Multiple routes of chemosensitivity to free fatty acids in humans

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Submitted 11 October 2006; accepted in final form 11 January 2007

Chalé-Rush A, Burgess JR, Mattes RD. Multiple routes of chemosensitivity to free fatty acids in humans. Am J Physiol Gastrointest Liver Physiol 292: G1206–G1212, 2007. First published January 18, 2007; doi:10.1152/ajpgi.00471.2006.—Selected free fatty acids (FFAs) are documented effective somatosensory and olfactory stimuli whereas gustatory effects are less well established. This study examined orthonasal olfactory, retronasal olfactory, nasal irritancy, oral irritancy, gustatory, and multimodal threshold sensitivity to linoleic, oleic, and stearic acids. Sensitivity to oxidized linoleic acid was also determined. Detection thresholds were obtained using a three-alternative, forced-choice, ascending concentration presentation procedure. Participants included 22 healthy, physically fit adults sensitive to 6-n-propylthiouracil. Measurable thresholds were obtained for all FFAs tested and in 96% of the trials. Ceiling effects were observed in the remaining trials. Greater sensitivity was observed for multimodal stimulation and lower sensitivity for retronasal stimulation. There were no statistically significant correlations for linoleic, oleic, and stearic acids and in 96% of the trials. Ceiling effects were observed in the remaining trials. Greater sensitivity was observed for multimodal stimulation and lower sensitivity for retronasal stimulation. There were no statistically significant correlations for linoleic acid thresholds between different modalities, suggesting that each route of stimulation contributes independently to fat perception. In summary, 18-carbon FFAs of varying saturation are detected by multiple sensory systems in humans.

gustatory; olfactory; sensory; multimodal; detection thresholds

There are multiple mechanisms by which dietary fats are detected. The most apparent cues are the textural properties they impart, particularly viscosity (thickness) and lubricity (slipperiness) (17, 49, 60, 63, 66, 78). However, fat may also be detected when textural attributes have been negated through the use of emulsifiers and thickening agents that mask viscosity cues derived from the fat (59, 65, 77) or through psychophysical comparisons between nutritive and nonnutritive oils to control for metabolic feedback and lubricity (27, 70). For example, aversions to a sucrose-corn oil (nutritive) mixture in rats generalize more strongly to the corn oil than to sucrose and the aversion to this nutritive mixture does not generalize to a sucrose-mineral oil (nonnutritive) mixture (70). Similarly, in a study in which postigestive cues were minimized, sham-fed rats consumed more corn oil than mineral oil (27). Furthermore, sham-fed rats are able to discriminate between 0.78% corn oil and tap water with similar tactile characteristics (50). In humans, oral exposure to butter, but not fat replacers that mimic the textural properties of fats, elicits a longer postprandial elevation in serum triacylglycerol (44), a biomarker for fat detection.

The acidic moiety of fatty acids may be an irritant that serves as a cue for their presence via irritancy (78). This route is supported by participants’ use of verbal descriptors for fatty acids such as “extremely strangled,” “pungent,” “warming,” “hot,” “burning-bitter” and “sour” (19, 28, 65) since these terms are suggestive of trigeminal involvement. Where this may occur in the oropharyngeal region is not known. Oral and nasal irritancy testing is required to clarify the location and contribution of this chemosensory system.

There are several observations supporting an olfactory contribution to fat perception. Olfactory bulbectomized chickens show no preference for a long-chain triacylglycerol-enriched diet in contrast to sham-operated and intact birds (42). Olfactory nerve sectioning in mice eliminates the preference for high-fat foods, and neuronal recovery results in a renewed preference for high-fat foods (38). Anosmic mice fail to show a preference for 1% and 3% corn oil in a 0.3% xanthan gum solution compared with normal mice (72), although they do so at higher concentrations. Olfactory stimulation may occur via orthonasal or retronasal routes. Othonasal stimuli reach the olfactory mucosa via the anterior nares or nostrils. Neurons in the orbitofrontal cortex of primates respond to orthonasal delivery of the odor of cream (63). Retronasal stimuli travel through the posterior nares or nasopharynx (18, 32, 54, 64). This pathway may be more important for accessing sensory cues from foods in the oral cavity and has been overlooked in prior studies on fat perception. Because there are differences in the processing of orthonasal and retronasal stimuli (32), their individual contributions to fat perception need to be assessed. However, there is also evidence that fat may be sensed without olfactory or tactile inputs. Anosmic rats are able to discriminate between oleate and triolein solutions suspended in 0.3% xanthan gum (22).

Data from behavioral and psychophysical studies conducted with animal models and humans suggest fats may be effective gustatory stimuli (9, 22, 34, 35, 45, 52, 66, 70, 77). In short-term trials recording tongue-flicking rates and bites, the lizard Podarcis lilfordi responds strongly to pure stimuli of pork fat and oleic acid, but not to cholesterol (in glycerol) or pure glycerol (9). However, vomeronasal, olfactory, and other cues were not excluded in this work. Rats prefer 1% oil-in-water (O/W) emulsions of long-chain fatty acids (LCFAs) relative to solutions of LCFA derivatives or xanthan gum (77), but it is unclear to what extent olfactory or other cues are involved. In humans, detection thresholds for emulsified oils are 5.3% (vol/vol) in young and 15.8% (vol/vol) in elderly adults (66). Reportedly, elimination of olfactory cues does not alter sensitivity. Other work also noted that the perceived fat content of a snack food (48) or dairy products (49, 66) is unaffected by minimizing olfactory input, but tactile cues remain possible cues. 6-n-Propylthiouracil (PROP) tasters, relative to nontasters, are able to discriminate between ice cream with added conjugated linoleic acid and unadulterated ice cream (52). Similarly, fat tasters, classified by their ability

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to discriminate between 10-µm linoleic acid emulsified in propylene glycol and demineralized water and a control of propylene glycol and demineralized water are able to discriminate between linoleic and oleic acids embedded in ice cream compared with nontasters (35). However, the effective free fatty acid (FFA) cue and/or the route of detection cannot be definitively determined in these studies. Furthermore, the relevance of sodium salts of FFAs (35), not found in foods (85), to fat taste is uncertain. Interestingly, unlike the other modalities, taste has been linked to postprandial lipemia (45). Both textural and orthograde olfactory stimulation have been discounted in this latter paradigm as oral stimulation with fat-free cream cheese (to control for texture), and sniffing the cream cheese had no effect on postprandial lipemia.

Evidence that “fatty” constitutes a basic taste quality has been accumulating over the past decade and has intensified since the identification of candidate fatty acid transduction mechanisms. Long-chain cis-polysaturated and monounsaturated FFAs inhibit delayed rectifying K⁺ channels in isolated taste receptor cells (23, 30). This results in depolarization and may be a primary signal for fatty acids or may facilitate effective stimulation by other taste stimuli (24). Additionally, the fatty acid transporter CD36 is present in the apical portion of circumvallate and foliate papillae and possibly in lower concentrations in fungiform papillae (21, 40). CD36 knockout mice lose sensitivity to fatty acids whereas sensitivity to other taste compounds such as sucrose and quinine is unaffected (40). Most recently, several orphaned G-protein coupled receptors (i.e., GPR40, GPR41, GPR43) have been isolated from taste receptor cells (31). Given the amphiphatic nature of fatty acids, diffusion across taste cell membranes is also possible.

A propos sensory mechanisms observed for fats is the phenomenon of multisensory integration (i.e., the integration of olfactory, gustatory, and somatosensory cues). Much research has focused on individual chemosensory routes for fat detection without consideration for the integration of the multimodal perception of fats. As yet, no study has directly examined multimodal stimulation by fatty acids.

The primary aims of this study were to further document a taste component to FFAs, to provide relative rankings for threshold sensitivity to fatty acids delivered to different sensory systems as well as the effectiveness of multimodal stimulation. In addition, sensitivity to fatty acids varying in saturation was explored. This information will help to clarify the attributes for fat that contribute to its detection and aid studies addressing the health implications (e.g., lipid mobilization and metabolism) stemming from exposure to dietary fats.

MATERIALS AND METHODS

Participants. Twenty-two men and women were recruited through public advertisements. Eligibility criteria included the following: between 18 and 40 years of age, healthy, nonsmoker, body mass index between 18.5 and 29.9 kg/m², no change of body weight >5 kg over the preceding 3 mo, nonrestrained eater (determined by the three-factor eating questionnaire) (71), not chronically taking medication (except oral contraceptives in women), maximal oxygen consumption equal to or greater than the 70th percentile for their age and sex (1), and measurable thresholds for common chemosensory stimuli (e.g., smell, butanol; taste, sucrose). Because of a reported association between PROP sensitivity and fat perception (73, 74), only PROP tasters were tested to maximize sensitivity. All gave written, informed consent. The study was approved by the University Institutional Review Board. All participants received financial compensation.

Participant sessions. Participants were scheduled for two ~60-min sessions per week for 9 wk. They were instructed to refrain from all food, beverages, and oral care products for a minimum of 2 h before testing. Four stimuli were assessed under four conditions: taste, orthonasal olfaction, retronasal olfaction, and multimodal (i.e., taste, olfactory, and tactile). Two additional conditions were assessed using linoleic acid: oral irritation and olfactory irritation. The stimuli include linoleic acid (a polysaturated fatty acid), oleic acid (a monounsaturated fatty acid), stearic acid (a saturated fatty acid), and oxidized linoleic acid.

Sensory testing procedures. Taste was assessed by having participants rate the samples with nares closed and the tongue desensitized to irritation. For orthonasal olfaction, odors were delivered through the anterior nasal cavity. The participant was first instructed to identify the most patent nare, which would be used for the duration of the sensory test. They were then presented with the stimulus in a 4-oz plastic squeeze bottle and asked to smell with noses open but mouth closed. Test condition contribution of retronasal olfaction was assessed by placing 35-mm film canister lids [or odorant presentation containers (OPC)] (29) on the dorsal side of their tongues with nares closed. In this condition, the stimulus would not be in contact with the tongue. Once the OPC was situated on the tongue, they were instructed to retract the tongue, at which point the nares were released to stimulate the retronasal route and allow the passage of air to flow directly through to the olfactory epithelium. Participants were instructed not to sniff but to take quiet and natural breaths (29) for both orthonasal and retronasal olfactory tests. The final condition determined the contribution of multiple cues. Here the participants sipped and swished the stimulus in their mouths with nares open.

Testing involved an ascending concentration, three-alternative, forced-choice procedure (3-AFC). Participants were given three samples and asked to choose which one of the three was different. Stimuli were presented in ascending order of concentration, that is, from the lowest to highest concentration. This procedure continued until the participant correctly identified the stimulus at a given concentration with another two consecutive correct judgments at the same concentration. Testing was conducted under red light (taste and multimodal conditions) or using bottle covers (orthonasal olfaction). For the retronasal olfactory sessions, stimuli were delivered directly into the mouth by the researcher. Stimuli were presented as 5-ml portions for tastes and multimodal stimulation, as 500 µl portions in OPCs for retronasal olfaction, or as 50-ml portions in plastic squeeze bottles for orthonasal olfaction. The interstimulus interval was 60 s, at which time an oral rinse was required (taste and multimodal). Stimuli exposures were 5 s for taste and multimodal or 10 s for olfaction. The stopping rule was three consecutive correct identifications of a target stimulus.

Screening stimuli. To verify participant sensitivity to taste and olfactory stimuli and ability to comply with the exposure protocol, taste thresholds were obtained for aqueous sucrose solutions (Spectrum Chemicals, Gardena, CA) using a concentration range of 0.0001 to 1.0 M, with dilutions differing by 0.25 log units. Sensitivity to butanol (Sigma-Aldrich, St. Louis, MO) was used to assess olfactory function with a concentration range from 0.0006 to 4.0% (vol/vol) and dilutions differing by a factor of three. PROP bitter taste was determined by the three-solution method (61), which entailed intensity scaling on the labeled magnitude scale of 3.2 × 10⁻⁵, 3.2 × 10⁻⁴, and 3.2 × 10⁻³ mol/l PROP (Spectrum Chemicals) and 0.01, 0.1, and 1.0 mol/l NaCl (Spectrum Chemicals) dissolved in deionized water. Taster status was assessed by visual classification whereby the subject’s PROP function was contrasted against their NaCl function. Only those participants whose PROP ratings were equal to or greater than those of NaCl were eligible to participate. Stimuli were presented at room temperature as 10-ml samples in disposable plastic cups.
Participants included 15 men and 7 women with mean age = 21.2 ± 0.6, body mass index = 23.6 ± 0.4 kg/m², body fat = 18.3 ± 1.3%, butanol olfactory threshold = 0.020 ± 0.007% (vol/vol), sucrose taste threshold = 0.021 ± 0.003 M, and maximal oxygen consumption = 58.6 ± 1.8 ml·kg⁻¹·min⁻¹. Seventeen of the 22 participants’ sensitivity to butanol fell between dilutions five and eight with dilution steps beginning from stimulus number zero, which is 4% vol/vol butanol (4, 20); however, five were slightly higher. All sucrose thresholds were within the normative value range of 0.00592–0.1 M (83).

Mean detection thresholds for linoleic, oxidized linoleic, oleic, and stearic acids for each route of exposure are presented in Figs. 1 and 2. Measurable thresholds were determined in 382 of 396 trials (96%). For the desensitization sessions, three participants failed to desensitize. However, excluding them from analyses did not alter the measured threshold for linoleic acid in this condition (0.033 ± 0.008% wt/vol).

There was a statistically significant difference observed in the detection thresholds for the routes of exposure for linoleic acid \([F(1,254, 26.339) = 7.699, P < 0.05]\). Post hoc tests revealed that the threshold for retronasal olfaction was significantly higher than for orthonasal olfaction, nasal irritancy, oral irritancy, taste, and multimodal stimulation. Additionally, thresholds for oral irritancy, nasal irritancy, and taste were significantly higher than for multimodal stimulation. Orthonasal olfaction failed to reach a statistically significant difference from the multimodal threshold, possibly because of the greater variance. There was a statistically significant difference in the detection thresholds for the routes of exposure for oxidized linoleic acid \([F(1,067, 22.399) = 8.026, P < 0.05]\). The threshold for retronasal olfaction was significantly higher than for orthonasal olfaction, taste, and multimodal stimulation, and the threshold for taste was significantly higher than for orthonasal olfaction and multimodal stimulation. Oleic acid thresholds differed significantly \([F(1.002, 21.052) = 7.353, P < 0.05]\). The threshold for retronasal olfaction was significantly higher than...
for orthonasal olfaction, taste, and multimodal stimulation, and taste was significantly higher than orthonasal olfaction and multimodal stimulation. There was a statistically significant difference in the detection thresholds for the exposure routes for stearic acid \(F(1.014, 21.300) = 67.664, P < 0.05\). The threshold for retronasal olfaction was significantly higher than for the other exposure routes. Comparisons between fatty acids detected statistically significant differences. Retronasal olfactory \(F(1.878, 39.446) = 37.162, P < 0.05\] and multimodal thresholds \(F(1.521, 31.936) = 7.248, P < 0.05\] were significantly higher for stearic than the other fatty acids. There were no significant correlations between thresholds.

DISCUSSION

These data demonstrate that 18-carbon fatty acids are effective chemochemical stimuli for multiple sensory systems in humans. Despite the similarity of absolute values for thresholds to most routes of stimulation, the lack of correlations between them suggests that they function independently. Retronasal olfactory thresholds were consistently the highest (i.e., lowest sensitivity) across fatty acids. Prior work with other odorants has also documented lower sensitivity following retronasal compared with orthonasal stimulation (32, 80). This has been attributed to an olfactory duality or perceptual independence between the two olfactory routes as a result of differences in olfactory processing of orthograde and retrograde stimulus delivery (64). This may be explained by differences in orthonasal and retronasal anatomy (12, 88) and adhesion patterns (33, 51) as well as retronasal adaptation to higher intraoral concentrations (3). In accordance with these observations, the data are consistent with a heightened sensitivity to FFAs for orthonasal rather than retronasal olfaction.

With the exception of oxidized linoleic acid, there was a trend for multimodal (i.e., combined taste and olfactory stimulation) thresholds to be lowest for linoleic, oleic, and stearic acids. This may represent a case of cross-modal integration as previously demonstrated by a subthreshold taste stimulus, such as saccharin, lowering the threshold for an orthograde olfactory presentation of benzaldehyde (11). Further psychophysical investigations have documented cross-modal additivity between congruent (acesulfame K and pineapple) and incongruent (monosodium glutamate and pineapple) taste-smell pairs (14, 15). In both human and nonhuman primates, neuroimaging studies have identified various regions in the brain, specifically the orbitofrontal cortex, amygdala, insular cortex, and cingulate cortex that respond to unimodal, bimodal, and multimodal inputs. However, the most robust responses are to bimodal and multimodal stimulation, consistent with taste-olfactory convergence (13, 62). PET scans (67) and functional magnetic resonance imaging (fMRI) (68) have also demonstrated taste-smell interaction in the insula, orbitofrontal cortex, and amygdala with odors presented orthonasally (PET) and retronasally (fMRI). However, the neural response resulted in suppression with orthonasally presented odors (67) and enhancement with retronasally presented odors (68). Alternatively, others have proposed that taste-smell additivity can be predicted through probability summation (15, 76). That is, there is a higher probability of stimulus detection with the presentation of multiple simultaneous cues rather than one (15, 76).

Sensitivity was greatest for orthonasal detection of oxidized linoleic acid. Observations with animal models indicate odor is the dominant sensory feature of oxidized oils (37) and that oxidized oils are discriminable relative to unoxidized sources (37, 59, 77). Furthermore, with few exceptions (59), unoxidized oils are preferred (37, 77). This may also apply to humans because the rancidity of oxidized oils is easily detected and is reportedly unpleasant (37). The heightened sensitivity to oxidized oils may be functional in an evolutionary context in that oxidized fats connotes a decrease in nutritional value and an increase in unsafe by-products (85). Thus their avoidance or reduced intake may be protective.

Localization of compounds presented ortho- or retronasally has been proposed as an index of whether a compound is an olfactory irritant or a pure odor. This approach was used here, and nasal irritancy thresholds were obtained. However, there is evidence of localization of pure odors as well (58, 81). Humans can localize pure odors, guided by temporal and spatial inputs. By use of fMRI, this observation was recently corroborated by Porter et al. (56), who identified contralateral (nasal-specific) responses in the primary olfactory cortex with bilateral convergence in the superior temporal gyrus, a region of the brain purported to represent multisensory inputs. In rats, spatial and temporal cues are also important in odor localization of pure olfactory stimulants (58). In light of this evidence, nasal irritancy and orthonasal olfactory thresholds are not indistinguishable. The thresholds reported here indicate that either they are similar or the most sensitive route of stimulation is being used as the cue for FFA detection. Thus the least sensitive cue remains unmeasured. Interpretation of the reported lateralization and orthonasal olfactory thresholds for linoleic acid must, therefore, be made with caution.

Oral irritancy and taste thresholds were nearly identical, suggesting that the irritancy threshold is either similar to taste or higher to the measured value and just reflects the taste component (determined following desensitization). Studies of fat perception have focused on isolated sensory properties with
limited attention to the relative sensitivity of humans to the different routes of detection. Reports indicate that nasal and oral irritation thresholds and intensity ratings are generally higher than odor (6–8, 43) and taste (25), respectively, for several compounds. Similar to nasal irritancy and orthonasal olfactory thresholds, it was not possible to completely separate oral irritancy from taste in the present investigation. Additionally, since the association between irritancy sensations from FFAs and capsaicin has not been addressed, we cannot confirm whether oral irritancy emanating from FFAs was indeed eliminated. In cases of weak or conflicting inputs, a focus on the gestalt (unitary perception) would predict that the cue from the most sensitive modality for that FFA will be the most likely to be detected, conveying modality dominance (79). This is an effective means of stimuli detection with relevance to investigations addressing health implications arising from fat perception.

With the exception of retrolental thresholds, the absolute threshold concentrations were similar across for the various sensory systems and fatty acids tested. However, this should not be interpreted as evidence of comparable physiological implications. For example, there is evidence that the taste component of dietary fat uniquely alters postprandial lipid metabolism (44–46, 75) and hormonal responses (10, 69). The role of these responses to fat taste in chronic disease risk (e.g., cardiovascular disease, obesity) has not been characterized. Unique roles have also been documented for other sensory systems. Olfaction has long been recognized for its influence on reproductive physiology and behaviors (39, 47, 53, 55, 57, 84, 86, 87). Furthermore, the somatosensory system plays a unique role in detection and avoidance of noxious stimuli (2, 36, 82).

This study used detection thresholds for the determination of sensitivity to FFAs. The measured values provide an indication of the level of sensitivity of our participants to these stimuli, but, because threshold values are always relative to the background signal, extrapolation of our reported concentrations to the level of sensitivity of our participants to these stimuli, but, because threshold values are always relative to the background signal, extrapolation of our reported concentrations to the dietary requirement for selected fatty acids, they provide energy and are carriers of fat-soluble vitamins. Thus a sensitive, redundant system for their detection would have adaptive benefit helping to meet and maintain energy stores and acquire needed nutrients (5).

GRANTS
This work was supported by National Institute of Diabetes and Digestive and Kidney Diseases Grant no. DK-045294.

REFERENCES


