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The Examination of Fatty Acid Taste with Edible Strips

Sahbina Ebba[a,c], **Ray A. Abarintos**[a], **Dae G. Kim**[a], **Melissa Tiyouh**[a], **Judith C. Stull**[b], **Ankur Movalia**[a], and **Gregory Smutzer**[a]

[a]Department of Biology, Temple University, Philadelphia, PA 19122

[b]Department of Sociology, La Salle University, Philadelphia, PA 19141

Abstract

The objective of this study was to determine whether humans could detect long-chain fatty acids when these lipid molecules are delivered to the oral cavity by edible taste strips. For suprathreshold studies, up to 1.7 *u*moles of stearic acid or linoleic acid were incorporated into 0.03 mm thick, one-inch square taste strips. Normalized taste intensity values for stearic acid were in the barely detectable range, with values equal to, or slightly above control strips. One-third of test subjects described the taste quality as oily/fatty/waxy. Approximately 75% of test subjects could detect the presence of linoleic acid when this fatty acid was incorporated into dissolvable strips. Normalized taste intensity values for linoleic acid were in the weak to moderate range. The most commonly reported taste quality responses for linoleic acid were fatty/oily/waxy, or bitter. When nasal airflow was obstructed, the perceived taste intensity of linoleic acid decreased by approximately 40 percent. Taste intensity values and taste quality responses for linoleic acid were then compared among tasters and non-tasters of 6-*n*-propylthiouracil (PROP). Individuals who could detect the bitter taste of PROP reported higher taste intensity values for linoleic acid compared with PROP non-tasters. However, taste quality responses for linoleic acid were similar among both PROP tasters and PROP non-tasters. These results indicate that humans can detect long-chain fatty acids by both olfactory and non-olfactory pathways when these hydrophobic molecules are delivered to the oral cavity by means of edible taste strips. These studies further show that genetic variation in taste sensitivity to PROP affects chemosensory responses to the *cis*-unsaturated fatty acid linoleic acid in the oral cavity.

1. Introduction

In humans, long-chain fatty acids not only supply substantial amounts of energy to the body, but these molecules are also important precursors for the synthesis of complex lipids. Fatty acids play critical roles in cell signaling, and are precursors for membrane phospholipids [1]. In addition, fatty acids modify the structure and function of plasma membrane lipid rafts, whose composition may be altered by the intake of dietary fats [2]. Elevated levels of serum fatty acids have been associated with an increased risk for insulin resistance and type 2 diabetes, cardiovascular disease, and abdominal obesity [3–5]. However, a high-fat

Correspondence to: Gregory Smutzer, 255 Biology Life Sciences Bldg., Temple University, 1900 N. 12th Street, Philadelphia, PA 19122, smutzerg@temple.edu; smutzerg@gmail.com 215 204-1236 [phone], 215 204-6646 [fax].
[c]Current Address Meharry Medical College, 005 Dr. D.B. Todd Jr. Blvd., Nashville, TN 37208

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(ketogenic) diet has been used as a nonpharmacologic therapy for some forms of epilepsy [6–7].

Studies that examine fatty acid taste perception are important for understanding how individuals make choices regarding the types and amounts of food that they ingest. Previous studies have shown that hedonic ratings for fats tend to correlate with increasing body mass [8], and that obese individuals have a stronger preference for high fat and sweet foods when compared to lean subjects [9]. In humans, hyposensitivity to fatty acids is associated with higher lipid intake and higher body mass index [10–12]. Thus, variations in fat taste perception could modulate the risk factors for cardiovascular disease and diabetes.

Humans detect both the odor and taste of long-chain fatty acids [13,14]. One hypothesis to explain fatty acid taste suggests that ingested fats are identified by their textural and odorant properties, rather than by their ability to elicit a specific taste response [14]. However, recent evidence suggests that fatty acids do exhibit a unique taste quality that is detected by humans [14–16]. These studies indicate that long-chain fatty acids are detected in the oral cavity when olfactory function is eliminated by blocking nasal airflow [15], or when the specific texture of fatty acids is masked by diluting the tastant in sonicated emulsions [15].

Studies using animal models have resulted in a variety of proposed mechanisms that may explain the initial stages of fatty acid taste [17–24]. One hypothesis suggests that fatty acids passively diffuse across the membrane bilayer of taste receptor cells [17], most likely as a protonated carboxylic acid [25]. Alternatively, a delayed rectifying K^+ channel may be inhibited by long chain *cis*-unsaturated fatty acids in isolated taste receptor cells [18]. Fat taste may occur via interactions with Cluster of Differentiation-36 (CD36) protein [19–23], a membrane glycoprotein that contains a large external domain that binds long chain *cis*-unsaturated and saturated fatty acids [19–23]. Finally, taste preferences for medium and long chain fatty acids may involve the G-protein coupled receptors GPR40 and GPR120 [24].

Recent psychophysical studies suggest that PROP taster status affects the chemosensory response of fatty acids in the oral cavity of humans. Duffy et al. reported that the liking of high-fat food and beverage groups decreased with increasing perceived bitterness of PROP in women [26]. Tepper and Nurse [27] reported that PROP non-tasters favored high-fat salad dressings more than did medium tasters or supertasters. On the other hand, Drewnowski et al. [28] and Yachinous and Guinard [29] both report no association between fat taste perception and PROP taster status.

Psychophysical studies of human fatty acid taste perception are limited in part because hydrophobic compounds are difficult to introduce into the oral cavity [14]. Hydrophobic tastants are generally presented to subjects as emulsions that are suspended in gums or mineral oil to mask their viscosity [14,15]. We minimized tactile responses by using edible taste strips that rapidly dissolve in the oral cavity [30,31], and we eliminated olfactory responses by blocking nasal airflow during presentation of edible strips [31].

The main goal of this study was to determine whether humans could detect long-chain fatty acids when these compounds are incorporated into edible taste strips. A second aim was to identify a possible role for *cis*-double bonds in fatty acid taste perception. A final aim was to identify a possible role for PROP taster status in fatty acid perception in the oral cavity.

Linoleic acid (18:2) was chosen for our studies because this *cis*-unsaturated fat is a representative fatty acid tastant [16]. In addition, linoleic acid is an essential fatty acid whose consumption would likely be monitored by the mammalian gustatory system [32]. Chemosensory responses from the linoleic acid study were then compared with the stearic

acid study (18:0) in order to identify a putative role for *cis*-double bonds in fatty acid taste perception.

2. Materials and Methods

2.1. Preparation of edible taste strips

Taste strips were prepared as previously described [30,31]. Pullulan (α-1,4-; α-1,6-glucan, NutriScience Innovations, LLC, Fairfield, CT), and was combined with the polymer hydroxypropyl-methylcellulose (Dow Chemical Co., Midland, MI) at a weight ratio of 11.5:1. Stearic acid, linoleic acid, and PROP were obtained from Sigma Chemical Co., St. Louis, MO. Mineral oil was added to control strips [13] at 0.005% w/v (CVS Caremark, Woonsocket, RI), along with food coloring to aid in visualization of taste strips. For film preparation, a flat casting surface was washed with 70% ethanol, dried, and wiped clean with a paper towel. The clear polymer solution was then poured onto a non-stick surface [30,31]. The solution was evenly spread over the enclosed area, and allowed to dry for 12 to 18 hours at room temperature in a glove box under a nitrogen atmosphere. After drying, the clear film was removed, cut into one-inch squares, and stored in the dark at 4 °C or −10°C in an airtight sealable bag for no more than three weeks.

2.2. Test subjects

Subjects were recruited through flyers posted on the Temple University campus and by word of mouth in the surrounding community. A total of 88 self-reported healthy volunteers who had no neurological disorders that would compromise their taste function participated (linoleic acid study n = 60 and stearic acid study n = 30, two subjects participated in both studies). Demographically, participants were: 37 male and 51 female; 46.6% Caucasian, 32.4% Asian, 17.6% African-American, and 3.4% Hispanic; and ranged in age from 18 to 74 years with a mean of 25.1 years. This was a sample of convenience. Confounding factors such as age, gender, and race/ethnicity were entered into the multivariate analysis as controls thus addressing possibilities of self-selection biases.

2.3. Trial design

Participants were told that each trial consisted of sampling two taste strips. Subjects were instructed that one strip contained a tastant that they may or may not be able to detect as a specific taste quality, and one strip did not. Control and fatty acid strips were randomized for each trial, and responses (taste intensity, taste quality, and hedonics) were recorded for each taste strip. The task of each subject was to rate the intensity of the perceived taste response, and to identify the perceived taste as sweet, sour, salty, bitter, no discernible taste, or “other” taste quality. Subjects who answered “other” had the opportunity to describe the taste quality further. Four different amounts of each fatty acid were presented to the subject (1.1, 1.3, 1.5, and 1.7 *u*moles), and the trial order was randomized. Finally, subjects reported a hedonic response. Participants rinsed with room temperature water after tasting every strip, and also rinsed with a mild salt solution followed by water between trials.

Subjects were presented with a control strip, a fatty acid strip, along with a sweet, sour, salty, and bitter strip prior to the start of the experiment. For consistency, subjects were asked to place the strip on the center of their tongue. Subjects were further instructed to touch the roof of their mouth with their tongue to allow the strips to fully dissolve, and to wait five seconds before reporting taste intensity, taste quality, and hedonics responses.

2.4. Effect of PROP taster status on fatty acid chemosensation

For PROP taster status, subjects for both the stearic acid and linoleic acid study were presented with taste strips that dissolved on contact with the tongue. Strips contained either

300 nanomoles of PROP, or no PROP. PROP non-tasters were classified as those who gave a normalized gLMS value of less than 6 (weak), and/or expressed an AVI/AVI genotype [31,33–36]. *TAS2R38* genotype data were identified in order to confirm the PROP taster phenotype for 14 of the 52 test subjects in the linoleic acid study. *TAS2R38* genotype and taster phenotype data for these 14 subjects had been obtained in a previous study [31].

2.5. Taste intensity and hedonics measurements

The general labeled magnitude scale (gLMS), a 100-point scale that measures perceptual intensity, was used for both taste intensity and light intensity measurements [37–39]. This scale contains labels at barely detectable (1.4), weak (6.0), moderate (17.0), strong (34.7), very strong (52.5), and the strongest sensation of any kind (100.0). All test subjects were trained in the use of the gLMS at the start of the study [39]. For taste strips, subjects were specifically instructed to report a taste intensity response and not a tactile response after five seconds.

For hedonics ratings of tastants, the degrees of liking–disliking of stearic acid and linoleic acid were rated on a horizontal (bipolar) hedonic gLMS (0 = neutral; ±6.0 = weakly like/dislike; ±17.0 = moderately like/dislike; ±34.7 = strongly like/dislike; ±52.5 = very strongly like/dislike; ±100.0 = strongest imaginable like/dislike of any kind) according to Duffy et al. [40].

2.6. Normalization of taste data

Taste data were normalized to a second sensory system that used visible light. Light intensity was varied with a rheostat (ISE Inc., Cleveland, OH), and test subjects verbally reported intensity values from the gLMS that were normalized according to Bartoshuk et al. [39]. Specifically, magnitude estimates were normalized by averaging two presentations of light at five different intensities, and the geometric mean of those averages was calculated for each test subject. A normalization factor was constructed for each subject by dividing the group median for the geometric mean by the geometric mean for each test subject [39]. Responses for each subject were then multiplied by the normalization factor [39]. One test subject in the linoleic acid study initially reported high values for control strips, was retrained in the gLMS, and retested at a later date for inclusion in the study.

2.7. *TAS2R38* genotyping

Genomic DNA was obtained from cheek cells with a sterile swab (Epicentre Biotechnologies, Madison, WI). DNA was amplified with TaqMan primers (Applied Biosystems, Foster City, CA) [35]. Alleles of *TAS2R38* were genotyped at each of the three variant single nucleotide sites [31,35] using allele-specific fluorescent probes from Applied Biosystems (Carlsbad, CA; reference SNP numbers include A49P = rs713598, V262A = rs1726866, and I296V = rs10246939). The ability of these nucleotide probes to fully hybridize to genomic DNA resulted in the loss of quenching of the terminal fluorophore, and was the criterion for identifying the specific nucleotide at each polymorphic site [31,35].

3. Results

3.1. Normalization of taste intensity and hedonics data

In magnitude-matching, test subjects judge intensities of sensations from two or more modalities on a single, common scale. Variable light intensity was used as the sensory modality for normalizing all taste intensity and hedonics data. Figure 1 shows a scatter plot of taste intensity responses to 300 nanomoles of PROP that were obtained with magnitude estimation and the gLMS.

3.2. Data analysis

Data are reported as mean ± standard error of the mean (SEM), and were analyzed using SPSS, version 17.0 for Windows (IBM, Armonk, NY). Significance was defined as $p < 0.05$. In order to understand the effectiveness of taste strips as a delivery system, and to quantify the sensitivity of PROP taster status to linoleic acid and stearic acid taste intensity responses, four regression analyses were completed for each fatty acid (one analysis for each of the four fatty acid amounts). In each regression analysis, the estimation of normalized taste intensity for each subject was the dependent variable. The following variables were entered into the analyses as controls: gender, age, and non-minority race/ethnicity status. Finally, the degree to which each subject found a taste strip to be pleasant or unpleasant was entered.

A statistical power analysis was completed to establish the sample size needed for robust analyses, and to accommodate for attrition. A required sample size of 25 was calculated using Cohen's method [41,42]. Specifically, a multivariate analysis (multiple regression, MANOVA, HLM) with $\alpha = .05$, u (the number of independent variables) = 10, $R^2 = .08$, and power = .80, a minimum sample size of 25 was needed in each of the two studies. According to these calculations, the sample sizes for both the stearic acid study and the linoleic acid study were of adequate size. For the two studies, linoleic acid subjects differed from stearic acid subjects in average age (26.5 vs. 23.4 years), and in the percentage that were male (38.3 vs. 50%).

3.3. Chemosensory responses to stearic acid

3.3.1. Perceived taste responses and hedonics responses for stearic acid—In order to examine the ability to perceive a fatty acid in the oral cavity, and to identify a putative role for *cis* unsaturated bonds on fatty acid taste perception, taste intensity, taste quality, and hedonics data were obtained for both a saturated fat (stearic acid), and a *cis*-unsaturated fat (linoleic acid) of identical carbon chain length.

The perceived taste intensity values for stearic acid were obtained at 1.1, 1.3, 1.5, and 1.7 *u*moles of tastant (n = 30 subjects). Correlation coefficients for each of the four duplicate taste intensity ratings for stearic acid were high (mean r = 0.72).

All four amounts of stearic acid yielded values that were at or near the "barely detectable range" for taste intensity, and were on average equal to, or slightly above the intensity values for control strips in our subject population (see Figure 2). Specifically, five out of 30 test subjects yielded intensity responses of zero for all four stearic acid amounts. Among the remaining 25 subjects, only eight subjects reported gLMS intensity values that were above control values for all four amounts of stearic acid. Finally, the mean suprathreshold taste intensity values for this fatty acid showed only a negligible increase as the amount of stearic acid increased in the taste strips.

As shown in Table 1, subjects were more likely to describe a taste quality for stearic acid than for control taste strips. This taste quality was most frequently described as "other" taste, while the highest amount of stearic acid showed a substantial number of bitter taste responses. Even with a barely detectable range for taste intensities, one-third of test subjects described the taste quality of stearic acid as oily/fatty/waxy (see Table 1). Taken together, these taste intensity and taste quality data indicate that some individuals in this study could perceive the taste of stearic acid at amounts up to 1.7 *u*moles.

3.3.2. The size of the effect - stearic acid—Single-factor ANOVA of taste intensity data obtained with control, 1.1, 1.3, 1.5, and 1.7 *u*mole stearic acid strips showed no significant differences among our test subjects. Furthermore, no statistical differences in

perceived taste intensity values were observed between males and females (P >= 0.05, two-tailed test). In the multivariate analysis, only two out of four regressions had any statistically significant covariates, and these regressions represented the two lowest amounts of stearic acid (1.1 and 1.3 μmoles). These statistical results are shown in Table 2.

For the lowest amount of stearic acid, the R^2 value indicated that 27.6 percent of variation in the dependent variable (the intensity rating) could be explained by the set of independent variables (age, gender, normalized PROP intensity ratings, minority status, and normalized hedonic ratings). At the lowest amount (1.1 μmoles), only hedonic ratings showed statistical significance. Specifically, for every unit increase in a subject's hedonics rating, the corresponding taste intensity rating decreased by 0.2 points. In other words, the more pleasant the subject rated a fatty acid strip, the lower the subject rated the taste intensity response.

For the second lowest amount of stearic acid (1.3 μmoles), the R^2 value indicated that 87.3 percent of variation in the dependent variable (taste intensity rating) could be explained by the entered independent variables. Gender and normalized PROP taste intensity scores for stearic acid were not statistically significant. However, taste intensity rating increased by 0.3 units for every one-year increase in the age of a test subject. In addition, the more pleasant a subject rated the fatty acid taste strip, the lower the subject rated the taste intensity. Finally, a one-unit increase in the hedonic rating correlated with a taste intensity rating decrease of 1.4 points for the 1.3 μmole stearic acid strips.

3.4. Chemosensory responses to linoleic acid

3.4.1. Perceived taste intensity responses and taste quality responses to linoleic acid—A total of 40 out of 52 non-smoking subjects could distinguish 1.7 μmole linoleic acid strips in the barely detectable to strong range (normalized gLMS values ≥ 1.4). Of the 12 subjects who rated the strips below the barely detectable range, eight subjects reported a gLMS value of zero for the 1.7 μmole strips. Overall, these results indicate that approximately 75% of non-smoking subjects could detect an intensity response in 1.7 μmole linoleic acid strips.

As shown in Figure 3, average taste intensity values for linoleic acid increased slightly as the amount of linoleic acid increased in the strips. On average, normalized gLMS values varied from 0.5 to 1.5 for control strips, and from 7 to 11 for linoleic acid strips.

When compared to control strips, subjects were more likely to report a taste quality response for linoleic acid. As shown in Table 1, approximately 50% of test subjects were able to associate a specific taste quality with linoleic acid when this stimulus was presented at amounts between 1.1 and 1.7 μmoles. In general, the percentage of subjects who reported a bitter taste response for linoleic acid increased as the amount of linoleic acid increased in the strips. As the amount of linoleic acid increased in the strips, the percentage of subjects who reported no discernible taste decreased. Finally, the percentage of respondents who reported the taste of this fatty acid as other than sweet, sour, salty, or bitter, did not vary appreciably in our study.

3.4.2. Statistical analyses of different subject sub populations to linoleic acid —The two regressions that proved to have variables that attained significance represented taste intensity values for the two highest amounts of linoleic acid (1.5 and 1.7 μmoles). These results are shown in Table 3. For 1.1 μmole strips, R^2 indicated that 24.1 percent of the variation in taste intensity could be explained by the entered independent variables taken together. Age and normalized PROP taste intensity ratings were not statistically significant. These variables were kept in the analysis as controls. However, being classified as male

added 3.5 points to the gLMS rating scale. Furthermore, for every one unit increase in the hedonic rating, the taste intensity rating decreased by 0.5 units on the gLMS. As with stearic acid, the lower the subject rated the taste intensity the more pleasant the subject rated the linoleic acid taste strip.

For the highest amount of linoleic acid in this study (1.7 *u*moles), the R^2 value indicated that 33.7 percent of variation in the taste intensity rating could be explained by the entered independent variables taken together. Age and gender were not statistically significant. Furthermore, for every one unit increase in a subject's hedonics rating, the corresponding intensity rating decreased by 0.9 points. (See Table 3).

In summary, the determinants of the subject's taste intensity ratings in the four linoleic acid analyses (1.1, 1.3, 1.5, and 1.7 *u*mole amounts) did differ from those in the four stearic acid analyses. However, some similarities were observed in hedonics ratings for these two taste stimuli. With both fatty acids, the pleasantness rating for the subject was most strongly related to that subject's taste intensity rating as evidenced by the size of the related betas (i.e., the standardized regression coefficients).

3.5. Effect of nasal airflow on linoleic acid oral chemosensation in the oral cavity

Linoleic acid is a liquid at room temperature, may volatilize in the oral cavity when taste strips dissolve, and may stimulate an olfactory response. In addition, Bolton and Halpern reported that humans could discriminate linoleic acid both orthonasally and retronasally via the olfactory system [13]. If volatilization of this unsaturated fatty acid occurs in the oral cavity, then blocked airflow through the nasopharynx would eliminate a potential olfactory component that might be perceived as a taste response by some test subjects. The goal of the final experiment was to determine whether the perceived taste intensity values for linoleic acid included an olfactory component. A group of 18 test subjects was instructed to report a perceived taste intensity response for linoleic acid both in the absence and presence of nose clips. For this study, the amount of linoleic acid in the taste strips was expanded to 2.8 micromoles. Figure 4 shows the effect of nasal airflow on linoleic acid perception. The results show that the perceived taste of linoleic acid decreased an average of 40% when nasal airflow was completely blocked.

3.6. Effect of PROP taster status on linoleic acid taste intensity responses

A goal of this research was to identify a possible role for PROP taster status on the ability to perceive the taste of fatty acids in the oral cavity. Analysis of the data indicated that the PROP taster status variable was statistically significant in the linoleic acid analyses, and was not significant in the stearic acid analyses. Figure 5 shows the effect of PROP taster status on taste intensity responses to linoleic acid under conditions of unobstructed nasal airflow. PROP tasters exhibited an increased ability to detect linoleic acid in the oral cavity when compared to PROP non-tasters. This difference was observed at all four linoleic acid amounts, and was most noticeable at the highest amount of linoleic acid. ANOVA results showed that the PROP variable was statistically significant in the four linoleic acid analyses (1.1, 1.3, 1.5, and 1.7 *u*mole strips). For every one unit increase in a subject's normalized PROP score, their linoleic acid taste intensity rating increased by 0.1 points.

Taste quality data were also separated into PROP tasters and PROP non-tasters (data not shown). Taste quality responses for linoleic acid between the two groups did not vary significantly among the categories of taste responses that were examined in this study.

4. Discussion

4.1. Chemosensory responses to fatty acids during unobstructed nasal airflow

Due to the fact that fatty acids are hydrophobic compounds, the gustatory component of fatty acid perception in the human oral cavity has been difficult to quantify. We examined this problem by preparing edible taste strips that rapidly and completely dissolve in the oral cavity. Two complimentary statistical analyses were completed for each fatty acid. The first analysis involved the verification of an effect, and the second analysis captured the size of the effect. Taken together, these statistical data provide support for the hypothesis that free fatty acids are readily incorporated into taste strips, and yield a measurable chemosensory response in the oral cavity of humans.

With unobstructed airflow through the nasal cavity, the perceived taste responses for stearic acid and linoleic acid indicated that most individuals could perceive a chemosensory response for these two fatty acids. Higher taste intensity responses were observed with linoleic acid than with stearic acid, and this finding may reflect the presence of *cis* double bonds in linoleic acid. These *cis* bonds depress the melting point so that linoleic acid (but not stearic acid) is a liquid at room temperature. This physical characteristic of linoleic acid may increase the volatility of this fatty acid in the oral cavity after the strips dissolve. In addition to a possible increase in volatility of linoleic acid over that of stearic acid for increasing olfactory responses, these *cis* double bonds could enhance chemosensory responses of linoleic acid in the oral cavity. These double bonds could increase accessibility and/or binding of fatty acid to taste receptors, or could enhance diffusion of fatty acid across the plasma membrane.

Some test subjects could not detect a chemosensory response to stearic acid or linoleic acid. These subjects could have taste thresholds greater than 1.7 *u*moles for these fatty acids, or may exhibit taste blindness to these fatty acids at physiological amounts. Although radically different delivery methods were used, the stearic acid amounts in this study were below the threshold values reported by Chalé-Rush et al. when emulsions were used as the delivery method [15]. However, threshold values obtained with edible strips are one to two orders of magnitude lower than thresholds obtained with aqueous delivery methods [30,31]. Therefore, direct comparisons between the two delivery systems are difficult to make. Nevertheless, our results are consistent with other studies that have also demonstrated that some individuals cannot perceive the taste of long-chain fatty acids [43].

The data for stearic acid and linoleic acid yielded a variety of taste quality responses. Variations in taste quality responses for these two fatty acids may reflect the unfamiliarity of test subjects to oral fat stimuli. Alternatively, fatty acids at or just above suprathreshold amounts may produce an aversive off-taste that may result in a bitter taste response in some subjects [44]. For example, linoleic acid is a major contributor to the bitter taste of poppy seeds [45]. However, these taste quality results are consistent with the observation that higher amounts of fatty acid tastants result in a decreased hedonics response.

4.2. Chemosensory responses to linoleic acid with obstructed nasal airflow

Our results indicate that the chemosensory response to linoleic acid in the oral cavity included both olfactory and non-olfactory components. The non-olfactory components could include a true taste response, a tactile response, or a combination of these chemosensory responses. The present results cannot determine what fraction of linoleic acid perception represents a true gustatory response. However, our taste quality data suggest that a component of the perceived taste of linoleic acid represents a true taste quality. Although chemosensory responses were reported after strips fully dissolved in the oral cavity, additional studies are required to determine whether a tactile response from dissolved strips

might contribute to the chemosensory response of linoleic acid. No matter what mechanism is used to detect fats in the oral cavity, the chemosensory response to linoleic acid may allow the body to finely regulate intake of this essential fatty acid for various physiological needs [10].

4.3. Putative role for PROP taster status in linoleic acid chemosensation

Although some disagreement exists [8,9,46], individual differences in fat taste perception have been associated with the ability to perceive the bitter taste of PROP [29,47–49]. A link between PROP taster status and fatty acid taste perception remains controversial. Nonetheless, our linoleic acid data with unobstructed nasal airflow supports a role for PROP taster status in the chemosensory response to linoleic acid in the oral cavity. These results agree with previous studies which demonstrated that PROP tasters had a greater ability to perceive bitter tastants than non-tasters [49,50]. If true, then one possible explanation for these findings is that the decreased ability to taste linoleic acid by PROP non-tasters may be caused by a reduced ability to detect a bitter component of fatty acid taste in these subjects. Alternatively, some PROP tasters have a higher papillae density in the oral cavity (i.e., supertasters) [51,52], which in turn could result in an increased ability to detect linoleic acid. Although the transduction mechanism for fatty acid taste in humans is not clear, increased papillae density, and an increase in the number of receptor cells that detect fatty acids, would be consistent with an increased ability to detect linoleic acid in the oral cavity. Finally, increased papillae density in some PROP tasters may allow an increased response to tactile cues from fatty acids in the oral cavity.

4.4. Biophysical aspects of fatty acid chemosensation in the oral cavity

When edible strips dissolve in the oral cavity, fatty acid is released. The fatty acid becomes hydrated, and may form micelles in the salivary fluid at amounts above the critical micelle concentration (cmc). In aqueous buffer, linoleic acid forms pre-micellar aggregates at approximately 120 *u*molar concentrations, and forms micelles near 150 *u*molar concentrations [53]. Since only polar carboxyl groups of fatty acids populate the surface of intact micelles, then fatty acid micelles that bind to receptor or carrier proteins are predicted to yield similar taste attributes in the oral cavity. However, fatty acids that are below their cmc in the oral cavity would have their entire molecule exposed for possible binding to a receptor or carrier protein. Thus, long-chain fatty acids such as stearic acid or linoleic acid below their cmc in salivary fluid would more likely yield different taste responses if the transduction mechanism activates either carrier proteins or receptor proteins.

Alternatively, fatty acid micelles might enhance chemosensory responses in the oral cavity via membrane fusion, followed by diffusion across the plasma membrane of taste receptor cells. In the latter case, lipid concentrations below the cmc might result in decreased plasma membrane integration, decreased diffusion, and a diminished taste response.

Recent evidence indicates that CD36 protein [23], along with rodent sweet taste receptors [54], may function within lipid raft domains. If the human fatty acid taste receptor or carrier protein functions within a plasma membrane lipid raft, then one plausible explanation is that free fatty acid tastants could disrupt the lipid raft at concentrations below their cmc. This disruption would in turn decrease the integrity and functional stability of the fatty acid receptor, and decrease the chemosensory response to long-chain fatty acids.

5. Conclusions

Our results indicate that free fatty acids are readily incorporated into taste strips, and these oral stimuli yield a measurable chemosensory response in humans. Higher intensity

responses were observed with linoleic acid than with stearic acid, and this finding may reflect the presence of *cis* double bonds, and increased volatility of linoleic acid in the oral cavity. This new delivery method should allow a direct measure of the taste component of long-chain fatty acids so that this unique taste quality can be examined in a quantitative manner.

At present, the incorporation of free fatty acids into taste strips is limited by the solubility of these hydrophobic molecules in aqueous solutions. Future studies will address this limitation by increasing the thickness of taste strips without adversely affecting the dissolving time or tactile response of strips in the oral cavity. Future studies will also examine the role of PROP taster status in fatty acid chemoreception when olfactory contributions are eliminated. Finally, future studies with edible strips will seek to identify a causal relationship exists between fatty acid taste, obesity, and obesity-related diseases [55,56].

Highlights

- Edible taste strips were used to examine fatty acid taste responses in humans.
- Taste intensity values for stearic acid were in the barely detectable range.
- Taste intensity values for linoleic acid were in the weak to moderate range.
- Taste intensity values for linoleic acid decreased nearly 40% when nasal airflow was blocked.
- PROP tasters reported higher intensity values for linoleic acid than did non-tasters of PROP.

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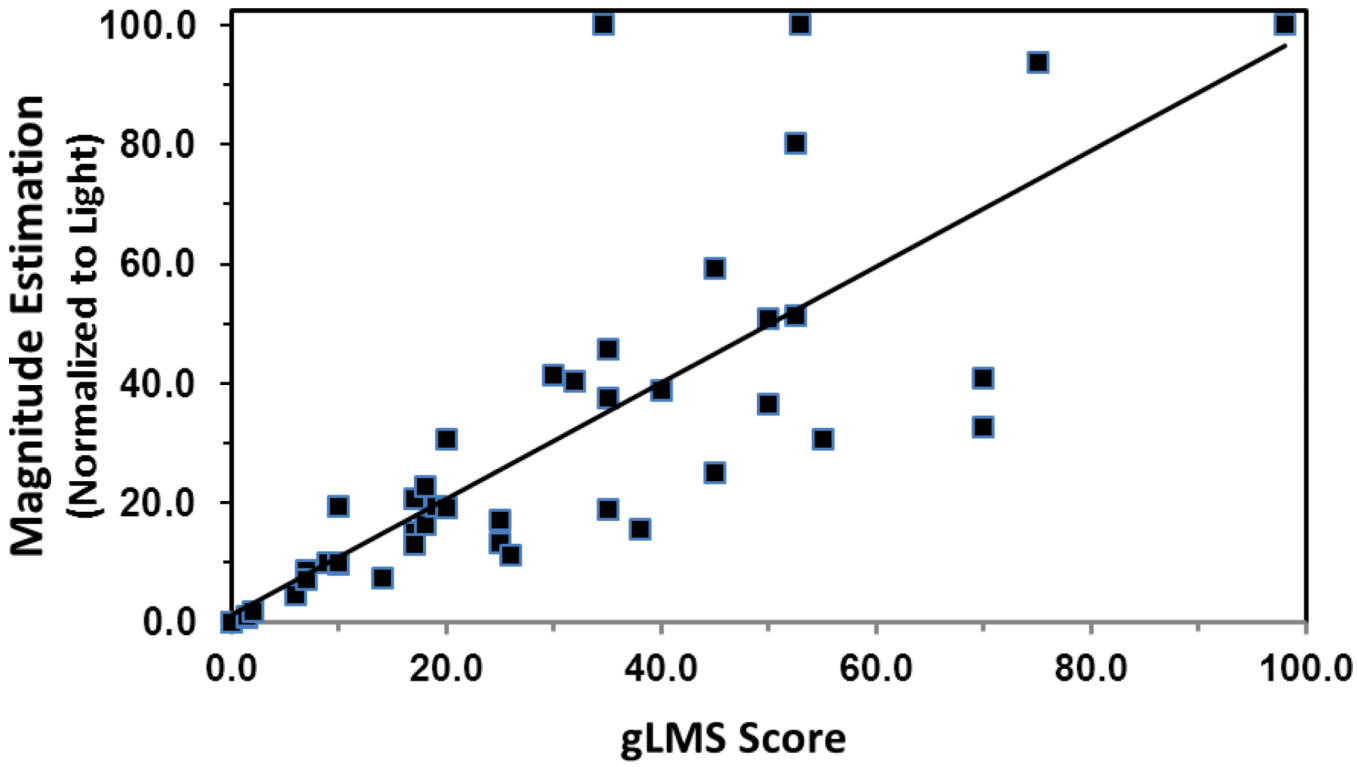

Fig. 1.

Comparison of taste intensity responses to 300 nanomole PROP strips that were obtained with the gLMS, and with magnitude estimation (n = 52). For light intensity measurements, subjects were asked to assign numbers to each light stimulus. Correlation coefficient = 0.82 for data points that included PROP tasters and nontasters, and r = 0.77 for the 43 individuals who gave a taste intensity response greater than zero for PROP strips.

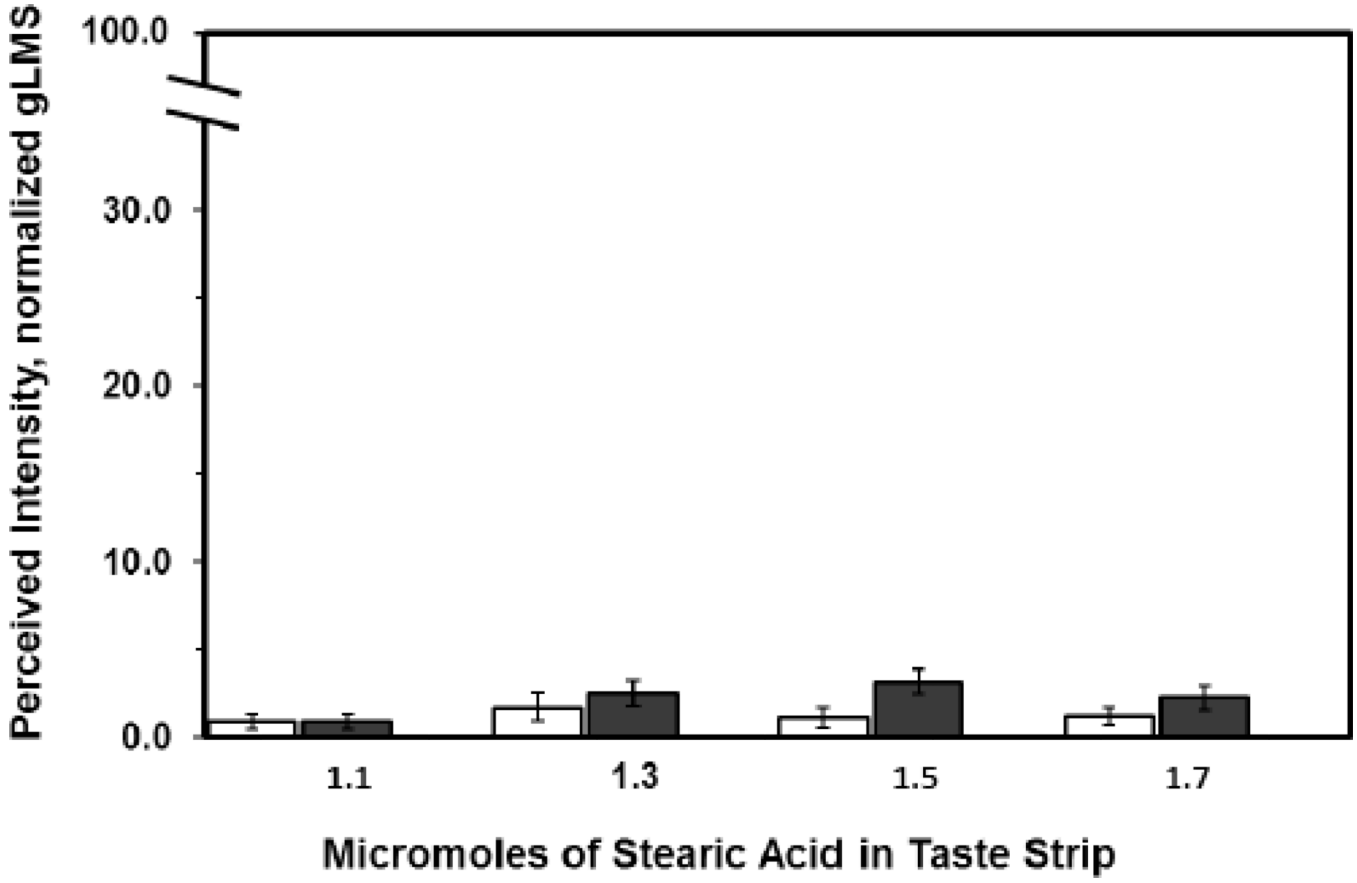

Fig. 2.

Perceived taste intensity values for stearic acid in a population of 30 test subjects. Intensity values were generated with the gLMS, and data was normalized as described in Materials and Methods. First column of each pair represents average taste intensity for control strips, and second column of each pair represents average taste intensity for stearic acid strip. Error bars represent standard errors for each data point.

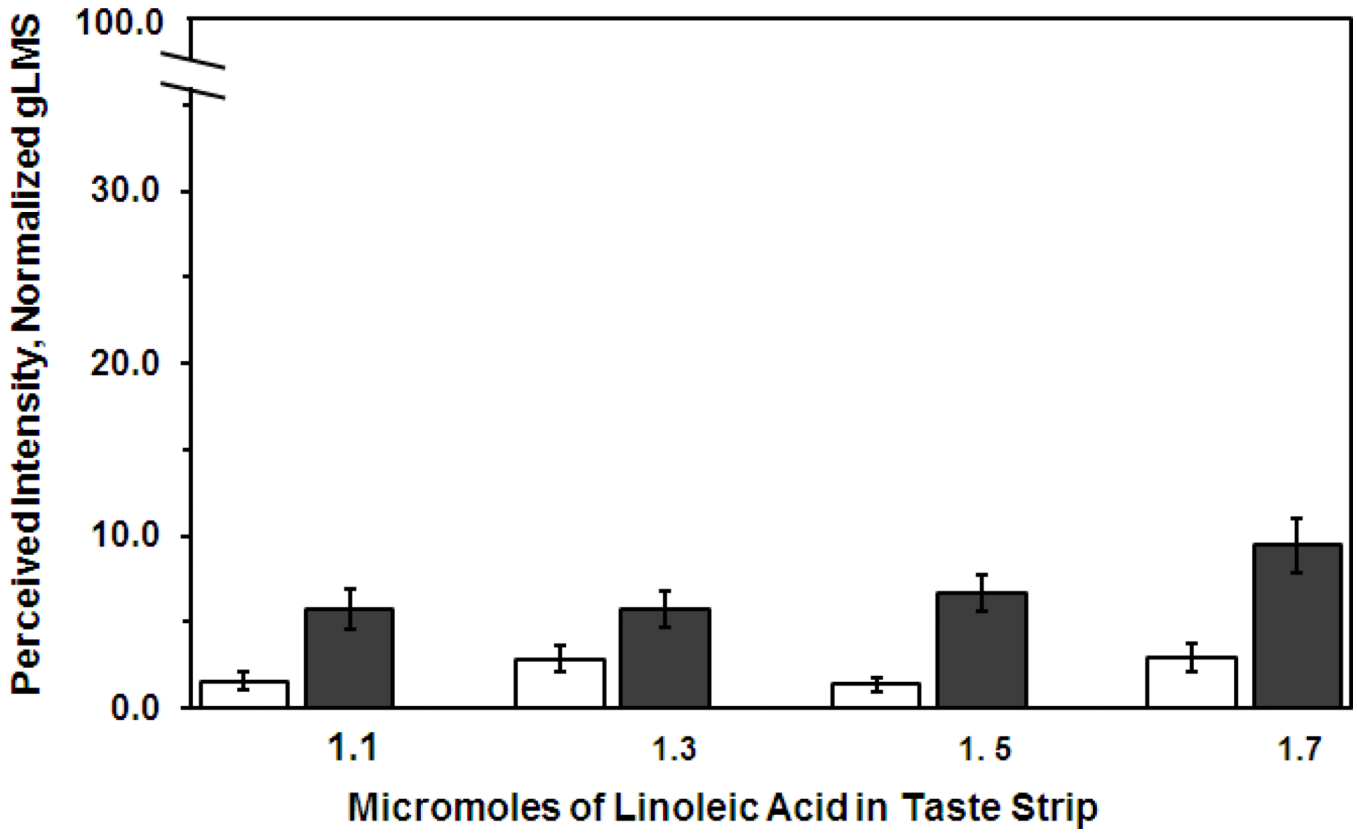

Fig. 3.

Perceived taste intensity values for linoleic acid in a population of 52 test subjects. All subjects were non-smokers. Intensity values were generated with the gLMS, and data was normalized as described in Materials and Methods. First column of each pair represents average taste intensity for control strips, and second column of each pair represents average taste intensity for linoleic acid strip. Error bars represent standard errors for each data point.

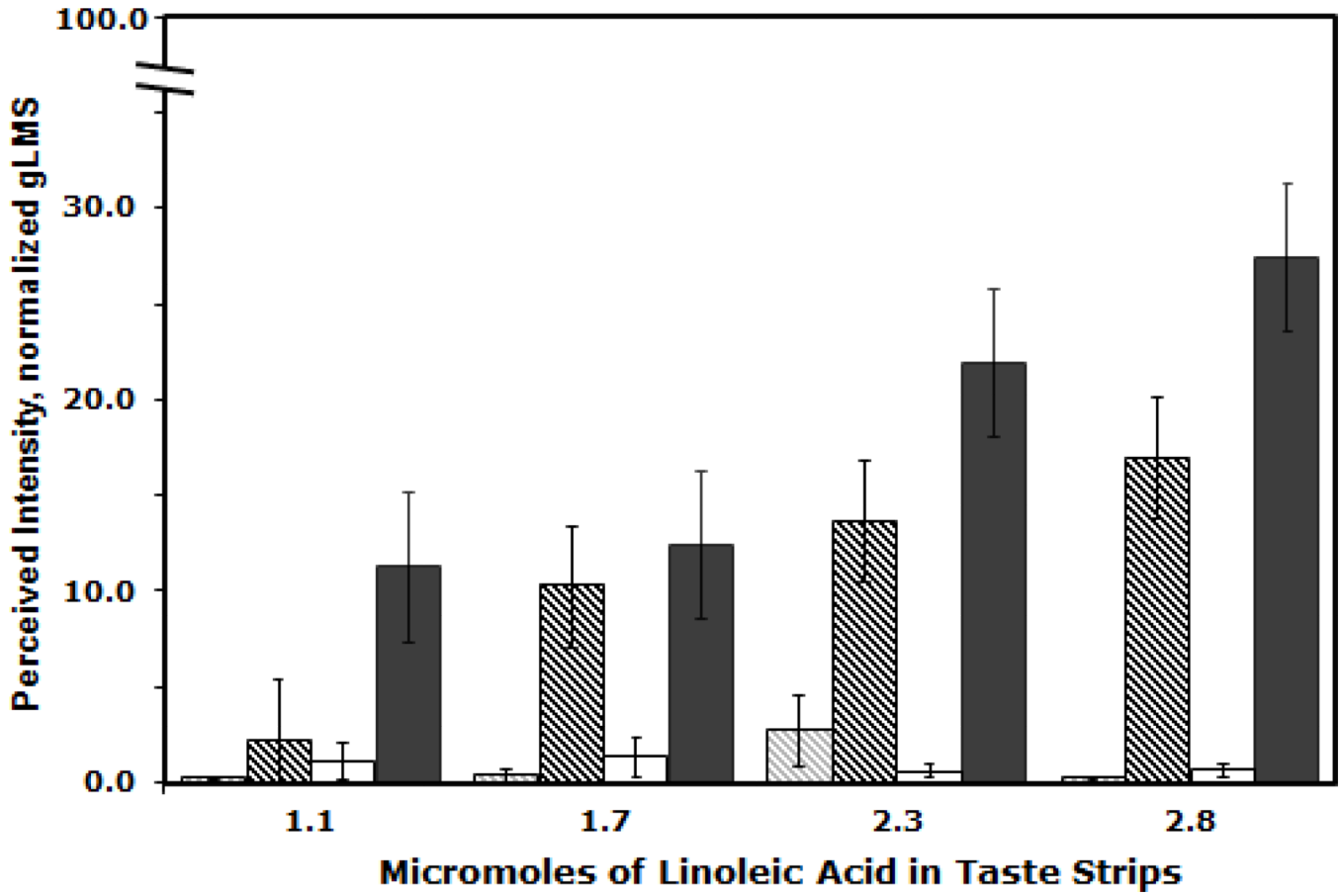

Fig. 4.

Perceived taste intensity of linoleic acid under conditions of obstructed and unobstructed nasal airflow [n = 16]. Subjects with blocked nasal airflow represent the two diagonally striped columns for each amount of linoleic acid, and subjects with unobstructed nasal airflow represent the two solid columns for each amounts of linoleic acid. First column represents control strips with nose clamps, second column represents linoleic acid strips with nose clamps, third column represents control strips with unobstructed nasal airflow, and fourth column represents linoleic acid strips with unobstructed nasal airflow. All subjects were non-smokers.

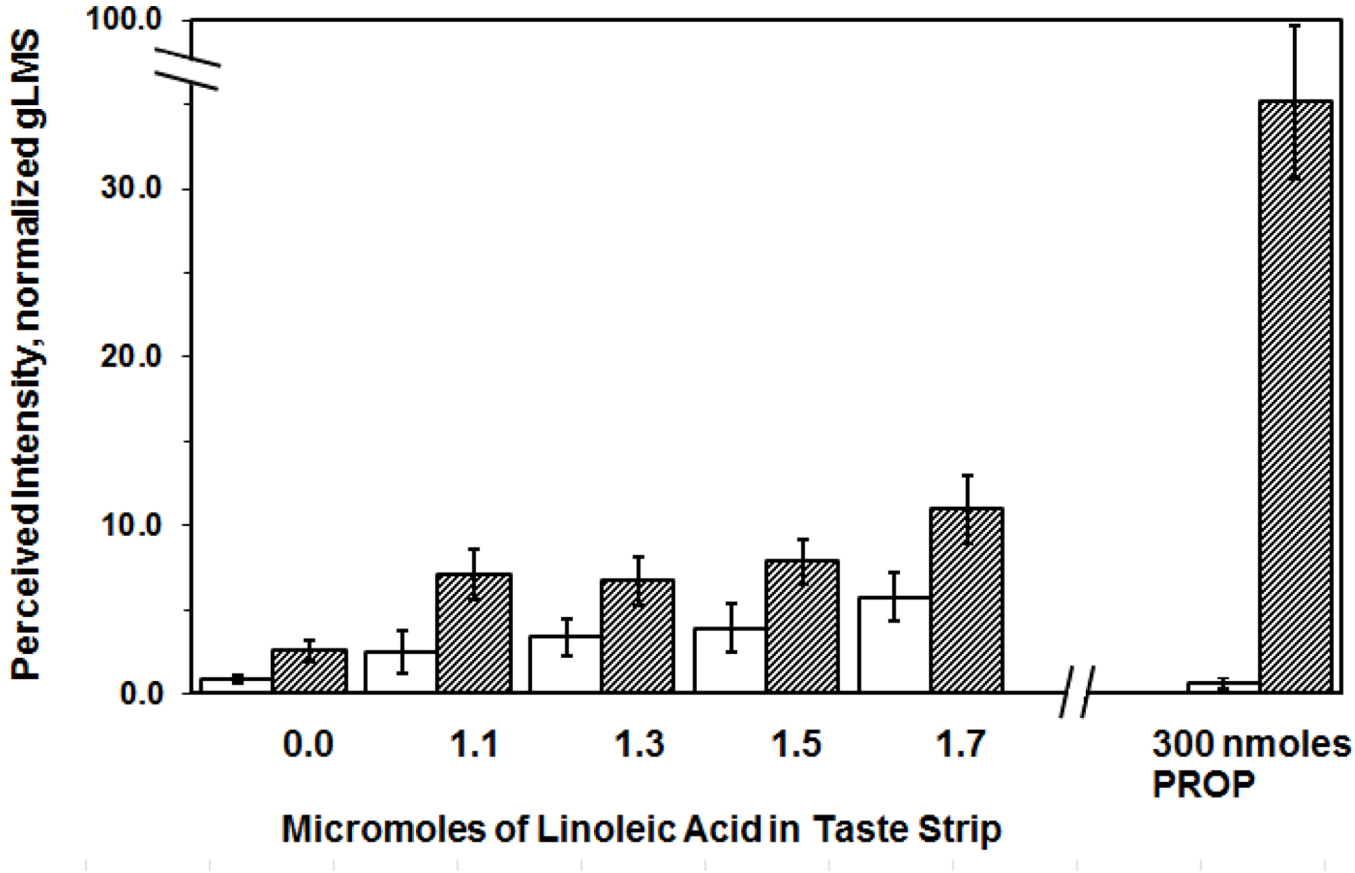

Fig. 5.

Comparison of linoleic acid taste intensity values for PROP tasters [n = 38] and PROP non-tasters [n = 13). All subjects were non-smokers. PROP non-tasters represent the clear columns for each amount of linoleic acid in the graph, and PROP tasters represent the diagonally striped columns for each amount of linoleic acid in the graph. The last two columns represent taste intensity values for PROP. The PROP intensity value for one non-smoking subject was not obtained.

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Table 1

Taste quality responses to various amounts of stearic acid and linoleic acid.

Stearic Acid, *u*moles	Sweet	Sour	Salty	Bitter	No Discernible Taste	Other
0.0	0	0	1	7	80	11 [46]
1.1	0	2	0	7	31	60 [48]
1.3	0	5	0	5	21	69 [79]
1.5	0	5	0	12	14	69 [48]
1.7	0	0	0	31	41	29 [67]
Linoleic Acid, *u*moles	**Sweet**	**Sour**	**Salty**	**Bitter**	**No Discernible Taste**	**Other**
0.0	3	1	3	5	81	7 [71]
1.1	2	2	9	13	34	40 [47]
1.3	0	0	9	23	23	45 [45]
1.5	2	9	9	17	21	43 [49]
1.7	0	4	6	28	19	43 [42]

All taste quality responses were normalized to 100%. “Other” taste represents a response that is not perceived as sweet, sour, salty, or bitter. Subjects who responded “other” were further asked to use a word description to identify the taste quality of fatty acid taste strips. Data in parenthesis in column seven represent the percentage of “other” tasters who gave a taste quality response of fatty/oily/waxy/sunflower seed taste. Data for stearic acid is from 30 subjects, and includes one light smoker (<10 cigarettes per day). Data for linoleic acid is from the 52 non-smoking subjects.

Table 2

Regression analysis for 1.1 *u*mole and 1.7 *u*mole stearic acid strips

	Unstandardized Coefficients (b)		Standardized Coefficients (Beta)		t		ρ	
	1.1 umol	1.7 umol	1.1 umol	1.7 umol	1.1 umol	1.7 umol	1.1 umol	1.7 umol
Subject's age	.048	.303	.037	.188	.164	1.947	.872	.069
Subject's gender (1= male, 0 = female)	−1.029	−.281	−.150	−.033	−.648	−.353	.526	.728
PROP rating normalized (0 – 100)	.007	−.008	.041	−.039	.186	−.423	.855	.678
Subject is not a racial/ethnic minority (1 = yes, 0 = no)	−2.494	−.096	−.359	−.011	−1.576	−.115	.135	.910
Hedonic rating normalized 1.1	−1.694	−1.403	−.489	−.965	−2.061	−9.525	.056	.000
(Constant)	2.066	−4.749			.314	−1.334	.758	.201

Table 3

Regression analysis for 1.1 *u*mole and 1.7 *u*mole linoleic acid strips (unobstructed nasal airflow).

	Unstandardized Coefficients (b)		Standardized Coefficients (Beta)		t		ρ	
	1.1 umol	1.7 umol	1.1 umol	1.7 umol	1.1 umol	1.7 umol	1.1 umol	1.7 umol
Subject's age	−.023	.129	−.043	.147	−.334	1.230	.740	.224
Subject's gender (1= male, 0 = female)	3.156	1.873	.230	.085	1.683	.658	.099	.513
PROP rating normalized (0 – 100)	.046	.132	.180	.321	1.326	2.487	.110	.016
Subject is a racial/ethnic minority (0 = yes, 1 = no)	.284	.606	.021	.028	.165	.234	.869	.816
Hedonic rating normalized 1.7	−.485	−.891	−.372	−.382	−2.827	−3.012	.007	.004
Test strips given in random order	−.304	−.494	−.022	−.023	−.162	−.175	.872	.862
(Constant)	25.958	42.058			2.979	2.781	.004	.008

1.1 umol: $R^2 = 0.241$

1.7 umol: $R^2 = 0.337$