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Evidence for Human Orosensory (Taste?) Sensitivity to Free Fatty Acids

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Abstract

Accumulating evidence suggests dietary fatty acids (FAs) may be sensed in the oral cavity. However, the effective cues have not been characterized. In particular, influences from other sensory cues have hampered identification of an independent gustatory contribution. Experiment 1 examined techniques to minimize the formation of FA oxidation products and improve the homogeneity of water/lipid emulsions to be used as stimuli in Experiment 2, a psychophysical study to determine FA detection thresholds in humans. Through sonication of chilled samples held in polypropylene labware and the addition of 0.01% ethylenediaminetetraacetic acid, calcium disodium salt, homogenous emulsions of unoxidized linoleic and oleic FAs were obtained. Spectrophotometric analysis revealed no oxidation product formation over a 24-h period. Coupled with these techniques, a masking approach was used to minimize other sensory cues imparted from linoleic, oleic, and stearic FAs. Concentration ranges from 0.00028% to 5% (w/v) were prepared in mixtures with 5% mineral oil (w/v) and 5% gum acacia (w/v) to mask lubricity and viscosity effects, respectively. Testing was conducted under red light with nares blocked to eliminate visual and olfactory cues. Oral rinses with 20 ppm capsaicin were administered to desensitize participants to selected irritation effects prior to remeasuring linoleic acid detection thresholds. To determine if the effective stimulus was an oxidation product, oxidized linoleic acid was included among the test stimuli. Detection thresholds were obtained using a 3-alternative, forced-choice ascending-concentration presentation procedure. The mean detection threshold for linoleic acid pre-desensitization was 0.034 ± 0.008%, for linoleic acid post-desensitization was 0.032 ± 0.007%, for oleic 0.022 ± 0.003%, for stearic 0.032 ± 0.005%, and oxidized linoleic 0.025 ± 0.005%. The results suggest that linoleic, oleic, stearic, and oxidized linoleic acids are detectable in the oral cavity of humans with minimal input from the olfactory, capsaicin, and viscosity-assessing tactile systems.

Key words: detection thresholds, free fatty acids, human, orosensory, taste

Introduction

Observations from cell electrophysiological, animal and human behavioral, and psychophysical studies suggest ''fatty'' may be a taste quality (Schiffman and Dackis 1975; Fukuwatari et al. 1997; Gilbertson et al. 1997; Schiffman et al. 1998; Smith et al. 2000; Mattes 2001; Nasser et al. 2001; Cooper et al. 2002; Kamphuis et al. 2003). However, preliminary human data (Schiffman and Dackis 1975; Schiffman et al. 1998; Nasser et al. 2001; Kamphuis et al. 2003) remain inconclusive because of the difficulty in isolating a taste component. There are many physical and chemical attributes that can provide a signal incorrectly interpreted as ''fatty'' taste. These include texture, both viscosity (thickness) (Mela 1988; Drewnowski 1992) and lubricity (slipperiness) (Ramirez 1994; Schiffman et al. 1998; Rolls et al. 1999; Verhagen et al. 2003), olfaction (Rolls et al. 1999; Takeda et al. 2001), and oral irritation (Verhagen et al. 2003). ''Sour,'' ''astringent,'' ''pungent,'' and ''burning bitter'' are common free fatty acid (FFA) flavor descriptors (Forss 1972; Schiffman and Dackis 1975; Grosch and Laskawy 1984). It is also possible that degradation products of FFAs (Ramirez 1992, 1993; Tsuruta et al. 1999), rather than a FFA itself, are the effective stimuli. FFAs may also have targets in the gut and may well generate signals analogous to taste. This has been reported for both sweet and fat stimuli in rodent models (Tracy et al. 2004). In humans, reports indicate that intravenous infusions of saccharin are detectable in the oral cavity (Fishberg et al. 1933). Technically, the problem of creating effective stimuli for fat is heightened by the difficulty of creating homogenous oil-in-water (O/W) emulsions of low lipid concentrations without surfactants; a problem that is encountered using conventional means of agitation. Traditional mixing with hot plate/stirrers and stirring rods not only serve to facilitate oxidation but also create samples with poor homogeneity.

The aims of this project were to develop a methodology that would address these issues and to measure fatty acid (FA) detection thresholds in humans. Experiment 1 focused on developing appropriate test stimuli, whereas Experiment 2 used these stimuli to document FFA detection thresholds in healthy adults.

Experiment 1

Reverted or oxidized FAs produce flavors reported to include a gustatory component (Ramirez 1992, 1993; Tsuruta et al. 1999). As such, there is the possibility that the effective stimulus in "fatty" taste may be the reverted or the oxidation product and not the FFA. Several approaches may be used to clarify the effective stimulus by reducing or eliminating oxidation products. An antioxidant (Lillard 1978; Sherwin 1985) or metal sequestrant (Lindsay 1976; Love 1985) and reduced agitation of FA emulsions through sonication are measures that may be taken to decrease the potential for oxidation. Metal chelating agents are not antioxidants by definition but, rather, bind metal ions that catalyze oxidation reactions (Lindsay 1976; Love 1985). This is an important factor to consider when using ingredients containing trace amounts of metal contaminants such as emulsifiers and thickening agents.

In addition to the need for FFA sample protection from oxidation is the problem of poor homogeneity in O/W emulsions. Sonication may be used to create stable O/W emulsions while posing limited risk of oxidation and ensuring sample homogeneity. Lack of homogeneity may also be attributed to adsorption of the test stimulus to glassware. Polypropylene surfaces favor desorption of FFAs during sample preparation and, therefore, limit loss to glass surfaces. Sonication of FFAs in polypropylene vessels should improve sample homogeneity and concentration accuracy. Thus, the objective of Experiment 1 was to document that through a rigorous sample preparation method, it was possible to prepare homogenous O/W emulsions of FFAs without oxidation products.

Materials and methods

FA samples

Linoleic and oleic acids (Nu-Chek Prep, Inc., Elysian, MN) were prepared at a concentration of 0.15 and 0.1 mg/ml, respectively, in a solution of 5% gum acacia (w/v, J.T. Baker [Mallinckrodt Baker], Phillipsburg, NJ) and demineralized water (Schiffman et al. 1998). Gums do not interact chemically with fats and are effective in masking textural attributes from viscosity (Ramirez 1992, 1994; Schiffman et al. 1998). A second set of FFA samples was prepared similarly, but 0.01% ethylenediaminetetraacetic acid (EDTA) (w/v, Spectrum Chemicals, Gardena, CA) was added directly to the FFAs. Samples were mixed either conventionally by a Corning stirrer (model 12V; Corning, Corning, NY) or by sonication using a Branson sonifier cell disruptor (model S-150D; Danbury, CT) for 12 min at an output frequency of 22.5 kHz and with the probe intensity gradation (i.e., amplitude of the ultrasonic vibration) set at 6. All samples were prepared, where applicable, in polypropylene labware.

FA analyses

FAs were extracted from test samples using the method by Folch et al. (1957) and, as part of the procedure, evaporated to dryness under nitrogen. To increase volatility before gas chromatographic (GC) analysis, methyl esters of FAs were prepared using boron triflouride (10–15%, VWR, Bridgeport, NJ) and analyzed by capillary gas–liquid chromatography (Li and Watkins 1998). The methyl esters were extracted in isooctane (VWR) for chromatographic analysis using a Varian 3900 gas chromatograph equipped with a flame-ionization detector, star workstation, and autosampler (model CP-8400; Varian Analytical Instruments, Walnut Creek, CA). To ensure efficient chromatographic separation, a wall-coated open tubular fused-silica capillary column (Varian Analytical Instruments; 30 m × 0.32 mm internal diameter, 0.25-μm film thickness) was used with helium as the carrier gas through the GC capillary column. The initial oven temperature of 175 °C was held for 4 min and increased at a rate of 3 °C/min until the final temperature of 240 °C was reached. The total GC run was 30.67 min. All samples were introduced by split injection (1:100). The split ratio is predetermined because the GC capillary column cannot handle the entire volume of one injection. An external standard mixture prepared from known amounts of methylated FAs (Supelco, Bellefonte, PA) was used to create a standard curve for each FA and convert peak areas to concentrations. Conjugated diene formation was used as a marker of FA oxidation. Spectrophotometric analyses were conducted on linoleic acid samples. This entailed scanning the hexane extract of each sample from 210 to 350 nm and quantifying conjugated diene formation by absorption at a wavelength of 230 nm. To test for homogeneity, the coefficient of variation (a measure of the variation between samples) was computed and compared between sonicated and nonsonicated samples.

Results

The initial concentration of linoleic acid was 0.15 mg/ml and oleic was 0.1 mg/ml. GC analysis revealed that a simple O/W solution of linoleic acid resulted in only 0.007 mg/ml recovery from the original concentration, and recovery of oleic acid was only 0.02 mg/ml (Figure 1). Addition of EDTA and sonication as well as preparation of FA emulsions using polypropylene vessels increased the recovered concentration of both FAs to 100%. Samples were made in duplicate and averaged. The mean concentration and standard deviation for linoleic acid was 0.154 ± 0.003% and for oleic 0.116 ± 0.011%. Similar FA preparation methods were also used in

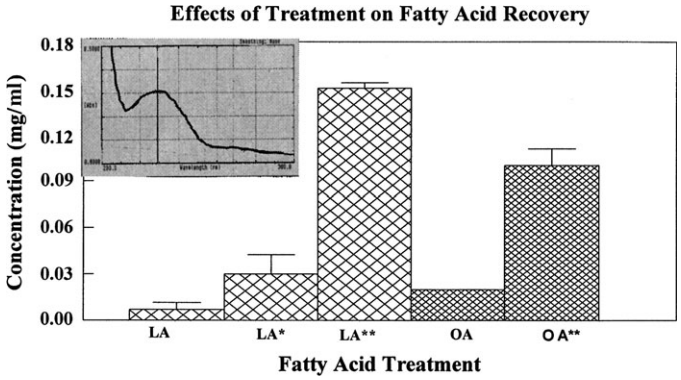

Figure 1 LA = linoleic acid; LA* = linoleic acid + EDTA; LA** = linoleic acid + EDTA + sonication; OA = oleic acid; OA** = oleic acid + EDTA + sonication. Far left inset of spectrophotometric scan showing conjugated diene formation at 230 nm in simple O/W emulsion of LA.

samples of linoleate at a concentration of 0.003 mg/ml. Linoleate was recovered with 100% efficiency and was comparable in both preparations, that is, with (0.0031 ± 0.0006%) and without EDTA (0.0028 ± 0002%). This is to be expected as linoleate is more soluble in water than linoleic acid. Spectrophotometric analysis of linoleic acid over a 5-day period confirmed conjugated diene formation after 24 h (reaching a peak after 3 days) resulting in only 73% recovery from the initial concentration at the end of the 5 days. Oleic acid samples remained stable over the 5-day period. The coefficient of variation between sonicated and nonsonicated samples was greater in samples that were not sonicated: oleic acid = 88%, linoleic acid = 66%, and linoleic acid + EDTA = 42%; in comparison with samples that were sonicated, oleic acid + EDTA + sonication = 9% and linoleic acid + EDTA + sonication = 2%.

Discussion

The results indicate the need for a better methodology in the preparation of lipid emulsions in model systems for investigation of human orosensory responses to FFAs; one that both minimizes FFA oxidation and achieves stable dispersions of FFAs in aqueous environments. This methodology has attempted to address these issues in developing appropriate stimuli to obtain human FA detection thresholds, which is the foundation for the next study.

Experiment 2

Experiment 2 sought to determine detection thresholds of three 18-C FFAs and an oxidized 18-C FFA by masking other sensory attributes. FAs were chosen as representations of those that commonly occur in food as well as representations of a polyunsaturate, a monounsaturate, and a saturate. To determine if an oxidized FFA was an effective stimulus, an oxidized polyunsaturate was included among the test stimuli. In order to eliminate confounding sensory cues, gum acacia (Schiffman et al. 1998) and mineral oil (used at a concentration typical for emulsions in industrial metalworking to reduce friction between particles) were used to minimize viscosity and lubricity, respectively; participants were required to wear noseclips to negate olfactory cues; oral FFA irritation was addressed through capsaicin desensitization (Green 1989; Lawless and Stevens 1989) of participants prior to testing; the potential for oxidation was decreased through the use of the methodology described in Experiment 1.

Materials and methods

Subjects

Twenty-two physically fit (maximal oxygen consumption [VO_{2max}] for men and women: ≥49 and ≥41 ml/kg/min, respectively) male (N = 15) and female (N = 7) adults (18–26 years old) with body mass indices (BMIs) ranging from 18.5 to 29.9 kg/m^2 participated. All had measurable thresholds for common chemosensory stimuli, were nonsmokers, weight stable (no change of body weight >5 kg over the last 3 months), not on a prescribed diet, not pregnant, and had no eating disorder (assessed through the Three-Factor Eating Questionnaire) (Stunkard and Messick 1985). Sensitivity to the taste of 6-*n*-propylthiouracil (PROP) has been directly associated with perception of fat-containing foods (Tepper and Nurse 1997, 1998) so, to optimize the chance of obtaining thresholds for FAs, participants were all PROP sensitive. Participants were recruited through public advertisements and received monetary compensation for their participation. The study was approved by the University's Human Subjects Institutional Review Board.

Screening stimuli

In order to verify normal chemosensory function, orthonasal olfactory thresholds were determined for butanol dissolved in deionized water ranging in concentration from 0.0006% to 4.0% (v/v), with dilutions differing by a factor of 3. Taste sensitivity for aqueous sucrose (Spectrum Chemicals) solutions was assessed using a concentration range of 0.0001–1.0 M, with successive dilutions differing by 0.25 log units. Classification of participants for bitter taste status was determined by their PROP/sodium chloride (NaCl) ratio using the 3-solution test (Rankin et al. 2004) consisting 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. Stimuli were presented at room temperature as 10-ml samples in 22.2-ml disposable plastic soufflé cups (sucrose and PROP tastes) or as 60-ml samples in 8-oz plastic squeeze bottles (butanol odor).

Experimental testing stimuli

Detection thresholds were determined for food grade linoleic acid (an 18-C polyunsaturated FA [PUFA]), oleic acid (an 18-C monounsaturated FA [MUFA]), and stearic acid (an 18-C saturated FA). FFAs were sealed under nitrogen,

in amber bottles, until the day of use. Linoleic was stored at −20 °C, oleic at 4 °C, and stearic acid at room temperature. Stocks were prepared fresh daily and consisted of FFAs sonicated in a vehicle of 5% gum acacia (w/v, TIC Gums, Inc., Belcamp, MD) (Schiffman et al. 1998), 5% mineral oil (w/v, Spectrum Chemicals), and 0.01% EDTA (w/v, Spectrum Chemicals) in deionized water. To verify the potential textural cues of the FAs were masked by the acacia and mineral oil, the flow rate of samples containing 0.03% linoleic acid, 1% linoleic acid, or just acacia and mineral oil (both at 5%) was measured on a Cannon-Fenske opaque (reverse flow) viscometer. There were no measurable differences between the samples containing a FA at the low or high concentration and the sample containing only the vehicle suggesting the FA did not contribute to this physical property of the stimuli. Additionally, the choice of instrument did not allow for direct measurement of lubricity. However, others have used a friction tester and report a reduction in friction with increasing fat content (de Wijk et al. 2006). The concentration of the FFA emulsions ranged from 0.00028% to 5% (w/v), with dilutions varying by 0.25 log units. Because stearic acid is a solid at room temperature, it was presented at 67–69 °C. All other FFAs were presented at room temperature. To determine if the effective stimulus was an oxidation product, oxidized linoleic acid was included among the stimuli. Oxidation was achieved by preparing linoleic acid 3 days prior to use (see Experiment 1).

Desensitization of the oral mucosa to irritation was achieved through exposure to capsaicin (Lawless and Stevens 1989). A 20-ppm capsaicin solution was prepared by dissolving 0.8 g capsaicin in 100 ml ethanol, and 0.05 ml of this stock solution was pipetted into 20 ml of deionized water just before presentation to the participant. All chemicals were purchased from Sigma-Aldrich Co., St Louis, MO, unless otherwise indicated.

Procedures

Normative tests. Participants were required to abstain from all food, beverages, and oral care products for at least 2 h prior to all tests. Olfactory function was assessed with butanol using an ascending-concentration, 2-alternative, forced-choice (2-AFC) procedure (Frank et al. 2003). Prior to testing, nasal patency for both nostrils was assessed by instructing participants to cover one nostril with a finger and to sniff through the other with their mouth closed. They repeated this procedure with the other nostril. The nostril with the self-assessed better airflow was used to assess sensitivity to butanol. Participants were then presented with 2 bottles in a random order, one with butanol and the other with deionized water. They were instructed to insert the bottle spout into the predetermined nostril, squeeze the bottle, and inhale through the nostril using normal resting breathing with their mouth closed. After repeating this procedure with the other bottle, they indicated which bottle had a stronger smell. An incorrect response resulted in the presentation of butanol at the next higher concentration. Testing was terminated when the participant made 5 correct choices in a row. This concentration was then compared with normative values (Cain et al. 1988; Frank et al. 2003). The interstimulus interval for butanol odor was set at 10 s and the intertrial interval at 60 s in order to reduce the likelihood of adaptation.

Concentrations of sucrose were presented to participants using a variation of the ascending-concentration, 3-AFC procedure (Weiffenbach et al. 1982; ASTM 2004). Participants were provided with noseclips and rinsed their mouth with deionized water before beginning. They were then presented with 3 soufflé cups positioned in random order, one with sucrose and 2 containing deionized water. They were instructed to swish the entire contents of one cup in their mouth for 5 s and expectorate. Before moving onto the next sample, they rinsed their mouth thoroughly with deionized water. After tasting all 3 samples, they were asked to choose which one was different. This procedure continued until the participant correctly identified the stimulus on 3 successive trials at the same concentration. Both the interstimulus interval as well as intertrial interval for sucrose taste sensitivity was set at 60 s.

To assess bitter taste function, participants rated the perceived intensity of 3.2×10^{-5}, 3.2×10^{-4}, and 3.2×10^{-3} mol/l PROP and 0.01, 0.1, and 1.0 mol/l NaCl dissolved in deionized water (Rankin et al. 2004). Participants were presented with 6 samples, 3 containing the PROP solutions and 3 containing the NaCl solutions. They were required to place the entire 10-ml sample in their mouth, evaluate the perceived intensity on a labeled magnitude scale (LMS) (Green et al. 1993), and expectorate. Participants rinsed their mouth thoroughly with deionized water between samples. The intensities of the 3 samples of NaCl were rated first followed by the 3 samples of PROP. Solutions were presented in random order within each taste quality. The interstimulus interval was set at 60 s. Results were plotted for each participant, and participants were assigned a PROP taster group by comparing their taste function for PROP with that of NaCl. If their rating for PROP relative to NaCl was lower, then they were classified as nontasters, if it was the same or higher, they were tasters. Only tasters were eligible to participate.

Experimental tests. Detection thresholds for linoleic, oleic, stearic, and oxidized linoleic acids were obtained using the same procedures outlined under normative testing procedures for sucrose taste sensitivity except that all testing was conducted under red light to minimize any visual cues. Participants completed a single sensory test in a given day. FFA detection thresholds were designated as the lowest concentration at which the participant correctly identified the target stimulus on 3 consecutive trials ($P < 0.05$). Missing values were replaced by the group mean for a FFA in analyses comparing threshold values. Outliers were identified

using the Dixon *Q*-test and excluded from statistical analyses (Dixon 1953). Thresholds were obtained from all but 3 participants (one missing for linoleic acid irritation, another for stearic acid, and one for oleic acid). There was, on average, only 1 outlier per FA.

In order to minimize oral FA chemesthesis as the source of stimulation, linoleic acid detection thresholds were remeasured following a desensitization pretreatment. Desensitization was achieved through the methods of Green (1989), Lawless and Stevens (1989), and Gilmore and Green (1993). Participants were presented with five 20-ml capsaicin solutions at 60-s intervals. They were instructed to swish the entire contents of the capsaicin sample in their mouth for 30 s and then expectorate. Immediately after expectoration, they rated the intensity on a LMS. The interstimulus interval was 30 s. No rinsing was permitted in between exposures. Upon completion of the task, participants were instructed to rinse their mouth thoroughly with deionized water. Capsaicin desensitization was then completed by interspersing a 15-min hiatus before remeasuring detection thresholds for linoleic acid. Another 20-ml test stimulus of the capsaicin solution was presented after linoleic acid detection thresholds were obtained to verify desensitization had occurred.

Results

Participants were young, physically fit individuals with predominately normal BMIs. Participants' sensitivity to butanol fell between the normative values of dilutions 5 and 8 (dilution steps are from stimulus number 0 which is 4% v/v butanol [Cain et al. 1988; Frank et al. 2003]) for all but 5 participants; 4 participants' had thresholds at dilution step 4 and 1 on dilution 3. Sensitivity to sucrose taste was comparable with normative values between 0.00592 and 0.1 M (Weiffenbach et al. 1982) for all participants.

Three participants (participants designated FE1–22, Figure 2) did not complete a LMS for capsaicin intensity during the desensitization session due to procedural error. Among the remaining participants, FE6, FE13, and FE21 failed to desensitize. Excluding them did not alter the measured threshold (0.033 ± 0.008% w/v). Their thresholds were 0.009%, 0.032%, and 0.0028%.

The means of the thresholds for each FFA are depicted in Figure 3. Thresholds were obtained from nearly all the 22 participants for each FFA tested: $N = 21$ for linoleic acid pre-desensitization, $N = 22$ for linoleic acid post-desensitization, $N = 21$ for oleic acid, $N = 21$ for stearic acid, and $N = 22$ for oxidized linoleic acid. The failure to obtain a threshold on the 3 trials was due to ceiling effects (i.e., failure to detect the highest stimulus concentration). The mean thresholds for linoleic pre-desensitization, linoleic post-desensitization, oleic, stearic, and oxidized linoleic acids were 0.034 ± 0.008% w/v, 0.032 ± 0.007% w/v, 0.022 ± 0.003% w/v, 0.032 ± 0.005% w/v, and 0.025 ± 0.005% w/v. A repeated measure analysis of variance with "stimulus" as a within-subject variable indicated no significant difference between the FFAs tested ($F(4,52) = 0.787$, $P = 0.495$).

Discussion

The present data reflect orosensory detection of FFAs in humans when inputs from olfactory, capsaicin, and viscosity-sensing systems have been minimized. The findings are consistent with a taste component for FAs but do not confirm this mechanism because of a lack of certainty that all other potential sensory cues did not contribute. Measurable thresholds were obtained from nearly all participants for each FA. The consistency of values suggests a common transduction mechanism for these long-chain FAs that is not influenced by saturation. Based on work with rats, there is specificity of sensitivity to FAs varying in saturation on different regions of the tongue (i.e., sensitivity to PUFAs on the anterior tongue and MUFAs on posterior tongue) (Gilbertson et al. 1997; Hansen et al. 2003) and an uneven distribution of CD36 in different papillae (i.e., concentrations for circumvallate > foliate > fungiform) (Laugerette et al. 2005). The present work involved whole-mouth stimulation, so any regional differences were not measured. However, the present findings raise several questions: 1) are current animal models reflective of human FA sensitivity, 2) do the DRK and CD36 mechanisms work together, and/or 3) are the thresholds measured here attributable to some other common transduction mechanism?

General discussion

Attempts to control textural attributes of fat in psychophysical experiments have involved the use of emulsifiers or thickening agents (Schiffman et al. 1992; Barylko-Pikielna et al. 1994; Tsuruta et al. 1999; Fukuwatari et al. 2003) to mask the contribution of viscosity from the FA. Ramirez demonstrated that xanthan gum, a carbohydrate polymer, effectively masks viscosity cues from FFAs (Ramirez 1992, 1994). Lubricity is another textural component proposed as a sensory cue for FAs (Ramirez 1994; Rolls et al. 1999; Verhagen et al. 2003) that has not been adequately controlled. In this study, mineral oil was incorporated into the FFA medium to add lubricity at a concentration used in industrial metalworking (from which the term "lubricity" is derived) to counteract tribological interactions. Our instrumental methods did not reveal a textural contribution of FAs from viscosity at the threshold level or even at a concentration approximately 2 orders of magnitude higher, but the possibility that humans have greater sensitivity than instrumental methods or are sensitive to textural qualities not masked by the acacia or mineral oil cannot be rejected. Olfactory input has been minimized in animals by olfactory bulbectomy, the use of $ZnSO_4$ (Ramirez 1993; Fukuwatari et al. 2003), or by asking human subjects to identify stimuli with nares closed (Schiffman et al. 1992; Mattes 2001).

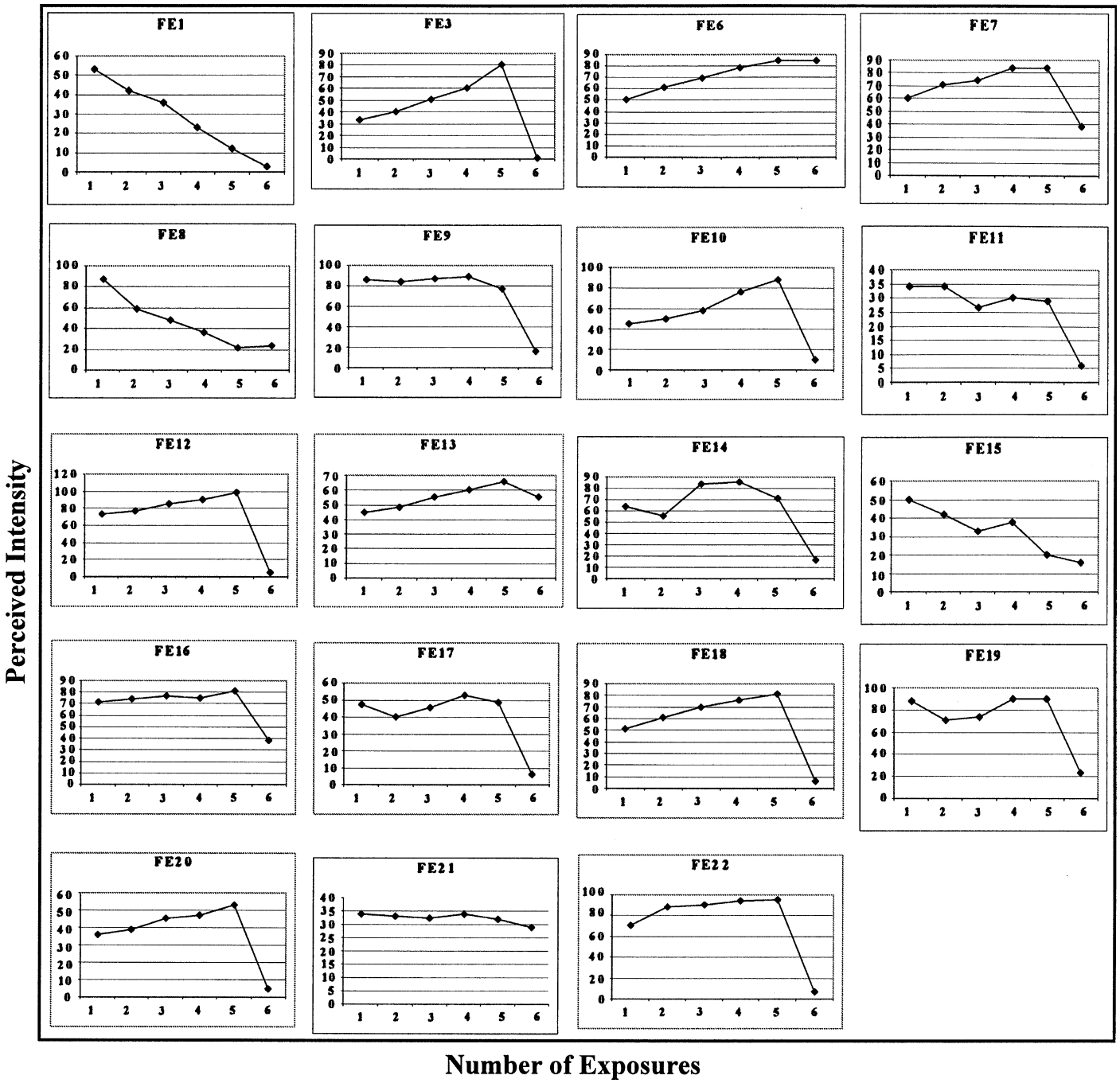

Figure 2 Perceived intensity of capsaicin irritation in the mouth as a function of number of exposures. Participants designated FE1–22. Five 20-ml 20-ppm capsaicin solutions were presented at 60-s intervals. A sixth solution was presented after obtaining linoleic acid taste thresholds to verify desensitization had occurred.

Visual identification has been diminished through the use of opaque cups and red cellophane (Schiffman et al. 1998) or by conducting all testing under red light. However, these approaches to minimize competing sensory cues from FAs remain incomplete. Various experimental methods to minimize oxidation product formation have been reported. One entails bubbling FFA samples with nitrogen (Gilbertson et al. 1997) or embedding the FFA in food (Nasser et al. 2001; Kamphuis et al. 2003). However, once exposed to air and the stresses of sample preparation, the potential for oxidation still exists. Alternatively, demineralized water may be deoxygenated by boiling and sealing until ready for use under nitrogen gas (Koriyama et al. 2002). Subsequent mixing and homogenization of FA samples is then

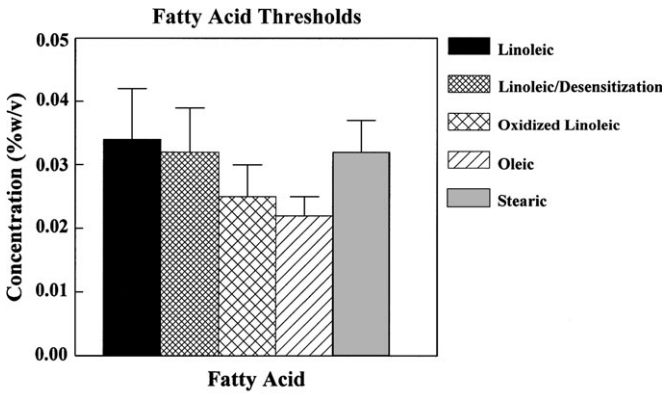

Figure 3 FFA detection thresholds.

performed under nitrogen gas (Koriyama et al. 2002). Although this method directly addresses the problem of oxidation, it is an elaborate method that may pose temporal difficulties in psychophysical studies employing large samples and still needs to consider the potential for nitrogen evaporation. Others have used sodium salts of FFAs (Kamphuis et al. 2003) known to be more soluble in water than their FFA counterparts. This eliminates solubility problems; however, sodium salts of FFAs are not widely present in foods (Weiss 1983), and thus, although they may in themselves present effective stimuli, their relevance to fat perception is uncertain. Recent evidence from rats (Clyburn and Pittman 2005) indicates linoleic acid and oleic acid are perceived similarly to linoleate and oleate, but the effective cue is not known or if it applies to humans.

Additionally, creation of a stable dispersion of an O/W emulsion requires consideration. Traditional mixing with hot plate/stirrers and stirring rods not only serve to facilitate oxidation but also create samples with poor homogeneity. The type of labware used in the preparation of low-concentration O/W emulsions is an additional potential problem in that it influences the degree of FA surface adsorption. This experiment used sonication to aid dispersion of FFAs in an aqueous environment that limited oxidation and created homogenous FA emulsions. Use of polypropylene labware also served to maintain stability by reducing adherence to the vessel walls. In our work, poorer recovery was observed in samples containing only linoleic acid and EDTA compared with the same samples when sonicated. However, linoleic acid samples were stable for only 24 h stressing the need for daily preparation of this FA in sensory studies.

Detection thresholds for humans, as observed in Experiment 2, are 2 orders of magnitude higher than those reported in cell electrophysiological studies (Gilbertson et al. 1997). However, methodological issues, including the medium in which the FFAs are suspended and delivered, as well as potential species differences in sensitivity probably render these comparisons meaningless. To date, psychophysical studies in humans investigating orosensory perception of dietary fat using detection thresholds are few. The detection threshold for linoleic acid emulsified in water with monolinolein is approximately 0.1%, only slightly higher than values reported here. In another human study (Schiffman et al. 1992, 1998), detection thresholds for oils (triglycerides) varying in chain length were much higher than reported here, ranging in young participants from 2.85% to 8.27% (v/v) and in the elderly from 9.77% to 25% (v/v). However, because this study used triglycerides without checking the accompanying FFA concentration, it is not possible to draw a direct comparison. It appears that thresholds for triglycerides are higher. Whether they are based on taste or other properties such as lubricity or irritation is not known. Detection and discrimination methodologies in humans employed by others (Nasser et al. 2001; Kamphuis et al. 2003) also make comparisons difficult because the effective FFA cue for detection cannot be definitively determined. Additionally, the stimuli were sodium salts, not present in foods (Weiss 1983), but were embedded in food. It is likely that orosensory cues emanating from dietary fat are dependent on the composition of the food stimuli used and the matrix in which they are embedded (Schiffman et al. 1998; Nasser et al. 2001).

We did not observe a difference between linoleic acid and oxidized linoleic acid. Animal data indicate that animals can discriminate between oxidized and unoxidized oils (Ramirez 1992; Tsuruta et al. 1999; Kimura et al. 2004). This is consistent with the production of highly potent odorants with very low thresholds produced presumably by lipid peroxidation (Blank et al. 2001; Lubran et al. 2005). Furthermore, unoxidized oils are preferred (Tsuruta et al. 1999; Kimura et al. 2004). Human reports also suggest that oxidized oils are unpleasant (Kimura et al. 2004). A difference between linoleic acid and oxidized linoleic acid may have been detected with a larger sample size. Additionally, we cannot rule out the influence of contaminants. The FFAs ranged from ≥95% to ≥99% in purity, so there is some chance that the trace amounts of other compounds were responsible for the measured thresholds. There is also evidence of peroxidase activity in human saliva (Cowman et al. 1983). Because salivary constituents were not analyzed in the present investigation, peroxidase activity could have contributed to our measured thresholds.

The proposal that humans may be classified as fat tasters and nontasters (Nasser et al. 2001; Kamphuis et al. 2003) is intriguing given a rodent model (Osborne–Mendel and S5B/P1 strains) suggestive of such a phenomenon (Gilbertson et al. 1998). We did not observe a bimodal distribution among any of the FFAs tested (data not shown). However, such a distinction may be based on other sensory properties, such as olfaction or texture, which were not completely controlled in the studies with suggestive results (Kamphuis et al. 2003). The use of oleic acid as a placebo in an earlier study (Kamphuis et al. 2001) is not consistent with later reports from cell electrophysiological studies (Hansen et al. 2003), animal behavioral studies (Clyburn and Pittman 2005), as well as our present study in which detection thresholds for oleic acid were obtained.

PROP tasters are purported to have a heightened sensitivity to fat-containing products (Tepper and Nurse 1997). However, this is not consistent (Drewnowski et al. 1998; Yackinous and Guinard 2001). Because our sample population purposefully consisted entirely of PROP tasters (to optimize the probability of obtaining detection thresholds), we cannot comment on the relationship between PROP and fat sensitivity except to state that tasters are able to detect low concentrations of FFAs.

Lastly, although it is not relevant here, where stimuli were only swished and expectorated, it is possible that FFAs have targets in the gut and may act via reflexes generating signals analogous to taste. This has been reported for both sweet and fat stimuli (Tracy et al. 2004).

In summary, using a paradigm that attempted to minimize input from olfactory, capsaicin, and viscosity-assessing tactile systems, the present data are indicative of orosensory detection of FFAs that does not appear to be dependent on degree of FA saturation. However, in the absence of complete certainty that competing orosensory cues were eliminated, coupled to the problems of attributing a quality to a yet-to-be elucidated oral sensation, we cannot confirm the effective cue for detection. Whether ''fatty'' is a taste quality in humans will depend on verification of transduction mechanisms and further psychophysical and physiological studies. Candidate FA transduction mechanisms have been proposed. Long-chain, *cis*-polyunsaturated and monounsaturated FFAs inhibit delayed rectifying K^+ channels in taste receptor cells (TRCs), (Gilbertson et al. 1997; Hansen et al. 2003). This may be the primary mechanism for FA transduction or may be a mechanism by which FFAs facilitate the transduction of other taste stimuli (Gilbertson 2005). The FA transporter, CD36, identified in circumvallate papillae a decade ago (Fukuwatari et al. 1997) and recently confirmed in circumvallate, foliate, and, to a questionable degree in fungiform papillae (Laugerette et al. 2005) may also contribute to fat taste transduction. Both these purported mechanisms as well as our detection thresholds are within the concentrations of FFAs found in foods (Weiss 1983). There have also been observations of orphaned G-protein–coupled receptors in TRCs (Hansen et al. 2006). Future confirmation of a ''fatty'' taste quality would not only contribute to our understanding of basic taste biology but also hold implications for understanding taste disorders, food preference and intake, as well as the development and acceptability of fat-modified products. Future studies should employ other approaches to verify these observations and explore sensitivity to FAs varying in chain length and in different media.

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