Effects of linoleic acid on sweet, sour, salty, and bitter taste thresholds and intensity ratings of adults

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Mattes RD. Effects of linoleic acid on sweet, sour, salty, and bitter taste thresholds and intensity ratings of adults. Am J Physiol Gastrointest Liver Physiol 292: G1243–G1248, 2007. First published February 8, 2007; doi:10.1152/ajpgi.00510.2006.—Evidence supporting a taste component for dietary fat has prompted study of plausible transduction mechanisms. One hypothesizes that long-chain, unsaturated fatty acids block selected delayed-rectifying potassium channels, resulting in a sensitization of taste receptor cells to stimulation by other taste compounds. This was tested in 17 male and 17 female adult (mean ± SE age = 23.4 ± 0.7 yr) propylthiouracil tasters with normal resting triglyceride concentrations (87.3 ± 5.6 mg/dl) and body mass index (23.3 ± 0.4 kg/m²). Participants were tested during two ~30-min test sessions per week for 8 wk. Eight stimuli were assessed in duplicate via an ascending, three-alternative, forced-choice procedure. Qualities were randomized over weeks. Stimuli were presented as room-temperature, 5-ml portions. They included 1% solutions of linoleic acid with added sodium chloride (salty), sucrose (sweet), citric acid (sour), and caffeine (bitter) as well as solutions of these taste compounds alone. Participants also rated the intensity of the five strongest concentrations using the general labeled magnitude scale. The suprathreshold samples were presented in random order with a rinse between each. Subjects made the ratings self-paced while wearing nose clips. It was hypothesized that taste thresholds would be lower and absolute intensity ratings or slopes of intensity functions would be higher for the stimuli mixed with the linoleic acid. Thresholds were compared by paired t-tests and intensity ratings by repeated measures analysis of variance. Thresholds were significantly higher (i.e., lower sensitivity) for the sodium chloride, citric acid, and caffeine solutions with added fatty acid. Sweet, sour, and salty intensity ratings were lower or unchanged by the addition of a fatty acid. The two highest concentrations of caffeine were rated as weaker in the presence of linoleic acid. These data do not support a mechanism for detecting dietary fats whereby fatty acids sensitize taste receptor cells to stimulation by taste compounds.

fatty acid; fat; human; taste transduction; chemosensory

THERE IS CONVERGING EVIDENCE for an oral chemosensory detection system for fatty acids in animal models and humans. Application of long-chain, unsaturated, fatty acids depolarizes isolated taste receptor cells from fungiform papillae (15). Behavioral animal studies have not adequately controlled all potential cues from dietary fats but suggest that animals prefer solutions containing low concentrations of unsaturated fatty acids when textural cues are masked (12, 48) and olfactory cues are eliminated (12). Studies of esphagostomized or esphaguss-ligated rats reveal orosensory exposure to free, unsaturated fatty acids elicits a rapid release of digestive enzymes (18, 23). Orosensory exposure to high-fat stimuli by rhesus macaque monkeys leads to a rapid activation of neurons in the orbitofrontal cortex and this is modulated by appetite (42). In humans, psychophysical data (20, 37, 43, 47), functional MRI scans (6), and studies of cephalic phase responses (30–32) suggest orosensory cues contribute to the detection and perceived intensity of, as well as the physiological responsiveness to, dietary fats. The latter demonstrates that oral exposure of humans to unsaturated dietary fats elicits a rapid rise of plasma triglyceride (TAG) that is only observed with a true dietary fat, compared with fat replacers. Furthermore, it appears to be based on taste since orthonasal olfactory stimulation is not effective for eliciting the TAG response, taste stimulation with nares closed is effective, and the TAG elevation occurs with foods varying in texture only if the stimulus includes a dietary fat. It is well established that textural and olfactory (ortho- and retronasal) properties may be used for the detection of fats, but additional evidence is required to substantiate a role for taste.

Identification of a transduction process for dietary fats would be an essential component for a taste mechanism. A number of candidate mechanisms have been identified. It has been known for some time that CD36 is a ubiquitous membrane protein capable of binding fatty acids and promoting their translocation across membranes (5). A decade ago, CD36 was identified in the apical membrane of circumvallate taste bud cells (12), and recently it was localized to foliate papillae as well (23). CD36 knockout mice cease to respond to fatty acid stimuli while maintaining normal taste responses to other common taste compounds (23). Whether CD36 acts as a docking protein or actually facilitates translocation of fatty acids in taste receptor cells is not established. There are also preliminary data for the presence of orphan G-protein coupled receptors (GPR40, GPR41, and GPR43) in taste cells that may play a role in fatty acid signaling (17). Because of their lipophilic properties, fatty acids may also diffuse across taste receptor cell membranes, and this may be augmented by intracellular fatty acid trapping through the action of long-chain and very-long-chain acyl-CoA synthetases (29). Other fatty acid transport proteins have been identified in various cells (51) but as yet not identified in taste cells. However, one of the best supported mechanisms for detection of fatty acids through the gustatory system involves their inhibition of delayed-rectifying potassium (DRK) channels (16). This mechanism accounts for the efficacy of cis-long-chain, polyunsaturated fatty acids to depolarize receptor cells in fungiform papillae and monounsaturated fatty acids to do the same in foliate and circumvallate papillae (16). The proposed mechanism holds that fatty acids block these channels, resulting in a cell that remains in an excited state. Thus it...
has heightened responsiveness to other taste compounds. In this view, “fatty” may not be a primary taste quality but a modulator of cells to other effective taste compounds. This has been demonstrated in rats, where the preference for a subthreshold concentration of saccharin was augmented by the addition of a low concentration of linoleic acid (16). Presumably, the augmented intake of the saccharin-sweetened solution stemmed from an enhanced sensitivity to the sweetener by the linoleic acid. More recently, the addition of linoleic, oleic, or a mixture of linoleic and olic fatty acids to suprathreshold concentrations of sucrose, glucose, sodium chloride, citric acid, or quinine hydrochloride was reported to enhance sensitivity to these common taste compounds as reflected by altered licking rates (38). A test of this proposed mechanism was the aim of the present study in humans. The hypothesis was that the addition of linoleic acid to solutions of other taste qualities would lower thresholds for these compounds and/or heighten suprathreshold responsiveness.

It is also possible that fats modify responsiveness to other taste compounds by physical processes, i.e., fat may act as a stimulant for salivation and thereby dilute stimulus concentrations; 2) a barrier that impedes access of taste molecules to their respective receptors or channels; or 3) a modulator of taste compound partitioning and effective concentration (11, 22, 26, 34). The effects of isolated free fatty acids on salivation have not been characterized, but oral exposure to foods of varying fat content, and presumably fatty acid concentration, does not elicit significant changes of salivation (19). Coating the oral cavity with oil to create a potential barrier decreases intensity ratings for subsequently presented sweet, sour, salty, and bitter stimuli (26). Using aqueous solutions, one study showed that 9% and 17% oil-in-water emulsions augmented sweet, salty, sour, and umami intensity ratings while suppressing bitter (34). This was attributed to partitioning effects because the taste augmentation was eliminated when the taste compound concentration in the aqueous phase was similar in the oil-supplemented and un-supplemented samples. The lower intensity rating for quinine was attributed to its lipophilicity and lower effective concentration in fat-supplemented samples. Intensity suppression was reported for salt after correction of partitioning effects (52) and for anethole (33). Other work, using 50% oil-in-water and water-in-oil emulsions revealed no effects on sweet, salty, or sour intensity ratings (2). Observations of fat influences on taste intensity in complex foods have yielded augmented (44, 46, 49), unchanged (25, 46, 50), and diminished (8, 10, 44) intensity reports for various qualities. These data have all been based on intensity ratings. Effects on threshold sensitivity have not been explored nor has the role of taste alone been determined. Fat in an oral stimulus at a concentration as low as 1% also alters the volatility of hydrophilic and hydrophobic compounds and suppresses the intensity of the odor they produce (35). Perceptual masking of odor intensity by fat has also been reported (41). The generally suppressive effects of fats on taste attributed to physical processes may require relatively high fat concentrations to occur. Stimuli with fat in concentrations in the micromolar range lead to little partitioning (22) and would not be expected to create an effective barrier. The present protocol allows an initial evaluation of the effects of fat concentration effects in the millimolar range on isolated taste responses to various compounds at the threshold and suprathreshold levels.

**METHODS**

Participant eligibility. Participants were recruited through public advertisements. They completed an online questionnaire eliciting health, demographic, and behavioral information as well as a personal interview and blood draw in the laboratory. Eligibility criteria included: between 18 and 65 yr of age, body mass index between 18 and 30 kg/m², good health, no change of body weight >5 kg over the last 3 mo, nonsmoker, no eating disorder or medically prescribed diet, resting serum triglyceride concentration below 250 mg/dl, and sensitive to the taste of propylthiouracil (PROP). The limitation on TAG values was imposed to permit direct comparison of these data with past and future studies of the TAG response to oral fat exposures.

General protocol. Following recruitment, participants were scheduled for two ~30-min test sessions per week for 8 wk. Sessions were scheduled for the same time of day and participants were required to refrain from ingestion of all food, beverages, and oral care products for a minimum of 2 h before arrival. Eight stimuli were assessed in duplicate. Sensory stimulation was provided by solutions of linoleic acid (an essential, polyunsaturated fatty acid present in food) with added sodium chloride (salty), sucrose (sweet), citric acid (sour), and caffeine (bitter) as well as solutions of these taste compounds alone. Testing involved an ascending, three-alternative, forced-choice procedure. Stimuli were presented as room-temperature, 5-ml portions in two vehicle cups and one target cup. The stopping rule was the correct identification of the target stimulus at a given concentration twice followed by correct identification at the next two higher concentrations. A rinse with deionized water was interspersed between taste samples. Testing was self-paced. The compounds were tested in random order, one quality per day. Participants wore nose clips to eliminate olfactory cues. Following completion of threshold testing, participants rated the intensity of the five strongest concentrations using the general labeled magnitude scale (1). The suprathreshold samples were presented in random order with a rinse between each. Subjects made the ratings self-paced while wearing nose clips. The protocol was approved by the University Institutional Review Board. All participants completed an informed consent form and received financial compensation.

Taste screening. Participants were required to be PROP tasters. This was determined by intensity ratings on the general labeled magnitude scale for solutions of 3.2 × 10⁻⁴ M PROP and 0.1 M sodium chloride. Individuals were classified as tasters if their PROP rating was more than 15.5 mm above the bottom of the 165-mm scale (39). The mean PROP-to-sodium chloride ratio was 4.1 ± 0.6. This was controlled because tasters and supertasters reportedly differ in the perception of and preference for dietary fats from nontasters (9, 47).

**Stimuli.** Eight series of solutions were prepared as serial half dilutions. Four contained 1% linoleic acid and four did not. All contained 5% wt/vol of gum acacia to mask potential viscosity cues from the fatty acids. The samples also contained 0.01% wt/vol EDTA and were sonicated on ice to reduce the formation of fatty acid oxidation products and to ensure a homogenous solution. The pH of the medium was 4.47 and did not change with the addition of the linoleic acid. The concentration ranges (and pH values) for the test stimuli were as follows: sucrose, 0.5–3.05 × 10⁻³ M (pH = 4.57); sodium chloride, 0.5–3.05 × 10⁻³ M (pH = 4.37); citric acid, 2.0 × 10⁻⁴–1.22 × 10⁻⁶ M (pH = 3.92); and caffeine, 1.25 × 10⁻²–7.65 × 10⁻⁷ M (pH = 4.57).

Statistical analyses. Taste thresholds were estimated as the mean of the lowest concentration where participants reliably detected the sample (two successive correct responses followed by correct identifications at the next two higher concentrations) on two occasions. Thresholds for taste samples with and without the addition of linoleic acid were compared by paired t-test. Sex differences were explored by Student’s t-test, and the association between body mass index (BMI) and threshold sensitivity was examined by Pearson correlation coefficients. To assess the effects of linoleic acid on taste intensity ratings,
mean responses for the five highest concentrations of a given quality were assessed by mixed-model repeated-measures analysis of variance with concentration and fatty acid content as within-subjects factors. When warranted, the least significant difference test was used for post hoc comparisons. Initial testing included sex as a between-subjects factor.

The criterion for statistical significance was \( P < 0.05 \), two-tailed.

**RESULTS**

Seventeen male and 17 female adults (mean ± SE age = 23.4 ± 0.7 yr) participated. They had a mean BMI of 23.3 ± 0.4 kg/m² and body fat of 19.7 ± 1.6%. Their mean triglyceride concentration was 87.3 ± 5.6 mg/dl.

**Taste thresholds.** Mean taste thresholds for sodium chloride, sucrose, citric acid, and caffeine in the presence and absence of 1% linoleic acid are presented in Fig. 1. Thresholds were significantly higher (i.e., lower sensitivity) for the sodium chloride, citric acid, and caffeine solutions with added fatty acid. The proportions of participants with lower thresholds (i.e., greater sensitivity) for the solutions containing linoleic acid were sodium chloride 32.4%, sucrose 35.3%, citric acid 5.9%, and caffeine 17.6%. There was no evidence of bimodal threshold distributions. There were no significant sex differences for any threshold, nor was BMI related to the sensitivity for any of the stimuli.

**Intensity ratings.** Taste intensity ratings for sodium chloride, sucrose, citric acid, and caffeine in the presence or absence of 1% linoleic acid are presented in Fig. 2. No significant sex effects were noted, so data from men and women were pooled. For the sodium chloride solutions, intensity ratings were greater for the samples without the linoleic acid \( [F(1,33) = 10.93, P < 0.005] \). The interaction between fat content and taste compound concentration was not significant. No significant effect of fat content was observed with the sucrose solutions and, although the interaction between fat content and taste compound concentration was significant \( [F(4,132) = 2.86, P < 0.05] \), this finding was not supported by post hoc testing. There was no main effect of fat content with the citric acid stimuli, but there was a significant interaction with citric acid concentration \( [F(4,132) = 3.41, P = 0.011] \). Post hoc tests indicated that the two least concentrated samples containing the linoleic acid were rated as more intense than the comparable samples without added fatty acid \( (P < 0.01 \) and \( P < 0.05 \), respectively). The fat-free samples of caffeine were rated as more intense \( [F(91,33) = 5.17, P < 0.05] \) and there was a significant interaction between fat content and caffeine concentration \( [F(4,132) = 3.92, P = 0.005] \). Post hoc tests revealed that the two highest fat-free stimuli were significantly more intense than their fat-containing counterparts \( (P < 0.05 \) both).

**DISCUSSION**

Dietary fats can be detected by the textural, olfactory, and visual properties they impart to foods (21, 40). A potential role for taste cues is under active investigation, and there is an emerging view that multiple mechanisms may be involved with different specificities. Taste receptor cells isolated from buds in the rat fungiform papillae depolarize in the presence of cis-long-chain polyunsaturated fatty acids but do not respond to
monounsaturated or saturated fatty acids (15). The putative mechanism involves inhibition of delayed-rectifying potassium channels by the former. In contrast, monounsaturated fatty acids are effective taste stimuli for receptor cells obtained from foliate and circumvallate papillae (16). These cells express CD36, a protein that binds fatty acids, whereas this may not be the case for cells in fungiform papillae (23). CD36 is also capable of binding saturated fatty acids and there are psychophysical data from humans that these too are detectable, on the basis of taste alone (23). The present study used a psychophysical approach to explore one potential mechanism in humans.

Gilbertson et al. (16) have suggested that fatty acids are not primary taste stimuli but rather sensitize taste receptor cells so they are more reactive to primary effective stimuli. To support this hypothesis, they demonstrated that a subthreshold concentration of saccharine (0.5 mM) becomes detectable, as evidenced by heightened preference responses to it in S5B rats, when presented in the presence of 5 or 20 mM linoleic acid. The enhancement did not occur with the 12-carbon saturated fatty acid, lauric acid. In the present study, it was hypothesized that if this also occurs in humans, taste thresholds for sweet, and possibly other, taste qualities, should be lower in the presence of linoleic acid than in its absence. However, no significant effect of the fatty acid was observed for sucrose and thresholds for sodium chloride, citric acid, and caffeine were significantly higher (i.e., sensitivity was lower) in the linoleic acid supplemented stimuli compared with the unsupplemented vehicle.

We further tested the effects of the linoleic acid on suprathreshold intensity judgments hypothesizing they may be augmented if the addition of linoleic acid heightens the reactivity of taste cells to other taste stimuli. Again little support for this mechanism was obtained. For sodium chloride and caffeine, the addition of linoleic acid led to a significant decrease in intensity ratings. Intensity ratings for the sucrose solutions were unaffected by the addition of the linoleic acid. Only for citric acid did the linoleic acid augment ratings, but this was limited to the two lowest concentrations and the shifts were small.

These observations of weak and suppressive effects are generally in agreement with other reports in the literature (reviewed in the introduction), although there were marked differences in testing conditions between this and earlier studies. In one comparable trial, several sweet, salty, sour, and bitter compounds were mixed with 100 mM linoleic and rated for maximum intensity (22). The ratings for sour and bitter compounds were suppressed whereas ratings only of inosine monophosphate + monosodium glutamate were enhanced by the fatty acid. However, testing did not control for olfactory stimulation so the effects cannot be attributed solely to taste. Another recent trial noted that licking responses of rats were increased to solutions containing suprathreshold concentrations of sucrose or glucose plus 88 μM linoleic, oleic, or mixed linoleic-oleic fatty acids compared with stimuli without the fatty acids (38) whereas licking response to mixtures containing sodium chloride, citric acid, and quinine hydrochloride.
were reduced. Again, olfactory cues cannot be excluded as possible contributors to the response.

Other work with aqueous stimuli indicated that 9% and 17% oil-in-water emulsions did not alter taste responses after correction for partitioning effects nor did 50% oil-in-water or water-in-oil emulsions (2).

These findings coupled with recent observations that humans can detect isolated fatty acids on the basis of taste properties alone (32), support a view that fatty acids may act as primary stimuli rather than a general modulator of taste receptor cell responsivity to other taste molecules. However, there are limitations to the present study. First, we tested only four prototypical taste compounds. They are not fully representative of the range of stimuli that elicit similar qualities nor the range of identified transduction mechanisms. Thus a modulatory role remains a possibility. The addition of 100 μM linoleic acid to a mixture of IMP and MSG did strengthen reported maximal taste intensity for this stimulus, although the effect cannot be unequivocally attributed to taste because olfactory cues were not controlled (22).

Second, Gilbertson et al. (16) documented their enhancement effect with saccharin whereas we used sucrose. Although they coexist in the same taste receptor cells, there may be different transduction pathways for nutritive and nonnutritive sweeteners (14, 27). Thus differential effects of fatty acids on responses to the two sweeteners are possible.

Third, in our trial, the concentration of linoleic acid was approximately three orders of magnitude higher than that used to augment responses with rats (36 vs. 0.02 mM). The rationale for this difference was twofold. First, without knowing the equivalence of rat and human transduction processes, it was believed that this assured adequate linoleic acid was available to block DRK channels and thereby adequately test the proposed mechanism. Second, this dose of linoleic acid was ecologically valid. Human linoleic fatty acid taste thresholds are in the low millimolar range in a complex fluid sensory medium (see Ref. 4), and naturally occurring free acid concentrations in high-fat fluid foods are in the millimolar (e.g., milk, ice cream) to molar (e.g., butter, lard) range and may be several fold higher in solid foods on a weight/weight basis (3, 7, 24, 28, 36). The lack of evidence for functional levels of lingual lipase in humans to hydrolyze triglycerides (45) indicates that the food source would be the determinant of the fatty acid concentration. The inconsistent suprathreshold ratings for the highly hydrophilic taste compounds suggest the acid was not physically impeding interactions between taste molecules and their transduction elements. Only free fatty acids were added to the medium, so the degree of disassociation did not influence the effective acid concentration and the quantity added did not alter the solution pH. Partitioning effects are improbable because, had they occurred, this would have concentrated the hydrophilic taste stimuli in the aqueous phase, leading to lower thresholds and/or higher intensity ratings, and neither effect was observed. The possibility of a masking effect cannot be excluded but seems unlikely given the low absolute fatty acid concentration. Nevertheless, it is possible that lower fatty acid concentrations will enhance the detection of subthreshold concentrations of other taste compounds, as demonstrated in rats (16). A dose-response study testing lower concentrations in humans would address this issue.

There have been suggestions of fatty acid tasters and non-tasters (20, 37, 47) with a proposed distribution of ~46:54% (20). The veracity of this characterization (37) and relevance to the present work is not known. Detection of fatty acid itself was not tested here, but, to the extent that differences in responsivity to free fatty acids could have led to bimodal distributions of the taste thresholds for the mixtures containing linoleic acid (e.g., lowered in tasters and unaffected or elevated in non-tasters) or slopes of the intensity functions, the findings do not lend support for such a distinction. There was no evidence of bimodal threshold distributions or intensity slopes for the sweet, sour, salty, or bitter taste compounds.

This work was prompted by the hypothesis that fatty acids may modify responses to other taste compounds through modulation of DRK channels (16). It was not possible to measure the effects of our stimuli at this level nor to exclude potentially confounding effects of other fatty acid transduction mechanisms (e.g., CD36, GPCRs, diffusion, fatty acid transporters, nutrient trapping). Whether rats and humans sense fatty acids by similar mechanisms has not been established, but the work documenting effects of fatty acids on taste responses to sweetness in rats (16) suggests that contributions of other mechanisms should not have masked effects.

Finally, it should be noted that extrapolation of the present findings to taste responses of humans to foods is tenuous. Triacylglycerol will be the dominant form of fat in foods that contain free fatty acids. This will alter the partitioning of free fatty acids so that concentration in the aqueous phase will be minimal. For this reason and the fact that taste responses must always be evaluated relative to the background signal (medium), the absolute values of thresholds and intensity ratings are of uncertain value.

In summary, these data in humans do not support observations in rats that linoleic acid heightens responsivity of taste receptor cells to sweet stimuli or prototypical salty, sour, and bitter compounds. Although this study does not definitively exclude the possibility that fatty acids are detected by their modulating effects on sensitivity to other taste compounds, evidence that humans can detect fatty acids on the basis of taste indicates that exploration of other mechanisms is warranted.

GRANTS

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REFERENCES


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