. 179-200.

553. Touzani, K., R.J. Bodnar, and A. Sclafani, *Dopamine D1-like receptor antagonism in amygdala impairs the acquisition of glucose-conditioned flavor preference in rats.* Eur J Neurosci, 2009.

554. Touzani, K. and A. Sclafani, *Critical role of amygdala in flavor but not taste preference learning in rats.* Eur J Neurosci, 2005. **22**(7): p. 1767-74.

555. Elizalde, G. and A. Sclafani, *Fat appetite in rats: flavor preferences conditioned by nutritive and non-nutritive oil emulsions.* Appetite, 1990. **15**(3): p. 189-97.

556. Diraison, F., et al., *Increased hepatic lipogenesis but decreased expression of lipogenic gene in adipose tissue in human obesity.* Am J Physiol Endocrinol Metab, 2002. **282**(1): p. E46-51.

557. Godbole, V. and D.A. York, *Lipogenesis in situ in the genetically obese Zucker fatty rat (fa/fa): role of hyperphagia and hyperinsulinaemia.* Diabetologia, 1978. **14**(3): p. 191-7.

558. Avram, A.S., M.M. Avram, and W.D. James, *Subcutaneous fat in normal and diseased states: 2. Anatomy and physiology of white and brown adipose tissue.* J Am Acad Dermatol, 2005. **53**(4): p. 671-83.