Multiple routes of chemosensitivity to free fatty acids in humans

Angela Chalé-Rush, John R. Burgess, and Richard D. Mattes
Purdue University, Department of Foods and Nutrition, West Lafayette, Indiana

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. 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. Sensitivity to oxidized 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 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 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). Anomelic 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

Address for reprint requests and other correspondence: R. D. Mattes, Purdue Univ., Dept. of Foods and Nutrition, 700 W. State St., West Lafayette, IN 47907-1264 (e-mail: mattes@purdue.edu).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
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-polyunsaturated 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 between 18 and 40 years of age, healthy, nonsmoker, body mass index 5 kg over 3 mo, nonrestrained eater (determined by the three-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...
which the participant made three consecutive correct identifications of fluidity. All FFAs were purchased from Sigma-Aldrich.

**Testing stimuli.** FFA emulsions ranged in concentration from 0.28 \(\times 10^{-3}\)–5% (wt/vol) and differed by 0.25 log units. Included in the FFA medium were 5% gum acacia (wt/vol, TIC Gums, Belcamp, MD) (66) to minimize viscosity cues (60, 66, 59) from the FFAs as well as 5% mineral oil (wt/vol, Spectrum Chemicals) as a control for lubricity and 0.01% EDTA (wt/vol, Spectrum Chemicals), which was added to prevent FFA oxidation. All samples were sonicated (Branson sonifier cell disruptor model S-450D, Danbury, CT) and chilled as further measures to reduce the potential for oxidation product formation, as well as to ensure sample homogeneity. Linoleic, oleic, and stearic acid samples were made daily. The pH of the aqueous phase of the vehicle alone and stimuli containing linoleic acid (0.03 and 1.0%) was 4.77, indicating the fatty acids were partitioned into the lipid phase. Oxidized linoleic acid was made 3 days before testing and stored at 4°C until use. Spectrophotometric analyses entailed scanning the hexane extract of this stimulus at a wavelength between 210 and 350 nm, and observation of absorption at 230 nm confirmed the presence of conjugated dienes, markers of FFA oxidation. Gas chromatographic analyses quantified oxidation product formation in these samples to be 27%. Stimuli were presented at room temperature, excluding stearic acid, which was presented at 67–69°C to maintain fluidity. All FFAs were purchased from Sigma-Aldrich.

**Oral desensitization and olfactory lateralization.** To control oral irritancy as a possible route of stimulation, oral rinses were implemented with 20 ppm capsaicin (Sigma-Aldrich) dissolved in ethanol (Spectrum Chemicals) and deionized water (25, 26, 41). Briefly, 0.8 g of capsaicin was dissolved in 100 ml of ethanol, and 0.05 ml of this solution was pipetted into 20 ml of distilled water. Therefore, each 20-ml sample contained 0.05 ml of ethanol (41). Participants swished five 20-ml samples of the capsaicin solution at 60-s intervals, holding each one in their mouths for 30 s. No rinsing was permitted between samples. However, after the fifth sample, a 15-min hiatus was inserted at which time the participants were required to rinse their mouths thoroughly with deionized water. Taste detection thresholds for linoleic acid were then remeasured. A final capsaicin solution was given after determination of linoleic acid taste thresholds to verify continued desensitization. Intensity ratings were obtained for each capsaicin stimulus using the labeled magnitude scale. Oral irritancy was assessed through olfactory lateralization using linoleic acid. Participants were required to simultaneously smell from two bottles. A port from each bottle was placed just inside each nostril. One bottle contained the linoleic acid stimulus and the other only vehicle. Testing terminated after participants correctly identified the bottle with linoleic acid on three consecutive trials.

**Statistics.** Thresholds were designated as the concentration at which the participant made three consecutive correct identifications of the target stimulus \(P < 0.05\). Missing values were replaced by the group mean for a particular FFA and condition in analyses comparing threshold values. Results are presented as means ± SE. Repeated-measures ANOVA was used for comparisons between conditions by using the SPSS statistical software package (version 14.0) with condition and FFA as within-subject factors. The relationship between thresholds for each FFA was examined with Pearson’s correlation coefficient. Outliers, identified by the Dixon Q-test (16), were excluded from statistical analyses. The criterion for statistical significance was \(P < 0.05\).

**RESULTS**

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. Oronasal 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 oronasal olfaction, taste, and multimodal stimulation, and the threshold for taste was significantly higher than for oronasal 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 olfac-
tory \(F(1.878, 39.446) = 37.162, P < 0.05\) and multimodal
thresholds \(F(1.521, 31.936) = 7.248, P < 0.05\) were signif-
ificantly 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 effec-
tive chemosensory stimuli for multiple sensory systems in
humans. Despite the similarity of absolute values for thresh-
olds to most routes of stimulation, the lack of correlations
between them suggests that they function independently. Ret-
ronasal olfactory thresholds were consistently the highest (i.e.,
lowest sensitivity) across fatty acids. Prior work with other
dorants has also documented lower sensitivity following ret-
ronasal compared with orthonasal stimulation (32, 80). This
has been attributed to an olfactory duality or perceptual inde-
pendence between the two olfactory routes as a result of
differences in olfactory processing of orthograde and retro-
grade stimulus delivery (64). This may be explained by differ-
ces in orthonasal and retronasal anatomy (12, 88) and ad-
sorption 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 sensi-
tivity 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 stimu-
lation) thresholds to be lower 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 investiga-
tions have documented cross-modal additivity between
congruent (acesulfame K and pineapple) and incongru-
ent (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 cingu-
late cortex that respond to unimodal, bimodal, and multimodal
inputs. However, the most robust responses are to bimodal and
multimodal stimulation, consistent with taste-olfactory conver-
gence (13, 62). PET scans (67) and functional magnetic reso-
nance 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 mul-
tiple 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), unoxi-
dized 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 connote 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 (nostril-specific)
responses in the primary olfactory cortex with bilateral con-
vergence (13, 62). PET scans (67) and functional magnetic reso-
nance 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 mul-
tiple simultaneous cues rather than one (15, 76).

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 retronasal 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, 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 food systems must be made with caution. Furthermore, there could have been some variation in the effective lipid concentration due to differences in the interfacial tension of the oil-in-water emulsion used in this work despite our attempts to create stable emulsions through sonication and the addition of gum acacia. Quality descriptors were not required or obtained with our testing protocol. However, unsolicited comments from participants included “disgusting,” “bitter,” and “cigarette-like.” None were of a positive affective nature. This is consistent with some previous work in rats and human (19, 24, 25, 28, 65), although there are also reports of indifference (35, 74) and preference (22, 77).

This study indicates that fat perception involves gustatory, olfactory, and somatosensory cues. Such broad sensitivity may reflect the importance of dietary fats to health. In addition 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).

**REFERENCES**


22. Frank RA, Dulay MF, Gesteland RC. Assessment of the Sniff Magni-


