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A Review of the Evidence Supporting the Taste of Non-esterified Fatty Acids in Humans

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Dietary fats contribute to the flavor of foods by multiple mechanisms. A role for their taste has only recently gained credence. Current evidence indicates non-esterified fatty acids (NEFA) are the effective stimuli for the taste component. CD36 and GPR120 are putative receptors, but may not fully account for the totality of the range of sensations elicited by fatty acids. The sensory quality of long-chain NEFA is not adequately characterized by commonly accepted taste primary qualities and has been termed oleogustus. There is marked individual variability in sensitivity to the taste of NEFA prompting hypotheses of genetic, and environmental determinants. Though an association with BMI has been proposed, the preponderance of evidence is not supportive. The importance of oleogustus has not been fully established, but likely contributes to flavor which influences food choice as well as lipid metabolism and chronic disease risk. A better understanding of oleogustus may provide insights useful for product formulation.

Key words:

fat taste, oleogustus, dietary fat, sensory, taste threshold, BMI, lipid, taste quality, lipid metabolism, food choice

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Introduction

Humans derive energy from three dietary nutrients; carbohydrate, protein and fat (alcohol is not a nutrient). Historically, each was primarily ingested as a macromolecule with limited taste activity (i.e., our evolutionary ancestors were unlikely to have the plethora of sweet carbohydrate foods that we have; even fruits would have been much less sweet before the advent of agriculture). The signal was amplified in the oral cavity through the oral phase of digestion, but to a large degree, decisions to swallow energy-yielding foods were based on subtle, transient taste sensations. This observation is relevant to any discussion of taste primaries as it indicates strength or clarity of sensation is not a critical defining property.

A compelling case can be made that sweet taste exists to facilitate the discovery of carbohydrate in the environment and its ingestion [1]. Carbohydrate generally, and simple sugars more specifically, are often vilified as contributors to chronic disease risk. However, it should be noted that detection and desired consumption of carbohydrate, including simple sugars, is highly adaptive. The sugar lactose constitutes about 40% of the energy of breast milk and glucose is used by every cell in our bodies. Though some natural products contain high concentrations of simple sugars (e.g., honey, some fruits), starch was, and is, the predominant form of dietary carbohydrate. Accumulating evidence suggests starch is an effective taste stimulus in rodents [2, 3] and possibly in humans [4], but it must be hydrolyzed by salivary amylase to mono or disaccharide units in the oral cavity to elicit a clear signal of sweetness. Among those subscribing to the concept that the sense of taste is comprised of a limited

number of primary qualities, sweetness is universally recognized as a taste primary, but the starchiness of carbohydrates is not.

In nature, proteins are also weak taste stimuli. The recent identification of the T1R1-T1R3 glutamate receptor is reported to code for a unique quality that aids identification of protein sources in the environment [5, 6]. The sensation quality is termed umami, translated from Japanese to English as "delicious taste." Importantly, that may be an apt description for low concentrations in appropriate food systems, but umami-tasting L-glutamate is not preferred over sucrose in aqueous solutions [7]. Further, higher concentrations in simple aqueous media are rated as unpleasant by most consumers. When proteins are hydrolyzed, the resulting mixture of amino acids is distinctly bitter and objectionable unless one becomes accustomed to them (e.g., infants regularly consuming formulas with hydrolyzed protein [8].The bitter amino acids (L-Leu, -Phe, -Trp, -Tyr, and D- and L-Cys, -Met) dominate the sensory profile of hydrolysates which also contain amino acids that are slightly sweet (e.g., Gly, D-His, -Phe, -Trp, -Tyr, and L-Ala, -Leu)) or neutral (e.g., (D-Ala, -G~u, L-His, and D- and L-Arg, -As~, -Ile, -Lys, -Pro, -Ser, -Thr, -Val). Some peptides such as aspartame [9] and proteins (monellin and thaumatin) [10] are sweet, but these are not strongly preferred. Due, in part, to questions about the quality of sensations derived from ligands for the T1R1-T1R3 receptor, the acceptance of umami as a basic quality remains less than universal.

The concept of fat taste is more recent, but acceptance is growing. There is little evidence that triacylglycerol, the predominant form of dietary (and body) fat is an effective taste stimulus [11-13]. However, its fatty acid constituents, non-esterified fatty acids (NEFA)

may be. These compounds are inherently present in fatty foods in low concentrations and occur in high concentrations in rancid products. They may also arise through the activity of endogenously secreted salivary lipase during the oral phase of digestion [14]. The latter mechanism is well accepted in rodents [11], but has only recently been supported in humans [14]. Prior views held that humans either lacked lingual lipase or had non-functional concentrations. Measurement of the enzyme has proven difficult, but documentation of the end product of its activity under physiological conditions strongly supports its role [14]. In simple aqueous solutions, NEFA are unpleasant. When present in high concentrations, they appear to act as warning signals to discourage ingestion of what may be unwholesome (rancid) foods. This seems inconsistent with the facts that there are essential fatty acids, dietary lipids are valuable dense energy sources, and fats serve multiple critical functions in the body. Their consumption should be encouraged. This may occur in humans for lower concentrations sensed by multiple cues, potentially including taste, but evidence is lacking on this point. The taste sensation elicited by long-chain, unsaturated fatty acids has been termed "oleogustus" based on the Latin roots; oleo – fatty and gustus – taste. Evidence further supporting the effectiveness of NEFA as gustatory stimuli is the topic of this review.

Transduction mechanisms

NEFA vs TAG

Throughout the body, fat is typically sensed as NEFA rather than triacylglycerides (TAGs). TAGs are the storage form of fat, but they are transported and packaged as larger lipid conglomerations, such as lipid droplets in adipose tissue or chylomicrons and lipoproteins in

blood. To serve as signaling molecules or to traverse cell membranes, TAG must be hydrolyzed. In the intestines or at the cell surfaces of peripheral tissues, TAG must be broken down so NEFA can bind to cell membrane receptors or be absorbed. In the oral cavity, NEFA are considered the likely stimulus for oleogustus. While TAGs certainly contribute to the structure, texture, and degradation qualities of food, NEFA are the likely chemical stimulus for taste because they are small enough to interact with chemoreceptors on taste cell surfaces. Indeed, NEFA, but not esterified fatty acids, can promote the depolarization of taste cells [12] and stimulate the release of digestive enzymes [15, 16]. Monoglycerides may also be small enough to be effective taste stimuli, but whether the presence of the esterified glycerol alters oleogustus sensation has not been tested.

Taste vs olfaction vs irritation

NEFA are multi-modal chemosensory stimuli. Much of the debate on the existence of oleogustus has focused on whether the sensation has a true taste component. It is well established that NEFA contribute to the textural properties of lipids and have odor and irritating qualities. Odors of NEFA change according to chain length and degree of saturation, a phenomenon that is now understood to be true in taste as well [17-20]. Long chain fatty acids can be discriminated by odor, both ortho- and retronasally, but using nose clips eliminates this sensation [21]; thus, to test NEFA taste, use of nose clips is recommended. Irritation is more challenging to eliminate, as putative NEFA receptors are present on trigeminal neurons [22] and there is rich somatosensory innervation around taste buds. However, individuals desensitized to chemical irritation through use of capsaicin were able to achieve similar detection thresholds

for NEFA, indicating a mechanism for detection that is likely independent of chemical irritancy [23]. Additionally, thresholds are lower for polyunsaturated fatty acids (linoleic, linolenic) compared to monounsaturated (oleic) fatty acids contrary to expectations that higher concentrations should be more easily detected only through their tactile properties [19].

Receptors

NEFA introduced into the oral cavity via food or generated by lipase within the oral cavity as transported to selected receptors, potentially added by salivary proteins. Currently the prime receptor candidates for oleogustus by humans are cluster of differentiation 36 (CD36) and G-protein coupled receptor 120 (GPR120), also known as free fatty acid receptor 4 (FFA4) [24, 25]. In mice, GPR40 also may function to detect NEFA [26], but this receptor has not been identified in the taste tissue of humans [17] or rats [27]. Cell culture studies indicate that CD36 responds to lower concentrations of NEFA while GPR120 is activated in the presence of higher concentrations [24], potentially yielding different responses across a range of NEFA that could be found in foods. How these different responses translate into the human experience remains untested.

CD36 is a scavenger receptor, detecting long-chain, non-esterified fatty acids and other lipids in a wide variety of human tissues [28-34]. Rodents that lack a functional CD36 receptor in the oral cavity have decreased affinity for NEFA and TAG [16, 35, 36], and exposure to NEFA and TAG may decrease expression of this protein in taste cell tissue of rodents [37, 38, 39]. If this is true for humans as well, and if CD36 is indeed a receptor for oleogustus, then exposure

to fat could modulate oleogustus sensation, a concept that will be discussed in a latter section of this review.

GPR120/FFAR4 is a g-protein coupled receptor (GPCR) that binds long and medium chain, non-esterified fatty acids. It would share more similarities in cellular signaling to the tastes of sweet, umami, and bitter, which are also detected via GPCRs [40]. Other GPCRs with different fatty acid specificities, such as GPR40/FFAR1, GPR43/FFAR2, and GPR41/FFAR3, and GPR84, have been suggested as receptors for oleogustus, but GPR120 is currently the only GPCR isolated from human taste cells [17].

Delayed rectifying potassium channels (DRK) have also been implicated in signaling oleogustus [12, 41], though these channels are integral to the signaling cascade for other tastes as well. DRK permit current flow from taste cells help to re-establish their resting energy state after depolarization in response to a stimulus. If current flow through these channels is blocked by a NEFA, potassium ions would accumulate in the cell resulting in depolarize and generation of a signal. NEFA blockage of DRK may lead to fat detection via their modification of sensations from other taste qualities [41]. Additionally, non-esterified fatty acids are capable of passive diffusion through cell membranes [42-44], and thus may not even need a receptor in order to influence cellular activity.

Notably, the identified receptors in humans (CD36 and GPR120) respond predominantly to long-chain fatty acids (C14+). While medium and short chain fatty acids are clearly sensed in the oral cavity [17, 18, 20], the mechanism(s) for their detection has/have been less thoroughly investigated. Presumably, the shortest chains could be detected through acid sensing

mechanisms as sour tastants, and these compounds have been described as sour [20]. All NEFA are fundamentally still acids, and yet the shorter chain lengths would have greater availability of protons on a mass basis.

Quality

While all NEFA are acids, in a sorting experiment, only the short chain NEFA (up to C6) were shown to have predominantly sour taste characteristics [20]. The sensation experienced from medium chain NEFA is unique from both shorter and longer chains (oleogustus), and may be more of an irritancy than a taste, at least for some individuals [18, 20]. Long chain NEFA are the dominant candidates for the true oleogustus sensation, though whether medium or short chain NEFA possess this quality at a lower intensity than their other characteristics is yet uncertain.

One of the prime considerations for the definition of a taste is that it should be unique from other taste sensations. Data from a variety of studies indicated that the sensations experienced from NEFA ranged from “stinging,” “soap-like,” “bitter,” “sour,” [17, 18, 20], to “dirty socks,” “moth balls,” and “grandma’s attic” (personal observations during studies [18, 19, 20]). Clearly, a lack of descriptive terminology exists to describe the sensation generated by NEFA. Due to this, a sorting experiment was devised [20] which allows participants to sort stimuli by similarity, rather than asking panelists to use labels to describe a sensation. This experiment demonstrated that short chain NEFA were most similar to sour stimuli such as citric acid, while medium and long chain NEFA were distinct. When all tastes were given, medium and long chain NEFA were grouped with other bitter (unpleasant) stimuli, but when only bitter

stimuli and NEFA were presented, the medium and long chain NEFA were sorted separately from the other tastants. This experiment demonstrated the sensation of NEFA was perceptually distinct from other tastes and was the impetus for generating the term "oleogustus" to describe the sensation of NEFA.

Individual variability

Given the subtlety of threshold sensations and lack of common awareness about the sensations evoked by oral NEFA exposure, it is essential to use tightly controlled testing protocols to answer mechanistic and functional questions about oral fat exposure. Failure to account for individual differences in sensitivity and responsiveness will yield higher variance and hamper identification of important outcomes. Several sources of inter and intra-individual variability are summarized below.

Genetics

Multiple groups report thresholds ranging over four orders of magnitude [45-47]. In some cases, distributions appear highly skewed or bimodal [48]. With repeated testing, thresholds decline markedly in some individuals, but remain relatively unchanged in others [49]. These results, coupled with increasing evidence of variability in many genes controlling lipid metabolism, (e.g. lipases and fatty acid binding and transport proteins [50-52], suggest a genetic basis to fat taste. This is bolstered by findings that thresholds in humans are altered by SNPs in the gene coding for CD36 [24]. In one trial of African American adults, three polymorphisms were related to the perceived creaminess and fat content of salad dressings [53]. Next, it was noted that one SNP (rs1761667) linked to reduced CD36 expression, was

associated with fat detection thresholds in obese adults [54]. Individuals homozygous for the G-allele were eight times more sensitive to oleic acid and triolein than individuals homozygous for the A-allele. This was followed by trials demonstrating the same relationship in obese Algerian children, but not their lean counterparts [55]; and Sardinian normal weight adults [56]. However, another trial failed to replicate these findings [57]. Analyses of SNPS in other purported fatty acid receptors (e.g., GPR120) and pedigree (family) studies are currently underway that will further determine the genetic basis for variation in fat taste responsiveness to NEFA.

BMI

Because dietary fat intake has been linked to energy intake, body weight and chronic diseases risk, there has been considerable interest in identifying the drivers of fat consumption. Increasing research activity is focusing on the oral sensory impression of dietary fats in people who are lean, overweight and obese. There is a large literature on dietary fat preference and intake that lies outside the scope of this review. We focus here on sensitivity and responsiveness (ability to scale sensory intensity with graded concentrations) to NEFA by people of different weight phenotypes.

Findings from multiple studies of taste detection thresholds in individuals who are lean, overweight and/or obese are summarized in Table 1. Differences in fatty acid stimulus, methodology, and stimulus delivery vehicle (water versus milk) and testing paradigm hamper direct cross-study comparisons. Some trials where testing was conducted with oleic acid justify

its use by noting its predominance in the diet. Others tested with linoleic acid because it is an essential fatty acid and sensitivity to it is greater than to oleic acid [19] and so it is a more sensitive index of the lower limits of sensory system capacity. Others have assessed multiple NEFA to gain insights on receptor mechanisms. Thus, there is a valid rationale for each. For most trials, the sample sizes are very small. Though some researchers reported BMI-related differences [55,57,62,63] a large body of evidence indicates there is no significant differences between BMI groups [47, 58-61]. Among the three larger trials, one [47] reports no difference and two [55, 57] observed higher thresholds for individuals with obesity compared to lean individuals. Interestingly, these two trials tested children and adolescents with BMI status determined by BMI z-scores whereas all other trials tested adults and used the standard BMI categorization. In one report, no difference was noted upon initial testing, but after six trials, the combined group of lean and overweight adults showed greater reductions of thresholds and values were lower than those with obesity [60]. There are no reports of greater sensitivity among those who are overweight or obese. Thus, presently there are insufficient data to draw firm conclusions about a relationship between BMI and threshold sensitivity. Even if differences exist, they are generally subtle. Thresholds have not been significantly associated with appetitive sensations [62, 60, 63]. Positive associations between fat taste and fat intake have been reported [60], but not consistently [62]. Taken together, the dietary and health implications of possible BMI-related threshold differences are uncertain.

Several studies have explored the association between BMI and intensity ratings for graded concentrations of NEFA. In the largest trial (N=735) no significant association was reported between percent body fat and intensity ratings, nor did groups categorized as lean,

overweight and obese differ [64]. Similar results have been reported by others [59]. One trial reported an inverse association between intensity ratings for linoleic acid and BMI [65]. However, the authors note that individuals with obesity were present in all categories of taste responsiveness (i.e., 8, 3, 3, 2 individuals in the low to high performance quartiles). Six weeks of energy restriction in individuals who were overweight or obese led to improved fat ranking accuracy relative to baseline when the diet was lower in fat, but not when energy restriction only entailed reduced portion sizes [66]. However, no comparison between the two diet groups was reported precluding assessment of a BMI effect. In a trial where participants were classified by taste sensitivity performance rather than BMI, those designated as hyposensitive were less accurate in ranking samples varying in oleic acid concentration [45]. The mean BMI value was significantly higher in the hyposensitive group, but this group included only 6 individuals who were overweight and one who was obese. Another trial reported hyposensitive individuals performed more poorly on a ranking task, but this trial included only 9 overweight individuals (4 in the hypersensitive and 5 in the hyporesponsive groups) and there were no individuals with obesity [45]. Therefore, the weight of evidence does not support a BMI-related difference in intensity judgments for NEFA.

Sex

Female rodents have lower NEFA detection and hedonic thresholds than male rodents [67]. Sex effects have not been systematically studied in humans, but observations generally fail to support differences in sensitivity between males and females [68-71]. One trial did note females showed greater declines in threshold values with repeated testing, but there were no

sex differences in threshold sensitivity at baseline [72]. Limited data suggest females assign higher intensity ratings for strong linoleic acid concentrations [73].Thus, the limited available evidence suggests no sex difference for thresholds and only very limited evidence of greater intensity ratings by females for higher of NEFA.

Age

There have been no studies of oleogustus across the lifecycle. However the limited available data suggests children assign higher intensity ratings to NEFA than older individuals whereas this was not the case for a sweet stimulus in the same study [73]. Thus, the reports were stimulus specific. It is also notable that the larger threshold studies that reported BMI effects were conducted with children and adolescents.

Recent diet

Adherence to a high fat diet reportedly alters taste responses selectively. In a murine model, 10 weeks of high fat feeding diminished responses to saccharin and acesulfame-potassium, altered signaling to denatonium benzoate and did not have an effect on monopotassium glutamate [74]. In rats, adherence to a high fat diet for 8 weeks resulted in greater weight gain than in control animals and lower mRNA expression levels of CD36 in circumvallate papillae [38]. Though obesity itself may alter taste responses [47, 54], effects of dietary fat intake, independent of body fat, on oral fat detection have been documented. CD36 expression in mouse circumvallate papillae is reduced following even low levels of oral fat exposure achieved by acute diet or direct oil deposition on the lingual surface [37]. This effect may only occur in lean animals [75]. In normal weight, but not obese mice, there is a down

regulation of CD36 one hour after oral fat exposure [37]. However, experimentally reducing the expression of CD36 in obesity-prone and obesity-resistant rats leads to comparable reductions of preference for linoleic acid [38]. In contrast, others report that adherence to a high fat diet for 3 or 14 days leads to increased expression of CD36 in circumvallate papillae in obesity-prone, but not obesity-resistant rats [76]. Collectively, these observations support an important influence of recent diet on oleogustus, though the directionality is not clear. It is hypothesized that recent diet may also alter human sensitivity to NEFA, possibly due to an effect on CD36 levels as compared to simple fatigue or adaptation, which would occur rapidly but transiently with repeated stimulation. Taste-active compounds may also gain access to taste cells via a vascular route and modify taste cell activity [77]. This has been proposed for fatty acids [12], but never tested.

Survey data in humans (N=223) indicate that total fat and monounsaturated fat intake at a meal prior to sensory testing was significantly negatively associated with intensity ratings for linoleic acid [64]. A trend was noted for polyunsaturated fat intake. Effects were strongest for obese individuals. In a controlled feeding trial, consumption of a high fat diet was not associated with a significantly higher oleic acid threshold, though there was a trend in this direction [59]. When participants were divided into those who were lean and obese, higher thresholds were only observed in lean participants. Consumption of a low fat diet was associated with lower thresholds for lean and obese participants. An additional trial reported that dietary energy restriction for 6 weeks in obese adults led to lower fat detection thresholds, but this did not differ with dietary fat composition [66].

Thus, oral fat exposure may alter sensitivity to NEFA by reducing apical receptor levels as well as adaptation and/or augmentation due to circulating NEFA that could modify taste cell responsiveness via effects on the basal region of the cell.

Threshold methodology differences

Threshold measures have inherently high variability in the taste and smell literature, as factors such as fatigue and false positives greatly influence the outcomes of the test [78], and many threshold measures originally designed for analysis of group data have been applied to individual assessments. For example, much of the research on oleogustus has used a variation of the ASTM E679 method. This method is designed to identify thresholds in groups. Each person is given an ascending series of pairs or trios of stimuli, and asked to identify the stimulus of interest. As this results in only a few data points per person, this method is not suitable for classifying individuals due to the high probability of false positives in the limited number of individual data points. Rather, this method should be used for studying larger groups of people, which yields a larger dataset that can be adjusted for chance. To compare groups of people with this method, the individuals should be assigned to groups before analyzing the data, and then data should be analyzed as a whole. If individual threshold measures are needed, then other methods, with more data points per individual and ideally more testing sessions, are more appropriate. Literature on threshold measurements also acknowledges that these measurements are expected to decrease with multiple tests, as individuals become better at the task (ASTM E679, [79]). Thus, the use of unsuitable methods creates even more variability in the research on oleogustus, as often too few points are available per person for confident assessments.

Metabolic consequence

Orosensory detection of dietary fats initiates neural signals that modify lipid ingestion, digestion and metabolism. This implicates taste in the risk for many current chronic diseases.

Intake

The interest in consuming high fat foods is largely based on the sensory properties they impart to foods. Dietary TAG contributes qualities that are often viewed positively, and this has been supported by brain imaging studies showing activation of reward centers to stimulus fat content [80], due to [81] or independent of texture [82]. However, NEFA add undesirable flavor notes to foods (e.g., rancidity) and can discourage consumption. Thus, the food industry attempts to keep concentrations in most foods below detection limits. Little is known about the oral conditions that influence taste responses to NEFA. They will likely be modulated by factors such as the matrix in which the NEFA occur, oral temperature, salivary flow rate/composition and recent diet.

Oral fat exposure may influence ingestive behavior by multiple mechanisms effecting host physiology as well. The presence of fat in the mouth, independent of ingestion, modifies appetite [83]; activates brain reward centers [84, 81, 80]; enhances gastrin, gastric acid and ghrelin secretion from the stomach [85, 86]; speeds gastric emptying [87, 83]; promotes release of gut "satiety" peptides such a CCK and GLP-1 [88, 85, 83, 86]; stimulates endocannabinoid mobilization in the small intestine [89]; alters lipid transport in the GI tract [90-93]; and augments pancreatic exocrine [15, 16] and endocrine [94] secretions. Additionally, enterocyte fatty acid oxidation, which may be modulated by oral fat signaling, may influence feeding [92,

95, 96]. An open question is what is the effective oral stimulus? It may be the molecule itself, its sensory property (intensity, quality, hedonic value) or its potential energy yield.

Recent evidence draws a distinction between a molecule's sensory quality and its energy content with respect to its reward value. Sensory qualities may hold innate or acquired information that guides ingestive behavior. An inherent liking for fat, let alone fatty acids, has been difficult to document. One report noted fetal exposure to oil led to decreased fetal drinking, but this could be attributable to the iodine added to the oil [97]. Studies of infant feeding have revealed no preference for higher fat milk [98-100]. Indeed, the hypothesis in this work was that a higher fat content would serve as a signal to terminate a feed. However, exposure of children to novel high fat foods leads to a low neophobic response [101] that would theoretically result in greater intake.

The importance of learning for fat preference has been documented. Participants placed on a reduced fat diet without access to foods containing fat replacers for 12 weeks exhibited a preference for lower fat concentrations in foods whereas those allowed to use products with fat replacers showed no change [102]. Given that the two groups were adhering to a comparable fat reduction (metabolic challenge), the shift only in the group deprived of orosensory exposure to fat indicates the preferred fat level is determined more by frequency of exposure or familiarity than metabolic cues. However, others using more naturalistic but less stringent experimental trials have not reported associations between dietary fat intake and fat preference [103].

More recent work suggests the presence of an energy sensing capability in the oral cavity. In the case of sweetness, at appropriate concentrations, sucralose and sucrose are

equally effective at stimulating dopamine release (an index of brain reward system activation) [82]. However, while sweet receptor knockout mice maintain responsiveness to sucrose they have a diminished response to sucralose. In other work, neural and behavioral responses to sweeteners persist after T1R3 is knocked out so no sweetness is being detected [104]. Further, iso-energetic sweet and tasteless stimuli activate reward centers comparably [105]. This all indicates that the energy value of the sucrose holds rewarding properties that are detected in the oral cavity. The mechanism for this effect is not known as there is no known energy detector in the oral cavity. It may entail preferential activation of an as yet poorly characterized secondary sweet taste receptor or transduction mechanism [104] by nutritive versus non-nutritive sweeteners except, the effect also seems to hold for NEFA. With dietary fat, administration of nutritionally inconsequential puffs of milk containing high fat stimuli led to greater brain reward activation than milk samples containing lower fat concentrations [106]. Early event-related potentials (ERP's), reflecting taste properties following oral exposure to graded fat concentrations revealed no differential responses. Reward signals, reflecting higher order processing, were altered by fat content, independently of taste intensity or discrimination. Taken together, the findings suggest discrimination of the energy value of the milk samples independent of their taste. While new, such findings should not be surprising as the importance of sensory qualities lies in their prediction of metabolic consequence while energy is actually required for life. What sensory system is involved in energy detection is not known.

Lipid Metabolism

At the level of gut physiology, emerging evidence indicates the intestine influences lipid metabolism by regulating the absorption efficiency of lipid from the lumen, its trafficking in the enterocyte (i.e., packaging into chylomicrons or storage as cytoplasmic lipid droplets) and the rate of lipid secretion into the circulation. An oral sensory signal is posited as modulating these processes [107]. Prior work reveals a substantive quantity of lipid consumed in one meal is stored probablhy in enterocytes until it is mobilized by sensory signaling, most effectively by dietary fat, at the onset of the next eating event [93]. These data raise new questions about the relationship between taste and gut function. In conjunction with new evidence for common transduction mechanisms in the oral cavity and GI tract (e.g., [108, 109]), there is great interest in NEFA as signaling molecules throughout the GI tract.

A key function of the sense of taste is to provide a pre-ingestive signal that allows the organism to decide whether or not to ingest a substance and to prime the body to initiate an appropriate physiological and/or behavioral response to the impending metabolic challenge. A rapid neurally-mediated reflex, termed a first or cephalic phase response serves this purpose. Gustatory stimulation generates an afferent signal that is conveyed to the cortex leading to activation of vagal efferent pathways that elicit responses including, but not limited to salivation, gastric acid secretion, gastric motility and pancreatic exocrine and endocrine secretions [110-113]. To our knowledge, the first evidence that oral fat exposure was an effective cephalic phase stimulus was provided by studies revealing rapid post-prandial elevations of vitamin A (a fat soluble substance) following sham feeding that were blocked by pre-treatment with atropine (a parasympathetic antagonist) [114]. Later studies in rats showed that a nutritionally inconsequential (0.1ml) oral exposure to corn oil provoked a prolonged

post-prandial rise of triacylglycerol (TAG) that did not occur with exposure to water or a saccharin solution [2] This early work set the stage for numerous studies exploring the effect of oral fat exposure on lipid metabolism

Phenomenologically, oral fat exposure results in augmented first and second phase elevations of circulating TAG. In a trial exposing 25 healthy adults to cream cheese that did or did not contain fat, participants exhibited a greater spike in circulating TAG in the 15-30 minute period following oral exposure to, but not ingestion of, the fat containing stimulus [115] This has been confirmed in subsequent studies [93, 47]. Further, it has been shown that a single 10 second oral exposure is sufficient to elicit the response, defined as a TAG rise of $\geq$10mg/dl, and the test-retest reliability was 75% [116].

The mechanisms underlying the oral fat exposure effects on lipid metabolism are not well characterized. The first phase response appears to reflect mobilization of lipid, probably stored in enterocytes from the prior meal into the circulation. Stable isotope studies document a sharp and rapid rise in TAG enriched with fat consumed the night before (14 hours prior to testing) following oral exposure to full-fat cream cheese the next morning that did not occur with oral exposure to non-fat cream cheese [93]. In other work, retinyl palmitate was administered at a breakfast and the lipemic response to a lunch was monitored [117]. Shortly after the midday meal, there was an abrupt rise of retinyl palmitate, ApoB48 and TAG, supporting a view that oral stimulation at one meal mobilized lipid stored in the intestine from the prior eating event. Though most work indicates fat is the most effective signal, there is some evidence that ingestion of a glucose solution leads to much the same response [118].

However, because the glucose was ingested, it is not known if this is a sensory signal or nutritive effect.

At another level, oral fat exposure alters pancreatic exocrine secretion via cephalic phase vagal activation. Oral exposure to linoleic acid in esophageal ligated mice enhances digestive enzyme secretions and this is blocked by sectioning the glossopharyngeal nerve alone or both the glossopharyngeal and chorda tympani nerves [36]. Sectioning of the gustatory nerves led to a lack of avoidance of an aversive linoleic acid stimulus paired with a control matched on textural properties indicating the primary signal was gustatory. Additionally, oral exposure to linoleic, linolenic and oleic acids in esophageal ligated or esophagostomized rats increases pancreatobiliary secretions [15, 16]. Methylated forms of these fatty acids were not effective, further supporting a taste mechanism [15]. Work in humans suggests there is a direct relationship between oral and gastrointestinal sensitivity to oleic acid [62].

Pancreatic endocrine responses to oral fat exposure have also been documented in humans. Sham feeding of a high fat meal suppressed ghrelin, but augmented insulin and pancreatic polypeptide in one trial [83]. Subsequent work confirmed an oral fat exposure effect on both insulin [93] and pancreatic polypeptide [94].

Finally, numerous trials have demonstrated that oral exposure to full fat versus non-fat foods [91, 90, 116, 119-122, 93, 83, 123] or linoleic acid alone [47] leads to a sustained elevation of TAG. This indicates the possibility of an effect on TAG clearance from the circulation. One plausible mechanism entails sensory modulation of insulin activity. The cephalic phase insulin response is augmented by palatable stimuli [125-128]. In this instance, the aversive nature of oral NEFA exposure could diminish insulin secretion with resulting

reduced peripheral lipoprotein lipase activity [129] and slowed TAG hydrolysis and clearance [130].

Collectively, these data document the existence of an oral gustatory signaling system that influences the digestion of fat, its absorption from the GI tract and its clearance from the circulation. The contribution of these processes to post-prandial TAG concentrations are not known, but warrant further study since circulating TAG is an important risk factor for cardiovascular disease [131].

Industry opportunity

Low concentration – flavor enhancement?

Very minimal work has been conducted on the perception of oleogustus in real foods at non-rancid concentrations. If oleogustus is a signal for the presence of fat in a food, then low levels could potentially contribute to palatability. Indeed, hydrolyzed fat can be used as a flavor additive (e.g., Butter Buds and Cocoa Buds), though the stated purpose of these products is to add aroma. NEFA have long been known as important flavor components of fermented products, such as cheese [132], and dairy products in general [133]. Medium chain fatty acids are believed to contribute to the "sweat-like" flavor of mutton [134], and differences in fatty acid composition contribute to variability in the flavor of animal fat (see [133]). Again, most of this work has been conducted from the perspective of NEFA aroma, rather than taste. However, NEFA have only recently been considered taste stimuli, so future work may demonstrate a role for oleogustus in the development of characteristic flavors of fatty foods.

High concentration – avoidance

The food industry has known for years that high concentrations of fatty acids are to be avoided, despite certain products whose flavor profile relies on high concentrations of NEFA (for example, strong cheeses). However, a high level of NEFA is generally a sign that a product is rancid. While the traditional assumption held that the odor of the NEFA and their subsequent degradation products was the aversive trait, new data indicate that high levels of NEFA may lead to rejection based on taste alone (Running, Hayes, and Ziegler, submitted). Preliminary results indicate adding linoleic acid above 0.5% and oleic acid above 2.3% to chocolate results in rejection of the sample compared to control chocolate by taste alone (participants wore nose clips). NEFA also influence the quality of frying oil, with decreased acceptability observed for NEFA concentrations as low as 0.02-0.2% for lauric (C12), linoleic (C18:2), and linolenic (C18:3) acids; some minor negative effects were observed at 2% for oleic (C18:1), palmitic (C16), and stearic (C18) acids [135]. While these data are based on sensory judgments without blocking the nose (meaning that aroma may play an important role in the flavor), participants in this study did note that the longer chain polyunsaturated NEFA imparted a “bitter” flavor to the food, which is in line with descriptors given in more recent work that did control for odor [20].

Fat replacers

Fat replacers have been developed largely to mimic the somatosensory properties of fats. There has been less effort directed at replacing their chemosensory contributions to foods. Though there are no relevant data to-date, it is possible the acceptability of fat replacers would be improved by capturing their full contribution to the flavor profile of foods and beverages.

Conclusion

Accruing evidence is driving greater acceptance of a view that NEFA may be detected in the oral cavity by the sense of taste. Research on the topic is now increasingly aimed at understanding individual differences in perception, sensory properties of varying fatty acids, sensory functions of NEFA in foods and beverages as well as the health implications of oral fat exposure. Additionally, recognition of fat as an effective gustatory stimulus has simultaneously raised claims that it is a sixth taste primary and that the concept of basic tastes warrants re-evaluation. Failure to recognize the full extent of chemosensory capabilities in humans will limit the ability to harness this rich source of information for improvement of health and well-being.

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Table Legend

Taste detection thresholds for people who were lean (L), overweight (OW) or Obese (OB). Threshold values from references 70 and 72 have not been previously been published according to L, OW and OB status.