Review Article
Mechanisms and effects of “fat taste” in humans

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Abstract
Evidence supporting a “taste” cue from fat in the oral cavity continues to accrue. The proposed stimuli for fat taste, non-esterified fatty acids (NEFA), are released from food through hydrolytic rancidity and lipase activity derived from foods or saliva. NEFA must then be released from the food matrix, negotiate the aqueous environment to reach taste cell surfaces, and interact with receptors such as CD36 and GPR120 or diffuse across cell membranes to initiate a taste signal. Knowledge of these processes in non-gustatory tissues should inform understanding of taste responses to NEFA. Additionally, downstream effects of oral triglyceride exposure have been observed in numerous studies. Data specific to effects of NEFA versus triglyceride are scarce, but modified sham feeding trials with triglyceride document cephalic phase responses including elevations in serum lipids and insulin as well as potential, but debated, effects on gut peptides, appetite, and thermogenesis. In this review, we highlight the mechanisms by which NEFA migrate to and interact with taste cells, and then we examine physiological responses to oral fat exposure.

Keywords: fat taste; non-esterified fatty acids; cephalic phase response; fatty acid diffusion; fatty acid receptors

1. Introduction
Research is continuing to uncover commonalities in cell surface receptors throughout the human body. Receptors mediating taste in particular are present in a wide variety of tissues where they serve different functions. The G protein-coupled receptors (GPCRs) identified as mediating bitter, sweet, and umami tastes have now been observed in numerous other locations, including the intestinal tract [1–3], lungs [3,4], brain [5], and heart [6]. Purported taste receptors for sour were originally identified in the kidneys [7–9]. The epithelial sodium channel, hypothesized as a salt taste receptor, is also found in multiple other tissues, including lungs, kidney, colon, sweat glands, and vasculature [10,11]. Although the ligand that binds to each receptor and depolarizes the receptor cell is common, the type of cell on which the receptor is located will determine its physiological role. Thus, it may be proposed that oral sensitivity might serve as a predictor for systemic reactivity to a given ligand stimulus.

Numerous recent reports indicate non-esterified fatty acids (NEFA) are effective gustatory (in addition to olfactory and somatosensory) stimuli in humans [12–24]. All the currently proposed receptors for oral NEFA detection are located in other tissues. More is presently known about the mechanisms and functions of the NEFA receptors in non-oral tissues, including strong evidence that they react differently to fats varying in chain length and/or degree of saturation. Thus, it may be posited that taste responses to different NEFA will vary, and indeed new data from our laboratory indicate that as methods for analyzing oral sensitivity to NEFA have improved, evidence of differences is emerging (unpublished results). Indeed, taste responses may further reflect differences in how NEFA of varying structure partition from foods to saliva to cell surfaces and intracellular compartments. Although much of the research linking NEFA structure with taste sensitivity and downstream effects has yet to be conducted, data are available on many of the mechanisms of NEFA solubility, diffusion constants across

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cell membranes, and interactions with receptors such as GPR120 and CD36 (present in many tissues including gustatory tissue). Research confirming these mechanisms in humans is scarce, but considerable data are available from rodent and cell culture studies. Additionally, downstream effects of oral triglyceride have been investigated, such as elevations in serum lipids and insulin as well as potential, but debated, effects on gut peptides, appetite, and thermogenesis. In this review, we highlight the mechanisms by which NEFA migrate to and interact with taste cells, and then we examine physiological responses to oral fat exposure. Although much more work has been conducted in rodents, the focus of this review is on human data due to potential species differences, for example, differences in lipoprotein metabolism [25], and rodents demonstrate positive hedonic responses to long chain NEFA [26] while humans do not [27].

2. NEFA Migration and Receptor Interactions
Non-esterified fatty acids (NEFA) are present naturally in foods and may be hydrolyzed from triacylglycerols by the activity of lingual lipase. Due to their hydrophobicity, NEFA are capable of passive diffusion through cell membranes, but numerous studies point to controlled, saturable systems for NEFA movement, indicating a more complex system than passive diffusion [28–33]. Numerous proteins have been identified as binders of NEFA in a wide variety of food products and human tissues, where they likely function to control concentrations of unbound fatty acids that can act as damaging detergents when concentrations are high [34]. Proteins localized to cell membranes have also been identified in the transport and detection of NEFA, and many of these proteins are found in multiple human tissue types. With the emergence of the concept of “fat taste” stimulated by NEFA, there is growing interest in identifying mechanisms for NEFA interactions with taste cells.

2.1. Extracellular Environment
2.1.1. Release of NEFA from food matrix and triglyceride
NEFA are released from triglycerides in foods due to hydrolytic rancidity and native lipase activity. In the oral cavity, NEFA are also released from triglycerides by the action of lingual lipase, the activity of which has been observed in saliva despite an ongoing debate about the specific protein in humans [22,35,36]. Additionally, mastication of food may be important for greater release of NEFA [37], perhaps because of increased secretion of lipase from the von Ebner’s glands co-located with circumvallate and foliate papillae or because of increased accessibility of the lipase to the triglyceride in the more homogenized sample. The concentrations of NEFA observed naturally after oral lipase action or after mastication are within the range of NEFA needed to elicit a taste response [37].

2.1.2. Access to the taste cell surface
In the intestine, fatty acids and monoglycerides must be incorporated into micelles to enter the unstirred water layer and be accessible to enterocytes. Likewise, oral surfaces are continuously coated in saliva, which changes the surface of gustatory tissue from hydrophobic (the natural character of the cell membrane) to hydrophilic. This occurs as salivary proteins adsorb to cell surfaces, orienting their hydrophobic portions toward the cell membrane and hydrophilic portions toward the saliva layer [38,39]. Similar to intestinal lipids with enterocytes, oral lipids must negotiate an aqueous layer (saliva) to interact with taste cells. Thus, lipid tastants must solubilize in saliva at a concentration high enough to induce a taste sensation, interact with some sort of emulsifying agent that can increase solubility of the lipid, or form droplets/micelles small enough to enter taste pores and interact with taste cells. Rodent literature indicates that natural saliva may play a role in NEFA taste, as partially desalivated rodents have higher discrimination thresholds for linoleic acid, and linoleic acid alone or with artificial saliva (lacking proteins) does not stimulate a chordi tympani nerve response [40,41]. The mechanisms for NEFA accessibility to taste cells have not been confirmed, but while longer chain NEFA are poorly soluble in water, they are capable of forming micelles and are bound by emulsifiers that increase their aqueous solubility [42–44]. Examples include lipocalin-1 (natural in human saliva, also known as von Ebner’s gland protein [45]) and food based emulsifiers such as β-lactoglobulin in dairy products.

2.1.3. Von Ebner’s gland protein/lipocalin-1
Lipocalin-1, also called tear lipocalin, human tear prealbumin, and von Ebner’s gland protein, was first found in tears in 1956 and then identified in saliva from the von Ebner’s glands in 1993 [46–48]. Lipocalin-1 can also be found in sweat, mammary glands, pituitary glands, nasal mucosa, prostate tissue, testes, tracheal cells, and skin [46]. Much of available literature on lipocalin-1 focuses on its function in tears, as this protein accounts for 15–33% of total tear protein [49].

Early research on this protein isolated from saliva showed it may assist with the clearance of denatonium benzoate, and so it was proposed to bind bitter molecules [50]. However, subsequent experiments revealed lipocalin-1 bound only fatty acids, rather than any of the tested bitter compounds [51–54]. Binding and structural analyses indicate lipocalin-1 has a single binding site [55] capable of binding cholesterol, retinol, phospholipids, fatty alcohols, and glycolipids in addition to NEFA [53,55]. Binding affinity of lipocalin-1 for fatty acids increases with alkyl chain length, and thus hydrophobicity, up to 18 carbons [54], but fatty acids with greater than 18 carbons show similar affinities [56]. This is likely because NEFA are bound in the calyx of lipocalin-1 with the carboxylic acid head oriented toward the exterior solvent and the tail extending into the interior cavity of the protein; thus after the cavity is filled, binding affinity no longer increases [56].

Lipocalin-1 has high structural homology with β-lactoglobulin [55]. As lipocalin-1 could have a function in taste, particularly in the binding of NEFA, the structural similarity to β-lactoglobulin could be important to this field, as some studies...
examining NEFA detection thresholds have used milk or whey protein (which contain β-lactoglobulin) in the emulsion preparation [18,20,21,23]. If lipocalin-1 does have a function in NEFA taste, then β-lactoglobulin would compete with lipocalin-1 for the binding of available NEFA. Whether either lipocalin-1 or β-lactoglobulin binding of NEFA would increase (by increasing NEFA solubility and delivery to the taste cell surface) or decrease (by binding NEFA and preventing them from interacting with taste cells) NEFA taste sensitivity is unknown. Also of potential importance to current NEFA taste studies, lipocalin-1’s isoelectric point is approximately pH 4.8–5 [55], and it undergoes conformational changes in more acidic conditions, losing affinity for fatty acid-type ligands at pH 4 [57]. If lipocalin-1 does have a function in NEFA taste, the use of high concentrations of gum arabic, pH of 4–5 in solution, in the tasting solution could interfere with the affinity of lipocalin-1 for NEFA.

2.2. Transport and Receptors

2.2.1. Diffusion

Fatty acids passively diffuse across cell membranes by the “flip-flop” mechanism [58–60]. This occurs primarily for the unionized (protonated) form of fatty acids, as a drop in pH is observed when the fatty acid moves into the intracellular space [61] and as the time required for flip-flop of ionized fatty acids is much longer [58,60]. NEFA pKₐ-s are typically reported between 4 and 5; however, the observed pKₐ of NEFA in an aqueous environment depends greatly upon the concentration of the fatty acid, especially for long-chain fatty acids [42,62]. Fatty acids bound by proteins such as albumin are also known to have lower pKₐ-s than fatty acids incorporated into phospholipid bilayers [43]. In the intestine, pH decreases near the brush border membrane, causing protonation of fatty acids and allowing diffusion of the non-ionized forms [63]. Such a mechanism has also been proposed for albumin, as albumin binds the ionized form of NEFA [64], and the pKₐ-s of fatty acids approach 7 in the vicinity of the phospholipid bilayer, again leading to protonation of the fatty acid and more efficient diffusion in the cell [60]. Thus, while saliva typically has near-neutral pH, in which NEFA may be expected to be ionized, the protonation status of NEFA would actually be determined by the proteins, lipids, or membranes associated with the NEFA. The effect of food pH on NEFA taste has yet to be tested systematically.

Passive movement across the membrane is inhibited by the presence of albumin, fatty acid binding proteins, or fatty acid transport proteins, which tend have greater affinity for longer chain (18+ carbons) fatty acids than medium or short chain fatty acids [58,61]. Affinity for the membrane itself is also lower for medium to short chain fatty acids, which are less hydrophobic, than long chain fatty acids [58]. Overall, shorter chain fatty acids diffuse more rapidly than longer chain fatty acids, as extra steps are required for long chain fatty acids (i.e., release from binding proteins, dimers, or micelles; slower desorption from the inner leaf of the membrane; movement into the water layer) [65]. Additionally, saturated fatty acids diffuse more slowly than unsaturated fatty acids of the same chain length, likely because the unsaturated fatty acids are more soluble than saturated fatty acids and also because the unsaturated fatty acids are already “folded” which may ease the movement from the outer leaflet to the inner leaflet of the phospholipid bilayer [59]. Generally, passive diffusion is capable of providing fatty acids to cells in normal physiological ranges (nanomolar and above) [61,66], but the level of control observed in many cellular models indicates diffusion gradients are not the only, or perhaps even primary, mechanism driving and regulating fatty acid uptake [28–33]. Nevertheless, diffusion could still play an important role for NEFA taste. In most studies of fatty acid uptake in cells, interstitial fluid or serum is the medium by which the NEFA are delivered to the cell surface. These matrices would contain albumin, which binds fatty acids and inhibits passive diffusion by limiting the amount of fatty acid available to freely absorb into the phospholipid bilayer [64,67–70]. To more accurately represent the physiological partitioning of NEFA in model systems, albumin is also frequently incorporated in the fluid media when studying NEFA diffusion through phospholipid bilayers. Saliva at taste cells would have an inherently different protein make-up from serum or interstitial fluid, lacking albumin but containing lipocalin-1 and proteins from the ingested food which may bind NEFA. Whether such proteins would bind NEFA and reduce passive diffusion of NEFA into taste cells (decreasing NEFA taste sensation) or help deliver the NEFA into the aqueous environment at the taste cell surface, increasing NEFA diffusion and/or receptor activation (both increasing taste sensation), is unknown.

2.2.2. CD36

Cluster of Differentiation 36 (CD36), also known as fatty acid translocase (FAT), is a glycoprotein with two transmembrane regions and both the N and C tails located intracellularly. This protein belongs to the class B scavenger receptor family and thus has a wide range of effective ligands and corresponding binding sites. The large, glycosylated extracellular portion of CD36 has several domains important to binding various stimuli, including domains that bind the antiangiogenic protein thrombospondin-1 [71,72], oxidized low density lipoproteins and apoptotic cells [72], and long chain fatty acids [73]. After stimulation by fatty acids, CD36 is ubiquitinated and degraded in cell culture models [74] and in rodent intestines [75], but this mechanism has not yet been tested in taste cells. Additionally in cell models, CD36 expression does not increase uptake of NEFA without co-location with caveolae [76], indicating that the mechanism of CD36 may require these lipid rafts. Additionally, taste bud cells cultured from both humans and rodents indicate that CD36 localization to lipid rafts is disrupted after NEFA exposure [77], which could explain decreased attraction for fat after oral fat exposure in rodents [78] and decreased sensitivity in lean humans on a high fat.
For rodents, CD36 expression is highest in circumvallate and foliate papillae, with limited expression in fungiform papillae [88,89]. In humans, CD36 has been located on the apical surface of taste cells in circumvallate and foliate papillae [90], but the protein may also be present in fungiform papillae [91]. CD36 null mice completely lose the innate attraction to NEFA emulsions [89,92], and do not display the activation of gustatory neurons in the brain that are observed upon oral NEFA deposition in wild-type mice [93]. These data indicate that CD36 is necessary for oral NEFA detection in mice. Additionally, the use of small interfering RNA to decrease tongue expression of CD36 also results in decreased preference for linoleic acid [94]. Some have shown that diet-induced obese rats have lower expression of CD36 in circumvallate taste buds than normal weight animals on a control diet [95]. Other mouse literature indicates no difference in fasting circumvallate papillae expression of CD36 between obese and normal mice but a difference in the down regulation of CD36 1 h after a meal, with normal mice showing decreased expression of CD36 and obese mice showing no change [96]. Similar studies indicate CD36 expression in mouse circumvallate papillae is directly correlated with oral fat exposure, as both acute diet and direct oil deposition on the lingual surface result in decreased expression [78]. One study also suggests a role of glucagon-like protein 1 (GLP-1) signaling in CD36 expression, as GLP-1 null mice also do not display downregulation of circumvallate CD36 postprandially [97]. Such changes in expression of the CD36 protein are potential mechanisms seen for differences in sensitivity to oral NEFA taste between lean and overweight/obese individuals [18,20,21], differences in learning observed between lean and obese individuals for repeated NEFA threshold testing [98], and improved NEFA sensitivity after a reduced-fat diet [19]. However, studies on human CD36 expression and NEFA sensitivity need to be conducted to confirm these hypotheses.

Human deficiency in CD36 has been studied because of implications for cardiovascular health. Two forms of human CD36 deficiency are known: type I, lack of CD36 expression in platelets and monocytes, and type II, expression in monocytes but not in platelets; type II is very rare among Caucasians (0.3%), but occurs at rates of 2–10% in Asians and Africans [99–102]. These deficiencies are due to genetic mutations in the CD36 gene that are different than those currently identified in fat taste sensitivity studies, so knowledge about any direct effect of type I or type II CD36 deficiency on taste is lacking [22,103,104]. Humans with CD36 deficiency have higher fasting and post-prandial FFA and TG and also are more likely to be insulin resistant, even without excess body fat accumulation [86]. Reports do not indicate reduced expression in other cell types in these CD36 deficient individuals, though it is unlikely that gustatory tissue has been tested. However, other variants in the human CD36 gene may be associated with fatness perception [103] and detection thresholds for oleic acid [22]. Thus, several genetic variants in CD36 do appear to influence oral perception of fat, but whether other clinically observed deficiencies in CD36 will also affect taste responses has yet to be studied.

2.2.3. GPCRs

Studies now indicate that previously orphaned GPCRs may be selective for fatty acids in various tissues [105]. These proteins have seven transmembrane domains with the N-terminus located extracellularly and the C-terminus located internally [106,107]. In taste epithelia, two GPCRs are of particular interest: GPR120, also known as FFA4, and GPR40, also known as FFA1. Both of these proteins have been isolated in rodent taste cells, but unlike GPR120, GPR40 does not appear to be present in significant concentrations in human gustatory tissue [23]. Synthetic agonists for both GPR120 and GPR40 [108,109] and antagonists for GPR40 [110–114] have been developed, but rodent data indicate that agonists unlike NEFA for GPR 120 and 40 do not elicit a preference, [115].

GPR120/FFA4

Two isoforms of GPR120, differing only in the addition of a 16 amino acid sequence in intracellular loop 3, have been identified in human tissue [116]. Human gustatory tissue expresses the short form [23], which activates G-protein and calcium signaling [116]. In cell culture, both isoforms undergo endocytosis upon receptor activation and subsequent interaction with β-arrestin 2 [116–118], though this mechanism has yet to be confirmed for gustatory tissues. Although GPR120 is often considered an ω-3 fatty acid receptor, research shows similar potency of ω-3 fatty acids for GPR40 [23,119–123]. The broader tissue distribution of GPR120 could potentially explain the tendency to classify this GPCR as the dominant ω-3 fatty acid receptor.

GPR120 is expressed in human intestine (ileum, colon, and rectum) and lung tissues [123] as well as the apical taste cell membranes harvested from human and rodent fungiform and circumvallate papillae and rodent foliate papillae [23,124]; human foliate papillae have not been tested for the presence of GPR120. Cell models indicate greater reactivity of GPR120 to longer chain, unsaturated fatty acids, with one study showing C8 < C10 < C18:3 < C18:2 [23] and another
C8≤C18=C18:1 < C16:1 < C22:6 < 18:3 [123]. Both studies indicate relatively lower activation by compounds such as docosahexaenoic acid (DHA C22:6) and arachidonic acid (C20:4). The carboxylic head appears to be important for fatty acid binding to the GPR120 receptor, as seen through computer modeling [125] and confirmed by cell models of activation and in vivo taste testing shows greater responses to fatty acids than to alcohols [23].

GPR120 knockout mice show decreased preference for linoleic and oleic acid [126] but similar preferences for soybean emulsion as wild-type mice [127]. The knockout animals also develop greater fat mass, likely due to a lower basal metabolic rate during the light period, than wild type when fed a high-fat diet, though no difference is observed with a normal diet [128]. GPR120 knockout mice also display diminished glossopharyngeal and chorda tympani nerve responses to oral NEFA [126].

Intestinal endocrine cells also express GPR120, and activation results in the release of GLP-1 both in cell models and in mice [123]. Mouse taste bud cells also release GLP-1 in response to NEFA and a selective GLP-1 agonist [97]. Unlike CD36, GPR120 circumvallate expression in rodents is not acutely regulated by dietary exposure to fat [78,97]. Additionally, mice display no preference for solutions containing synthetic agonists of GPR120, indicating either a different ligand-receptor interaction and signaling role for these synthetic compounds or that activation of this receptor alone is not enough to stimulate a palatable NEFA taste [115].

**GPR 40/FFA1**

GPR40 is highly expressed in the human and rodent pancreas [120,121,129,130] and has also been detected in the human and rodent brain [120]. The high expression of GPR40 in the human pancreas and its important role in insulin secretion has led to numerous attempts to make synthetic GPR40 agonists, with the hope of finding new diabetes treatments [106,131]. Native ligands for GPR40 include NEFA of medium to long alkyl chain lengths, with pentadecanoic and palmitic acids (C15 and 16, respectively), showing the greatest affinity among the saturated fatty acids and the unsaturated fatty acids palmitoleic (C16:1), oleic (C18:1), linoleic (C18:2), α-linolenic (C18:3), and γ-linolenic (C18:3) all displaying similar potencies [120]. Human GPR40 appears to be more strongly activated by medium chain fatty acids than GPR120, though activation by long chain fatty acids is similar for these two proteins [23].

GPR40 has not been found in gustatory epithelia (at least at higher concentrations than surrounding non-gustatory epithelia) in humans [23] or rats [124] but has been found in mouse circumvallate, foliate, and to a lesser extent fungiform papillae [126]. Knockout models for GPR40 in mice display lower attraction to linoleic acid solutions in brief access tests than wild-type mice, indicating this protein may have a function in NEFA taste for this rodent [126]; however, other research shows no loss of preference for soybean oil emulsion by GPR40 knockout mice [127]. Like GPR120 null mice, GPR40 null mice show diminished glossopharyngeal nerve responses to NEFA stimulation, but chorda tympani nerve responses are unchanged in GPR40 knockout mice compared to wild type [126]. As with GPR120, activation of oral GPR40 in mice with synthetic agonists does not stimulate a taste preference for these solutions [115]. Additionally, as this protein has yet to be isolated from human gustatory cells, despite active testing for the protein in gustatory epithelia [23], the relevance of the mouse model data to humans remains unclear.

### 2.2.4. DRK channels

When taste cells are activated, they depolarize, which leads to release of synaptic transmitters and activation of the sensory neuron [132]. The depolarization and repolarization of the cell is controlled by voltage-gated ion channels. One class of these channels is delayed rectifying potassium (DRK) channels, which can be inhibited by polyunsaturated fatty acids [133–136]. Experiments with rat fungiform papillae indicate that this inhibition occurs only for fatty acids with two or more double bonds, with linoleic and docosahexaenoic acids being the most potent, followed by arachidonic, eicosapentaenoic, and α-linolenic acids [134,137]. DRK channels in human gustatory tissue have yet to be studied, but as these channels are important for basic maintenance and function of cellular electrical gradients, they are likely present in human taste cells. Obesity-prone rats express higher concentrations of DRK channels than obesity-resistant rats, and DRK currents are inhibited more strongly in obesity-resistant rat fungiform papillae. Additionally, linoleic acid increases preference for a subthreshold concentration of saccharin only in obesity-resistant rats [138]. However, it is important to note that these channels are involved in the signaling cascade and taste cell repolarization of multiple primary taste transduction mechanisms [132,139], and that direct stimulation of DRK channels, in the absence of other membrane receptor proteins such as CD36 or GPRs, leading to a NEFA taste signal has not been shown.

Thus, numerous methods are available for NEFA to access taste cell surfaces and initiate a taste sensation. Research on the implications for factors such as salivary or food based NEFA carriers (such as lipocalin-1 and β-lactoglobulin) and influence of pH remains to be conducted. Further studies should explore these extracellular effects on taste sensation, as these elements could be confounding comparisons across NEFA taste studies. Research in animals and cell cultures has shed light on the mechanisms of proposed NEFA taste at the taste cell surface. Evidence currently seems to favor CD36 as a prime NEFA taste receptor, especially at low concentrations of NEFA [77], but other mechanisms and receptors may still be important for various sensation dimensions.

### 3. Physiological Responses to Oral Fat Exposure

The following sections provide an overview of select physiological effects of oral fat exposure based on differences in chain-length and saturation as revealed by modified sham-feeding
(MSF) of stimuli containing fat or fatty acids. MSF studies involve oral exposure only, without swallowing, and provide strong evidence of orosensory signaling that initiates cephalic or first-phase responses. These cephalic phase responses are sensory stimulated physiological processes that initiate and modulate digestive, absorptive, and metabolic events associated with eating. They trigger and work in concert with post-ingestive responses that allow the body to mount an appropriate response to the physiological challenges posed by eating. The cephalic phase response is generally correlated with the post-absorptive response [140,141]. MSF studies document numerous and diverse important roles for orosensory stimulation by fat.

3.1. Serum Lipid Responses

Oral exposure to fat via MSF frequently results in two serum triacylglycerol (TAG) peaks—a small cephalic, or first phase, TAG peak and a larger, post-absorptive peak when paired with consumption [142]. Experimental protocols usually provide a fat load either orally or via capsules and subsequently require participants to MSF. This load, typically 30–50 g of fat, ingested immediately prior to MSF is key to seeing cephalic phase TAG elevations. Though lipid ingested 12 hours before MSF appears in the first phase response [143], loads less than 10 g or ingested more than 5 h prior to MSF have not generated a cephalic phase response [142]. With this protocol, cephalic phase elevations of TAG usually occur within 15–60 min of exposure [141,144,145] and decline, often not fully to baseline, within 60–90 min. MSF of full-fat stimuli generates stronger, more reliable cephalic phase TAG responses, for example, higher peaks and/or more sustained elevations, compared to MSF of low-fat, fat-free, or fat-mimetic products [146–149]. Although early studies on cephalic phase TAG responses relied upon MSF exposures of 2 h to examine responses, one study demonstrated a significant cephalic phase TAG response after one 20 min exposure, comparable to the duration of a meal [148]. A rise has also been noted with a single 10 sec fat exposure compared to no exposure while a significant rise was not observed after a single 10 sec exposure to a non-fat stimulus, but the responses to the non-fat and full-fat exposures did not differ from each other [148]. A combined MSF and feeding protocol mimics a meal consisting of multiple courses, so the higher and more rapid serum TAG peaks observed under this condition [150] suggests an eating pattern involving multiple oral exposures to fat may be especially problematic for individuals predisposed to cardiovascular disease (CVD). Taken together, MSF is sufficient to produce significant lipid responses, especially when exposure uses full-fat products, and these responses are greater when paired with larger but still commonly consumed amounts of fat [148].

The composition of the fat used in MSF studies, for example, saturated (SFA), monounsaturated (MUFA), or polyunsaturated (PUFA), influences TAG excursions. MSF of PUFA lead to significantly greater changes in serum TAG as measured by percent change in AUC [144] or by peak response [149] compared to MUFA. It is important to note that studies showing greater responses to PUFA collected samples for less than 8 h, but responses further out from exposure might differ as one consumption study found higher TAG concentrations in response to PUFA ingestion at 4 h but higher TAG at 8 and 24 h after MUFA and SFA exposure [151]. Thus, the time course of experimental trials likely impacts findings.

Chain length of ingested fatty acids modifies TAG responses. Long chain triglyceride (LCT) consumption, with the exception of stearic acid, increases serum TAG post-prandially; whereas, this is not reported for medium chain triglycerides (MCT) [152–154]. A longer-term study of 6 d reported that MCT did not elevate post-prandial TAG but LCT did [155]. However, fasting TAG may be differentially affected as another study lasting 6 days demonstrated an increase in fasting TAG after a diet high in MCT but not LCT [156]. We know of no MSF studies examining the effect of chain length on serum TAG responses, so the effects of chain length and fat taste are unknown.

In addition to increased serum TAG, serum NEFA and other blood lipids are impacted after MSF although the direction of change is mixed. There are reports of increased NEFA concentrations [143,157], no changes [147,158], or decreases [159]. Differences in findings likely result from the time course of the measurements, whether specific NEFA or total NEFA concentrations were measured, as well as the stimulus used. One study compared meals containing different oils: olive oil, an oil high in linoleic acid, and an oil high in oleic acid, and reported MSF of meals with olive and linoleic acid oil elevated serum NEFA but not MSF of a meal with oleic acid oil [144]. As with serum TAG concentrations, elevated NEFA concentrations are associated with increased risk of CVD [160] and lipotoxicity [161].

Genetic differences may also explain some of the variability in reported plasma TAG results, especially if participants are CD36 deficient. As described previously, CD36 deficiency is characterized by a lack of the CD36 antigen on monocytes or platelets. Although CD36 deficiency has not been described in taste receptor cells to-date, CD36-deficient individuals have elevated triglycerides, apoB-48, and NEFA concentrations after an oral fat load compared to healthy controls [86]. These individuals also typically present with metabolic syndrome [162,163]. Genetic differences in CD36 contribute to altered responses to lipid intake in general (see [164] for a review).

3.2. Serum Glucose and Insulin Responses

In healthy people, elevated blood glucose triggers insulin secretion leading to cellular glucose uptake, synthesis of fatty acids and TAG as well as fat storage. Ingestion of carbohydrates increases blood glucose concentrations with the subsequent increase in insulin, while protein and fat intake make lesser or negligible contributions to elevations in glucose [165,166]. Although fat ingestion does not stimulate glucose production, both MSF and ingestion of fat led to elevated
insulin release in a number of studies [158,167,168]. However, elevations in insulin after MSF are not uniformly observed [169,170]. Lack of findings in these experiments may stem from the fact that one study did not use a sampling protocol designed to specifically investigate cephalic phase insulin responses and may have missed the response [169] while the other tested only lean women [170] whose cephalic phase insulin responses may be lower compared to individuals with higher body mass index (BMI) [171].

Just as lipemic responses differ according to the degree of saturation of the stimulus, glycemic responses also differ. MSF as well as consumption of a mixed-macronutrient meal containing oil high in linoleic acid (PUFA) generate higher insulin concentrations between 15 and 90 min post-prandially than water [144]. MSF studies examining chain length and insulin responses have not been completed at this time.

3.3. Gut Peptides
A number of gut peptides are released upon both consumption and MSF of fat and fatty acids. The peptides that are best characterized are briefly discussed below. These include: cholecystokinin (CCK), glucagon-like peptide-1 (GLP-1), and ghrelin. A recent in-depth review of CCK and GLP-1 and high-fat diets is available [172].

3.3.1. CCK
CCK is a gut peptide secreted by the small intestine that plays a role in meal termination [173] by delaying gastric emptying and reducing GI motility [174,175]. Elevations in CCK concentrations result in decreased food intake [176,177]. In general, CCK concentrations are typically elevated after a meal, especially a meal containing fat and/or protein [178–183]. CD36 on enteroendocrine cells contributes to CCK secretion, with CD36 knock-out mice producing significantly less CCK than wild type controls after an intragastric load of fat [87]. CCK elevation occurs after MSF with fat [184,185], although not always [142,181,186], and is a less potent stimulus compared to fat consumption [184]. MSF studies exploring the effects of chain length and saturation remain to be conducted.

3.3.2. GLP-1
GLP-1 is secreted primarily by the distal small intestine and colon. It enhances the action of insulin as well as meal termination [187]. GLP-1 concentrations increase following carbohydrate and fat consumption [172,188] and also increase in rodents in anticipation of a meal [189]. The pattern of GLP-1 release differs by macronutrient, with maximal GLP-1 release after fat ingestion occurring much later than maximal concentrations after carbohydrate consumption [188,190]. Previous work found little support for a cephalic phase GLP-1 response upon ingestion [191], yet others report evidence of such a response in humans [190] and mice [192]. MSF studies of cephalic phase GLP-1 release in humans using fat stimuli are lacking, but oral stimulation with glucose of anaesthetized mice demonstrated increased circulating GLP-1 concentrations [192]. As both carbohydrate and fat are potent inducers of GLP-1 release in the intestine during ingestion, fat may also induce GLP-1 cephalic phase release, an hypothesis that needs testing.

GLP-1 is also secreted by taste receptor cells and enhances sensitivity to sweet, sour, and fat taste in rodents [97,193,194]. Data in humans are lacking; however, long-chain fatty acid treatment of mouse circumvallate cells resulted in increased GLP-1 concentration in culture, with no effect of saturation (MUFA vs. n-3 PUFA) [97]. As with CCK, a chain length of greater than 10 is necessary to evoke a rise in GLP-1 in the gut [195,196]. Whether this is the case in the oral cavity remains to be determined. In rodents, oral fat exposure decreases CD36 protein levels [78], but in GLP-1 receptor knock-out animals, CD36 receptor levels do not decrease after fat exposure [97]. Thus, GLP-1 may play a role in CD36 activity.

3.3.3. Ghrelin
Ghrelin is a gut peptide released from the stomach [197] as well as the small intestine [198], brain, and other organs [199]. Unlike the other gut peptides, increased ghrelin concentrations are believed to stimulate eating [200–202]. Although there is some debate about whether ghrelin is truly an orexigenic compound and not simply a marker of hunger [203,204], ghrelin concentrations typically [205,206], but not always [204,207], decrease after mixed meals high in carbohydrate and fat. Mixed results (lower, higher, or no change) are reported for high protein meals [205,206,208,209]. MSF of a mixed meal depressed ghrelin concentrations to the same extent as ingestion [210], while MSF prior to ingestion of a pure fat load decreased ghrelin concentrations more than ingestion alone [157]. Studies on the effect of NEFA chain length and saturation on ghrelin suppression are needed.

In summary, oral fat exposure through MSF indicates that the sensory effects of fat are capable of modulating gut peptide concentrations. Further examination of whether these changes translate into differences in the timing of or total peptide release and their impact on ingestive behavior is warranted.

3.4. Appetite
Appetitive sensations, like hunger and fullness, frequently fail to be strong predictors of time to next meal, energy intake, or weight loss [211–214] as these sensations can be ignored or overridden. Yet minimizing feelings of hunger and maximizing feelings of fullness are the goals of many weight loss strategies [215]. Conventional wisdom suggests that there is a macronutrient hierarchy for satiety and satiation sensations; namely, that fat is the least satiating and protein the most satiating with carbohydrate in between, but these relationships are not always borne out [180]. Some of the physiological changes discussed throughout this paper have also been associated with appetitive sensations, for example, CCK and gastric emptying, but these changes are best thought of as part of an appetitive cascade. A change of one peptide within physiological ranges may not be sufficient to drive the system [216].

With these caveats noted, MSF has independent effects as well as additive effects on appetitive signals when paired with
ingestion. MSF plus an oral fat load produces greater feelings of satisfaction and fullness than an oral fat load alone [157]. MSF of high-fat meals leads to reports of decreased hunger and increased fullness [144] compared to water, but these effects may be specific to the fats used, as only oleic and linoleic acid rich oils induced these changes while olive oil did not. The differences in FA composition between the stimuli were not reported, only that the macronutrient profile was similar, so differences in FA composition might explain differences observed among the stimuli. In contrast, one MSF study comparing a non-fat stimulus to a full-fat stimulus reported no differences in hunger or fullness between treatments [170]. This study examined lean women who have demonstrated reduced cephalic phase insulin release [171]. It may be the case that appetitive sensations are less sensitive to the effects of fat in this population. Specific examination of chain length and saturation effects using MSF, to our knowledge, has not been undertaken.

### 3.5. Thermogenic Effects

Diet-induced thermogenesis (DIT) is the energy expended after eating due to digestion, absorption, and, predominantly, the storage of nutrients [217,218]. DIT accounts for up to 10% of total energy expenditure [219] and is influenced by the macronutrient composition of the diet [220,221]. When measured separately, DIT from protein consumption elevates energy expenditure by 20–30% [222], alcohol by 10–30% [223], carbohydrate by 5–10% [222], and fat by 0–3% [222]. Although it is interesting to note the differences between individual macronutrients, it is rare to consume macronutrients in isolation. In one trial, MSF compared to ingestion of a high-fat meal did not result in differences in DIT, and neither condition elicited responses that varied from water exposure [224]. No differences in DIT were noted when MSF of butter (high SFA) was compared to MSF of margarine (high MUFA) [169]; however, this study should be interpreted cautiously as during the time it was conducted, margarine typically contained a great deal of trans-MUFA, which might not allow for a true comparison between SFA and MUFA. Studies on the role of MSF and chain length with regards to DIT effects are lacking.

### 3.6. Gallbladder/Bile Acid

As noted above, MSF with consumption of fat triggers cholecystokinin (CCK) release [178], which in turn causes the gallbladder to contract and bile acid to be released. Ingestion of food provides a stronger stimulus to induce CCK release, but both feeding and MSF result in significant reductions in gallbladder volume [181,225]. In mice, MSF of long-chain MUFA and PUFA increase bile acid release [89]. This change does not occur with either saturated long-chain or medium-chain fatty acids. The bile response is also abolished in CD36 knock-out mice exposed to linoleic acid, suggesting that the release of bile is mediated by CD36 activity in the oral cavity [89].

This section summarizes a number of the physiological effects of oral exposure to fat and fatty acids. One of the criteria suggested as necessary for classifying a taste quality as a basic taste is that it should generate a physiological change [226]. Clearly, oral exposure to fatty acids generates cephalic phase responses that have implications for macronutrient digestion, absorption, and metabolism. Although previous study designs have focused on chain length and saturation as variables, rather than lumping fatty acids into broad categories of chain length and saturation, future research may benefit from contrasting responses to individual fatty acids. That is, the classification of PUFA does not capture the differential effects on health outcomes between the 20-carbon eicosapentaenoic and arachidonic acids, the former is largely anti-inflammatory while the latter is largely pro-inflammatory [227], while stearic acid, a SFA, does not follow the typical pattern of saturated fat-induced serum TAG concentration elevation [153].

### 4. Outlook

Our sense of taste serves as a sentinel, promoting acceptance or rejection of foods. Fatty acids interact with taste cells in multiple ways, playing important roles in gustation, as well as olfaction and, somatosensation, thereby contributing to the overall flavor perception of foods. Gustation also triggers multiple important physiological responses downstream from the oral cavity. These responses are necessary for the digestion of foods and the absorption and metabolism of essential nutrients. New research in this field will likely focus on translation of our basic knowledge about NEFA taste receptors and mechanisms into how genetic differences may influence the human taste response to fat, how the matrix of a food alters the oral sensation of NEFA, and how both of these factors influence human physiology and overall health. Understanding of the individual variability attributable to genetics and environmental factors on physiological responses to oral fat will aid in dietary recommendations to control serum lipids and reduce cardiovascular disease risks. Additionally, isolating the effects of food constituents and chemical properties, like pH, on the taste system will aid in development of functional foods to control physiological responses. Consequently better characterization of how NEFA interact with the taste system and the responses to this interaction will provide insights for dietary practices to optimize health.

### References


[116] Tucker et al.


Witteman, B. J., Jebbink, M. C., Hopman, W. P., Masclee, A. M., Lamers, C.
Bachman, J. L. and Raynor, H. A. (2012) Effects of manipulating eating fre-