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Different oral sensitivities to and sensations of short-, medium-, and long-chain fatty acids in humans

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1.   Abstract

Fatty acids that vary in chain length and degree of unsaturation have different effects on metabolism and human health. As evidence for a "taste" of nonesterified fatty acids (NEFA) accumulates, it may be hypothesized that fatty acid structures will also influence oral sensations. The present study examined oral sensitivity to caproic (C6), lauric (C12), and oleic (C18:1) acids over repeated visits. Analyses were also conducted on textural properties of NEFA emulsions and blank solutions. Oral thresholds for caproic acid were lower compared with oleic acid. Lauric acid thresholds were intermediate but not significantly different from either, likely due to lingering irritating sensations that prevented accurate discrimination. From particle size analysis, larger droplets were observed in blank solutions when mineral oil was used, leading to instability of the emulsion, which was not observed when emulsions contained NEFA or when mineral oil was removed from the blank. Rheological data showed no differences in viscosity among samples except for a slightly higher viscosity with oleic acid concentrations above 58 mM. Thus, texture was unlikely to be the property used to distinguish between the samples. Differences in oral detection and sensation of caproic, lauric, and oleic acids may be due to different properties of the fatty acid alkyl chains.

2.   Introduction

Structural features of fatty acids, predominantly chain length and degree of unsaturation, determine their physiological role in preventing, promoting, or alleviating disease states (3, 16, 29, 42). Generally, polyunsaturated fatty acids and *cis*-monounsaturated fatty acids are associated with improved health outcomes when substituted for saturated fatty acids (3, 16, 29, 42). Chemically, unsaturation and shorter chain length lead to faster diffusion through cell membranes (30), and long-chain polyunsaturated fatty acids have greater affinity for certain fatty acid receptors, such as G protein-coupled receptor (GPR)120, than saturated or short-chain fatty acids (18, 27).

Definitions of "short-chain," "medium-chain," and "long- chain" fatty acids vary, but generally short-chain fatty acids are composed of 2 to 4, and sometimes up to 6, carbons, medium- chain fatty acids are composed of 6 or 8 to 10 or 12 carbons, and long-chain fatty acids are composed of 12 or 14 to longer carbon chains. As the alkyl chain length increases, the molecules become less water soluble. Short- and medium-chain fatty acids also diffuse more rapidly across cell membranes than long-chain fatty acids (17). Short-chain fatty acids, such as butyric (C4) and caproic (C6) acids, are present in dairy products, but the bulk of these fatty acids in the human diet are actually byproducts of dietary fiber fermentation by bacteria in the colon (11, 12, 26, 65, 66). Medium-chain fatty acids of 8–12 carbons are found in foods such as palm kernel oil and coconut oil, with some lower concentrations in dairy products (1). Long-chain fatty acids are the most abundant fatty acids in the human diet, as they are prevalent in most triglycerides in food and are vital components of cell membranes.

Knowing that structural differences influence the absorption (38) and physiological roles of fatty acids in nongustatory tissues, and given the accumulated evidence that non-esterified fatty acids (NEFA) are effective taste stimuli in humans and rodents (for recent reviews, see Refs. 20, 39, 44, and 59), the concept that structure may alter the taste sensation of NEFA seems probable. While numerous studies have been conducted to investigate the role of different types of NEFA on health outcomes, few have investigated their differential impacts on oral chemosensation in humans. One study (51) showed lower thresholds for linoleic (C18:2) than oleic (C18:2) or lauric (C12) acids, whereas another study (36) showed no differences in

thresholds for caproic (C6), lauric, and stearic (C18) acids. Additional studies have reported caproic acid thresholds are lower than linoleic, stearic, and lauric acid thresholds (35) and no difference in sensitivity among oleic, linoleic, and stearic acids (8). However, all of these studies only tested each participant once. New research has shown wide within-subject variability and/or learning effects over time, indicating a need for multiple testing visits to establish reliable taste thresholds for these compounds (57, 58). A study (18) that used a trained panel, who presumably had numerous exposures to the NEFA, tested a variety of NEFA (C10, C12, C18:1, C18:2, C18:3, and C20:4), but that report did not indicate whether the thresholds differed significantly. Thus, clarification is needed for whether oral sensitivity to NEFA differ by fatty acid structure and whether multiple tests per participant are required to document accurate limits of detection for each NEFA (57, 58).

Additionally, most NEFA taste studies have used carbohydrate gums and/or mineral oil to mask the textural contribution of NEFA to the blank sample (for a review, see Ref. 44). Textural properties and physical characteristics, such as particle size and emulsion stability, of NEFA emulsions are rarely reported, yet such parameters contribute to the oral sensation of emulsions (13–15, 49, 62, 64). While there is evidence that carbohydrate thickeners mitigate the increase in perceived thickness caused by unstable emulsions (64), the efficacy of mineral oil as a textural masking agent for NEFA has not been studied. Given that mineral oil, unlike NEFA, contains no hydrophilic moieties, this lipid does not form natural micelles. Thus, the physical structure formed in a mineral oil emulsion is different from an emulsion containing NEFA. We thus tested emulsions of NEFA with and without mineral oil as well as "blank" solutions of carbohydrate gums with and without mineral oil to determine what physical effects this lipid has on the samples.

The present study was designed to investigate the differences in oral taste thresholds of caproic (hexanoic, C6), lauric (dodecanoic, C12), and oleic (*cis*-9-octadecenoic, C18:1) acids as well as assess the potential differences in viscosity and particle size for NEFA emulsions with or without mineral oil. The stimuli examined here were 6, 12, and 18 carbon fatty acids and are referred to as short-, medium-, and long-chain fatty acids. While stearic acid would have been a more ideal candidate to maintain the same level of saturation among the tested NEFA, stearic

acid is solid until 69°C, a temperature at which sustained exposure could cause thermal burns. The hypotheses tested were 1) emulsion particle sizes would be smaller for mixtures with NEFA than mixtures with mineral oil alone, 2) viscosity would be greater for emulsions containing mineral oil than emulsions not containing mineral oil, 3) viscosity would not be significantly different among NEFA emulsions and the blank, 4) human oral sensitivity to NEFA would increase with decreasing alkyl chain length (sensitivity to caproic acid > lauric acid > oleic acid), and 5) human oral sensitivity to all NEFA would improve over multiple testing sessions.

3. Materials and methods

Participants

Participants were recruited through the Laboratory for Sensory and Ingestive Studies participant pool and public announcements. To be eligible, participants had to be between 18 and 60 yr of age, in good health, available to complete 21 study visits within 3 mo, and provide written informed consent. Participants who had been in other fat taste studies in the past 6 mo were excluded. The protocol was approved by the Human Subjects Institutional Review Board of Purdue University and was registered at www.clinicaltrials.gov (NCT01996566).

Additionally, potential participants were screened for their ability to detect emulsions orally. Pilot data with a 5% (wt/wt) mineral oil emulsion in carbohydrate gum solutions indicated that 5 of 50 people could accurately discriminate, presumably by tactile cues, between the mineral oil and carbohydrate-only (blank) solutions (see below for details of solution and emulsion preparation). Thus, in an attempt to eliminate textural discriminators from the present study on NEFA taste, all potential participants were screened on their ability to distinguish a 5% mineral oil emulsion from blank solutions. At the screening visit, potential participants donned a blindfold and nose clips and were presented with three samples, only one of which contained mineral oil. After tasting all samples, participants were asked to identify the different sample. This was repeated in triplicate. Any individual who successfully identified the mineral oil sample all three times was excluded from the study. All participants completed a validated food frequency questionnaire for habitual fat intake (4). Participants' height and weight were measured at the screening visit, and age, sex, and self-reported ethnicity were

recorded. Body mass index (BMI) was calculated from the height and weight measurements. Nineteen individuals were screened for the present study; two individuals were ineligible due to their ability to detect the mineral oil emulsion. Thus, 17 participants (5 men and 12 women) enrolled in and completed the study. The average age was 24.9 ± 5.4 yr (range: 19–38 yr); BMI was 22.4 ± 3.2 kg/m2 (range: 18.3–31.1 kg/m2). Two participants were overweight (BMI 25.6 and 25.9 kg/m2), and one participant was obese (BMI 31.1 kg/m2).

Study design

A randomized crossover design was used. All participants were tested for their thresholds for all NEFA types (caproic, oleic, and lauric acids). Participants were randomly assigned to an order for NEFA testing, but a restriction on randomization was used to ensure that each NEFA type was tested first, second, or third in approximately equal proportions. Seven threshold visits were conducted per NEFA, for a total of 21 threshold tests/participant.

Samples

Oleic acid (O1014, Spectrum Chemicals), lauric acid (W261408, Sigma-Aldrich), caproic acid (W255904, Sigma-Aldrich), mineral oil (M1180, Sigma-Aldrich), ethylenediaminetetraacetate acid (EDTA, E1001, Spectrum Chemical), *tert*-butylhydroquinone (TBHQ, T1073, Spectrum Chemical), gum arabic (TIC Gums Pre- Hydrated Gum Arabic Spray Dry FCC Powder), and xanthan gum (TIC Gums Pre-Hydrated Ticaxan Rapid-3 Powder) were used to create samples. The blank vehicle was made first by dissolving 10% (wt/wt) gum arabic, 0.05% (wt/wt) xanthan gum, 0.01% (wt/wt) EDTA, and 0.01% (wt/wt) TBHQ into deionized water. This solution was allowed to rest for at least 45 min to hydrate the gums. The solution was then mixed for 4 min at 14,000 rpm with a T18 Ultra Turrax homogenizer equipped with an S18N-19G dispersing element. To make the emulsions, appropriate amounts of oleic acid, mineral oil, caproic acid, or lauric acid were added to yield the concentrations shown in Table 4-1. The concentrations for lauric and caproic acids were selected based on pilot tests indicating these concentrations were of similar potency to the 5% (186 mM) oleic acid. Additionally, caproic and lauric acids are potent irritants at higher concentrations, making the test less relevant to the

concept of NEFA taste. Lauric acid mixtures were placed in a 49°C water bath before emulsification to melt the NEFA. Mixtures of vehicle and NEFA were emulsified with the T18 Ultra Turrax with S18N-19G element for 8 min at 14,000 rpm. Lauric acid mixtures were emulsified in a hot water bath (~85°C) to keep the samples liquid. To make the blank, solutions of gums and antioxidants were homogenized for an additional 8 min at 14,000 rpm (for a total of 12 min for all solutions/emulsions). To eliminate any confounding influence of temperature for the lauric acid sample, all samples were placed in 49°C water baths and maintained at this temperature for all threshold tests. All NEFA and mineral oil emulsions were prepared fresh each day. Dilutions of the NEFA emulsions were prepared in quarter-logarithmic (base 10) steps. All samples (with or without NEFA) had a pH of ~4.3, which did not vary depending on NEFA type or concentration.

Particle size data

Particle size distributions of 5% (186 mM) oleic acid, 5% oleic acid plus 5% mineral oil, and 5% mineral oil emulsions were obtained in duplicate using a Mastersizer 2000 with a Hydro 2000MU dispersion unit. The dispersant was deionized water. A refractive index of 1.458 (per manufacturer) and absorption of 0.005 (measured at 632 nm) was used for oleic acid. For mineral oil, a refractive index of 1.467 (per manufacturer) and absorption of 0 (measured at 632 nm) was used. Lauric acid emulsions were not tested due to the solid nature of this NEFA at room temperature (leading to crystallization and inaccurate particle size readings), and caproic acid samples were too dilute to give any measureable particles.

Rheology

For liquid samples, viscosities measured at a shear rate of 50 s^{-1} give predictive values for oral thickness perception; however, oral shear rates may range from 10 to 1,000 s^{-1} (47, 48, 56, 67). Consequently, for the present study, viscosities were evaluated over the range of 1–300 s^{-1}. Preliminary tests showed that below 1 s^{-1}, measurements included large amounts of noise, and above 300 s^{-1}, the same trends were apparent as measured at lower shear rates. Viscosity was analyzed using an ARG2 Rheometer from TA Instruments (New Castle, DE) equipped with a

40-mm 2° cone and plate geometry, a water solvent trap to minimize evaporation, and a Peltier plate for temperature control. Shear rate was increased logarithmically from 1 to 300 s^{-1} at 37°C, with 10 data points/decade. Measurements were collected in duplicate, and viscosities were analyzed at each shear rate. Comparisons were made between 5% oleic acid plus 5% mineral oil emulsions and 5% mineral oil-only emulsion and between the blank and 5% (186 mM) oleic acid, 1.58% oleic acid (58 mM, second quarter-logarithmic dilution), 0.137% (34 mM) caproic, and 0.708% (59 mM) lauric acid emulsions.

Threshold testing

Participant thresholds were determined using an ascending three-alternative forced-choice test. Briefly, participants were given three samples, one with NEFA (stimulus) and two without NEFA (blank). Participants wore blindfolds (to limit visual cues) and nose clips (to limit olfactory cues) during the tests and were not allowed to retaste samples. After tasting all three samples, participants would say which sample they thought was different (contained NEFA). If the participant was correct, the test was repeated with the same concentration of NEFA. If the participant was incorrect, the test was repeated with a quarter-logarithmic step higher concentration of NEFA. This was repeated until either the participant gave three correct responses sequentially or until the maximum concentration of NEFA was reached. The concentration at which a participant identified the NEFA correctly three times was deemed the threshold. If a participant reached the highest concentration of NEFA and still did not give three correct responses, that visit was designated as a "no threshold" visit and treated as right censored data in the statistical analysis.

Each participant began the study at dilution step 18 (4.5 logarithmic dilutions below the maximum concentration). To minimize fatigue by reducing sample number while still allowing for observation of learning effects, after the first visit, participants began the next test seven dilution steps below their previous threshold. When changing to a different NEFA, participants were started at seven dilution steps below their average performance (rounded up) on the previous NEFA (for example, if a participant's average threshold on visits 1–7 was dilution step 4.6, they began the next NEFA test at dilution step 12). If a participant gave three correct

responses on the first concentration tested during any visit, the test was restarted at four concentration steps (one logarithmic dilution) below the initial start point (which occurred 11 times out of 357 total trials; a χ^2 goodness of fit test indicated that these were likely due to chance as P = 0.47). At the end of each visit, when participants had either identified the NEFA successfully three times or had reached the maximum concentration of NEFA, participants were asked what seemed different about the samples they chose as containing NEFA.

The mineral oil discrimination test, the same as described for the screening visit, was repeated on visits 7, 14, and 21 to determine if participants were learning a texture component of the emulsions over the course of the experiment.

Statistics

SAS 9.3 was used for data analyses. Data were analyzed for differences in thresholds over repeated visits using the NLMIXED procedure. Visits where participants did not successfully achieve a threshold were incorporated into this model as right censored data. Because visit number was not found to be significant (no learning effects over multiple testing sessions), main effects of BMI, habitual fat intake, and NEFA type on thresholds were analyzed. As NEFA type was found to be significant, post hoc comparisons using the Bonferroni correction were conducted (for three comparisons, α = 0.05/3 = 0.017). Additional analyses indicated that overweight/obese individuals (n = 3) were not significantly different from the other participants, nor were results significantly different when these three individuals were excluded from the analyses; thus, data were combined for all BMI classes. For the rheological data, ANOVA was used to compare 5% oleic acid plus 5% mineral oil to 5% mineral oil only as well as the blank to 5% oleic acid, 1.58% oleic acid, 0.137% caproic acid, and 0.708% lauric acid (comparisons corrected using Dunnett's test with the blank solution as the control).

4.   Results

Particle size

Oleic acid plus mineral oil emulsion (5% of each) averaged a volume-weighted mean droplet diameter [D(4,3)] of 3.61 μm and a surface-weighted mean droplet diameter [D(3,2)] of

0.45 µm. Oleic acid emulsions [5% (wt/wt), 186 mM] averaged D(4,3) = 1.69 µm and D(3,2) = 0.68 µm, whereas mineral oil emulsions [5% (wt/wt)] averaged D(4,3) = 19.79 µm and D(3,2) = 5.89 µm. The means of duplicate measurements of distributions of droplet diameters for all three emulsions are shown in Fig. 4-1. Lauric acid emulsions are not included as this NEFA is solid at room temperature. Caproic acid emulsions showed no measurable particles, indicating this NEFA was mostly dissolved in the vehicle or had too few droplets to measure. Creaming was observed in the 5% mineral oil emulsions as early as 3 h after sample preparation. No creaming was observed in the combined 5% mineral oil plus 5% oleic acid emulsion nor in the 5% oleic acid emulsion even after storage at room temperature for over 48 h.

Rheology

Figure 4-2A shows the mean viscosity from 1 to 300 s^{-1} for the 5% mineral oil plus 5% oleic acid emulsion as well as the 5% mineral oil emulsion. Viscosities of these two emulsions were significantly different between 2 and 200 s^{-1} ($P < 0.05$). As shown in Fig. 4-2B, above 39 s^{-1}, the 5% (wt/wt) (186 mM) oleic acid emulsion had significantly higher viscosity than the blank solution ($P < 0.05$), but the difference was eliminated upon dilution to 1.58% (58 mM, two quarter- logarithmic dilution steps). As shown in Fig. 4-2C, caproic and lauric acid emulsions were not significantly different from the blank solution in viscosity (all $P < 0.05$).

Differences in NEFA oral thresholds

As shown in Fig. 4-3, group data indicated mean oral thresholds for caproic acid (mean ± SE: -2.86 ± 0.17 logM, or 1.45 mM) were significantly lower than for oleic acid (mean ± SE: -1.59 ± 0.29 logM, or 25.70 mM, $P = 0.002$). Lauric acid thresholds (mean ± SE: -2.27 ± 0.27 logM, or 5.37 mM) fell in between caproic and oleic acids but were not significantly different from either ($P = 0.0989$ and $P = 0.1032$, respectively). Table 4-2 shows a summary of how many visits for each fatty acid resulted in no threshold (participant never had three correct identifications sequentially), which were incorporated into the statistical model as right censored data. Whereas oleic and lauric acids had similarly high numbers of no threshold visits, lauric acid's 30 no threshold visits were due to 7 people, whereas oleic acid's 27 no threshold visits were due to

11 people, 5 of whom only had one no threshold visit each. Table 4-3 shows a summary of the dominant qualitative descriptors given by participants from each visit. These data are included for the purpose of demonstrating the large number of participants experiencing a burning, irritating, and/or spicy sensation from lauric acid. Caution should be taken when interpreting these data as they are clearly subjective measures, and participants supplied their own descriptors instead of rating the NEFA solutions for specific qualities.

Learning effects

Thresholds did not improve over the seven visits for any of the NEFA tested. Overall, participants also did not improve in their ability to detect the mineral oil emulsion compared with the blank. Only two participants showed possible learning effects for the mineral oil emulsion, not correctly identifying the mineral oil on the screening visit and visit 7 but correctly identifying it on visits 14 and 21.

Within-subject SDs by NEFA type are shown in Fig. 4-4. While there were no learning effects over multiple testing sessions, a large degree of variability was observed within each subject in their measured thresholds to each NEFA. These data are generated from the NLMIXED model's parameters for variability about a mean threshold for each participant for each fat. The results indicated an average SD of nearly 1 logM for each NEFA, which, when interpreted, is equivalent to 10 times (plus side) or 1/10th (minus side) of the mean thresholds in molar concentration.

Fat intake

There was no significant main effect of habitual fat intake on taste thresholds and no interactions between fat intake and NEFA type (overall correlation between taste thresholds and fat intake $P = 0.08$, between caproic acid threshold and fat intake $P = 0.36$, between lauric acid thresh- old and fat intake $P = 0.31$, and between oleic acid threshold and fat intake $P = 0.30$).

5. Discussion

Our hypothesis that oral sensitivity increases with decreasing alkyl chain length is supported by the finding that caproic acid thresholds were lower than those for oleic acid. However, learning effects and associations with dietary fat intake were not observed as posited. Mineral oil produced larger emulsion droplet sizes in the absence of NEFA, yet did not contribute to emulsion viscosity, at least in vitro. The observation of larger droplet sizes and creaming in this emulsion could lead to different oral sensations than imparted by NEFA. NEFA form micelles and more stable emulsions, yet few participants, either in pilot data or in the study on NEFA taste, were able to detect the mineral oil emulsion compared with the blank solution. Viscosity measurements indicated that emulsions with higher concentrations of oleic acid are significantly more viscous than the blank solution, regardless of whether mineral oil was added to the mixture. Each of these findings warrants further consideration.

Differences in oral thresholds

Most prior studies have reported no differences (36) or did not give *P* values for differences (18, 51) in oral sensitivity between caproic, lauric, and oleic acids. We observed lower oral taste thresholds for caproic acid compared with oleic acid, with lauric acid in between but not significantly different from either caproic or oleic acids. Only one previous study (35) noted a significantly lower oral threshold for caproic acid compared with lauric acid. Several factors may account for the lack of difference with lauric acid in the present study. First, it may be attributable to lingering irritating qualities of lauric acid that hamper sensitivity (discussed below). Second, there is large daily variability, even within each subject, in sensitivity to each NEFA, as shown in Fig. 4-4. Without multiple tests per participant, this variability is difficult to capture, reducing both the accuracy of the results as well as the power for finding a difference. Furthermore, in the present study, all samples were presented warm. Since other studies have served caproic and oleic acids at room temperature but lauric acid warm, differences in results could be due to temperature confounds. The results of this study are difficult to compare to other NEFA taste studies, as the effect of temperature on NEFA taste has not been tested.

Despite these methodological issues, we did observed significant differences in oral sensitivity between caproic and oleic acids that could reflect the effects of chain length on NEFA affinity for receptors, ability to diffuse through the cell membrane, and solubility. Caproic acid is much more soluble than lauric or oleic acids, allowing easier access to taste cell surfaces as it can more freely partition into the aqueous environment of saliva. Notably, caproic acid is also more volatile than oleic or lauric acids. While nose clips have been shown to block retronasal identification of long-chain fatty acids (5), it is possible that the higher volatility of caproic acid would make it more likely to reach the olfactory epithelium in very small amounts. Additionally, affinity of von Ebner's gland protein, more commonly called lipocalin-1, is greater for oleic acid followed by lauric acid and then caproic acid (19). Potentially, lipocalin-1 could bind the longer-chain NEFA and clear them from cell surfaces, reducing their interactions with taste receptors (33, 45, 46). Caproic acid also diffuses more rapidly across cell membranes (17). Importantly, affinities of CD36 and GPR120, putative NEFA taste receptors, are much higher for oleic acid than for medium-chain (low affinity) or short-chain (almost zero affinity) fatty acids like lauric and caproic acids (6, 18, 25, 27). Work in rats has shown that myristic (C14), oleic, arachidonic (C20:4), docosahexaenoic (C22:6), and linoleic (C18:2) acids all activate trigeminal lingual neurons, but caproic acid does not (68). Other research on NEFA taste has reported a "fatty" sensation at lower concentrations and a "scratchy" sensation at higher concentrations for oleic, linoleic, linolenic, and arachidonic acids but only a "scratchy" sensation for lauric and decanoic (C10) acids (18). The data indicating a lower oral threshold for caproic acid may be reflective of a diffusion-based mechanism for this NEFA's oral sensation, as fatty acid receptors for short-chain fatty acids, such as GPR40, GPR41, and GPR43, have not been identified in human or primate oral tissue (7, 18, 37, 60), although these receptors may function in rodents for NEFA taste (21).

Oleic acid was more viscous at high concentrations (the top two dilution steps) than the blank solution, whereas caproic and lauric acids were not significantly different from the blank. Thus, individuals who did not detect oleic acid until these concentration steps may have been distinguishing the emulsion from the blank based on a textural sensation. Out of the total 17 participants, 10 participants had visits where they detected the oleic acid emulsion above 58

mM (1.58%, two quarter-logarithmic dilutions below maximum; the concentration at which the difference in viscosity from the blank was eliminated). However, only five participants had a mean threshold above 58 mM. Most NEFA taste studies have reported average taste thresholds for oleic acid much below 58 mM, usually in the range of 0.5–4 mM (9, 18, 51–54). However, some studies have observed much higher mean or median thresholds, from 20 to 150 mM (40, 57, 58). Differences in the preparation of the emulsion could contribute greatly to these observed differences between studies (44). Few studies have actually reported information on viscosity of the emulsions and blanks or data on emulsion stability. Without this information, it is unclear whether or not participants may have detected the higher concentrations of NEFA orally through a textural sensation. However, the differences observed in our analysis of oleic acid emulsion compared with blank, while significant, were small: 20 compared with 35 mPa·s (similar to 55% and 59% sucrose in water at room temperature) at low shear rates and 10 compared with 14 mPa·s (similar to 46% and 50% sucrose in water at room temperature) at high shear rates (22). While there is some evidence that humans can distinguish between these viscosities, the data available are from a sorting task and the solutions used had greater differences than those found between the oleic acid emulsion at 5% (186 mM) and blank solution in the present study (50). Consequently, while some participants may have been able to distinguish a textural difference in our samples, it is unlikely that this was the dominant sensation for most participants. Viscosity of emulsions can increase when mixed with saliva, although this effect varies among individuals and can be mitigated by the addition of carbohydrate gums (62, 64).

In the qualitative data collected at the end of each threshold test, irritancy/burning sensations were reported to be the dominant quality of lauric acid on 50 visits, of caproic acid on 32 visits, and of oleic acid on only 10 visits (total of 119 visits per NEFA). Notably, participants were not asked to describe the level of irritancy of each NEFA but rather were only asked to report the dominant sensation. Thus, it is not possible to determine with certainty whether lauric or caproic acids were sensed as more irritating than oleic acid. Nonetheless, the stronger irritant quality of lauric acid compared with caproic and oleic acids has also been noted in skin tests, although concentrations tested were higher than physiologically relevant for

an intraoral sensory cue (0.5–1 M) (32, 43, 55). Many compounds have both taste and irritant qualities, and usually detection thresholds for taste are lower than for irritation (23). Increased solubility of the caproic or lauric acids, compared with oleic acid, would also increase access of these NEFA to nociceptors conveying irritant sensation. Potentially, fatty acids such as lauric acid may interact with both the gustatory system and trigeminal system, as discussed in another NEFA taste study (18). Additionally, the temperature at which the NEFA was tested may have contributed to their potency as irritants. Previous work has indicated that the sensation of chemical burn of capsaicin, a transient receptor potential vanilloid 1 agonist, increases with increasing temperature (24), and monoglycerides are also known to activate transient receptor potential vanilloid 1 (28). Thus, while it was necessary to test the samples at 49°C due to the solid nature of lauric acid at room temperature, this may have increased the irritant qualities of the NEFA.

In the present study, lauric acid had a large number of right-censored thresholds, meaning on these study visits the participant never had three sequential, correct identifications of the NEFA sample. With lauric acid, four participants had two or fewer visits where a threshold was successfully obtained, whereas oleic acid had only two participants and caproic acid zero participants with two or fewer successful threshold visits. While in the data analysis we interpreted this to mean the threshold for the NEFA was greater than the range of concentrations tested, for lauric acid in particular this was likely not the case. Rather, the chemestetic sensation of lauric acid was difficult to clear from the oral cavity. When questioned about how they were making the decision of which sample seemed different, all participants who were unable to identify lauric acid reported they experienced a lingering burning, irritating, or spicy sensation from all samples, not just the NEFA sample. As the blank samples were the same for all visits and NEFA types, the burning sensation must be attributable to lauric acid. Lauric acid thus may have lingering trigeminal qualities, particularly at warmer temperatures, which may mask or overwhelm any potential NEFA taste. Additionally, lauric acid melts at 44°C (38a), which is above body temperature. Potentially, lauric acid could be solidifying in the mouth during taste testing, leading to deposition on the oral surfaces. When individuals who had five or more "no threshold" visits for lauric acid were removed from the

analysis (leaving n - 13), mean oral thresholds for lauric acid were significantly lower than for oleic acid (P = 0.0143) and the observation of caproic acid thresholds lower than oleic acids was maintained (P = 0.0076). Thresholds for caproic and lauric acids were still not significantly different. Similar trends were observed when all no threshold visits for lauric acid, where irritancy was reported, were removed. This could be a truer representation of the relationships among oral taste threshold for these NEFA. It may be better to remove, or analyze separately, individuals who experience a dominant burning sensation from further analysis of NEFA taste, as the irritancy sensation is not experienced among all NEFA equally. However, to accomplish this, participants may need more training to accurately identify and distinguish trigeminal and gustatory sensations.

The observed taste thresholds in our study are markedly higher for oleic and lauric acids than in other studies, including some studies from our own laboratory. Many studies have reported thresholds in the millimolar range and below (9, 18, 35, 36, 51–54), but there are reports of thresholds closer to the ranges observed in the present study or even some participants unable to detect the NEFA above 100 mM (10, 57, 58). Much of this variability could be explained by the preparation of the vehicle or by actual differences in sensitivity among subjects studied. The carbohydrate gums used in all studies will vary by source, and preparation methods could also lead to different emulsion characteristics. For reviews of how sample preparation and individual variability could influence NEFA taste studies, see Refs. 44 and 59.

Learning effects

The lack of learning effects observed in our data compared with other studies may be due to several factors. First, participants were not all naïve to the testing procedure. Screening criteria required that participants not have been in a NEFA taste study only within the previous 6 mo; thus, some participants may have had previous experience with the method and NEFA taste. Published work demonstrating the learning effect with oleic acid taste thresholds was conducted entirely with naïve participants (personal communication). Additionally, learning effects may differ across fatty acids.

Physical properties of NEFA and mineral oil emulsions

The larger droplet sizes for mineral oil compared with oleic acid or mineral oil plus oleic acid emulsions were expected, as NEFA can form micelles (31) and mineral oil, a mixture of alkanes lacking any hydrophilic moieties, cannot. The observed decrease in particle size of mineral oil emulsions with addition of oleic acid was also expected, as oleic acid would function as a surfactant. Mineral oil has been used in many NEFA taste studies with the intent to mask lubricity contributions of NEFA (8, 9, 18, 35, 36, 51–54). Lubricity is the decrease in friction caused by a substance and is a tribological property, reflecting thin film rheological behavior. However, whether mineral oil is effectively achieving the goal of masking lubricity has not been studied. Indeed, the study often cited for the need of a lubricity control (41) in NEFA taste studies only hypothesizes, rather than actually tests, that a property such as lubricity could be an oral cue to the presence of an oil.

Fats do indeed act as lubricants in the oral cavity, but to do so, the emulsified droplets must shear and spread across the oral surface. Coalescence of lipid droplets (small droplets combining into large droplets) can lead to greater lubricity in the oral cavity (14, 15), whereas flocculation of droplets (droplets that adhere together but do not combine into a larger single droplet) contributes to oral perception of thickness rather than to lubricity (61). As observed in particle size data in the present study, mineral oil and oleic acid form different emulsions. While the ability to detect the mineral oil emulsion was used in our study to eliminate textural discriminators of emulsions, we cannot be certain that mineral oil and oleic acid emulsions would have been detected by the same oral mechanism. The creaming and larger particle sizes observed in the mineral oil samples indicate these emulsions are much less stable than the oleic acid emulsions. Thus, it is possible that the mineral oil emulsions were detected by some individuals orally due to increases in perceived thickness from saliva-induced flocculation (13–15). Published data have indicated that emulsions varying in average droplet size from 0.5 to 6 μm are not rated substantially differently for mouthfeel attributes (63). However, this study did not examine larger particle sizes and also did not try to mask the texture of the emulsion by adding carbohydrate gums. In the present study, the specific textural contributions of NEFA to

emulsions and the effectiveness of using mineral oil as a masking agent are still unclear. However, the instability of mineral oil-only emulsions should be of great concern. Potentially, the creaming would not only affect the textural sensation of the mineral oil-only emulsion but could also affect the release of tastants and odorants from the emulsions. Some may have greater affinity for the oil droplets that rise to the cream layer and others to the more aqueous phase. While no creaming was observed in the 5% mineral oil plus 5% oleic acid emulsion, the physical distribution of the NEFA with the oil in this system could still potentially affect tastant partitioning from the emulsion, to saliva, to taste cell surfaces. At a minimum, studies using mineral oil should confirm that the emulsions are stable and that NEFA are distributed evenly before and after making dilutions to use for taste experiments. Additionally, caution should be used with adding mineral oil to the blank, as this emulsion creamed very quickly. Uneven distribution of mineral oil in the blank could potentially contribute to even larger rheological and droplet size differences compared with emulsions containing NEFA. More work should be conducted to determine the implications of adding nonnutritive lipid such as mineral oil to the blank solution for NEFA taste testing, and a better understanding of textural contributions of NEFA is still desirable.

1.   Conclusions

Humans are more sensitive to caproic acid than oleic acid orally, with lauric acid intermediate but not statistically significantly different from either of these other two NEFA in the present trial. Habitual fat intake had no effect on oral taste thresholds for any of the NEFA tested; however, the analysis was only conducted using a food frequency questionnaire, where high consumption of high fat foods may be regarded as socially undesirable, leading to underreporting. Further analysis of NEFA taste thresholds using a controlled study where the previous meal or meals are provided would be valuable. The greater sensitivity to caproic acid may be due to the shorter chain length, and thus increased solubility and faster diffusion across the cell membrane, with this NEFA compared with oleic acid. Orally, NEFA are sensed both as tastants and irritants. Which of these is the dominant sensation, particularly for lauric acid, remains unclear. Furthermore, the mechanism for sensing caproic acid is also unclear as it has

not been demonstrated to activate either trigeminal or taste receptors. Participants did not appear to be detecting textural attributes of any of the NEFA tested except perhaps at higher concentrations of oleic acid. Use of mineral oil in NEFA taste testing should be approached cautiously, as this lipid forms unstable emulsions that could lead to discernable textural sensations for a small percentage of individuals.

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9. Disclosures

No conflicts of interest, financial or otherwise, are declared by the author(s).

10. Author Contributions

Author contributions: C.A.R. and R.D.M. conception and design of research; C.A.R. performed experiments; C.A.R. analyzed data; C.A.R. and R.D.M. interpreted results of experiments; C.A.R. prepared figures; C.A.R. drafted manuscript; C.A.R. and R.D.M. edited and revised manuscript; C.A.R. and R.D.M. approved final version of manuscript.

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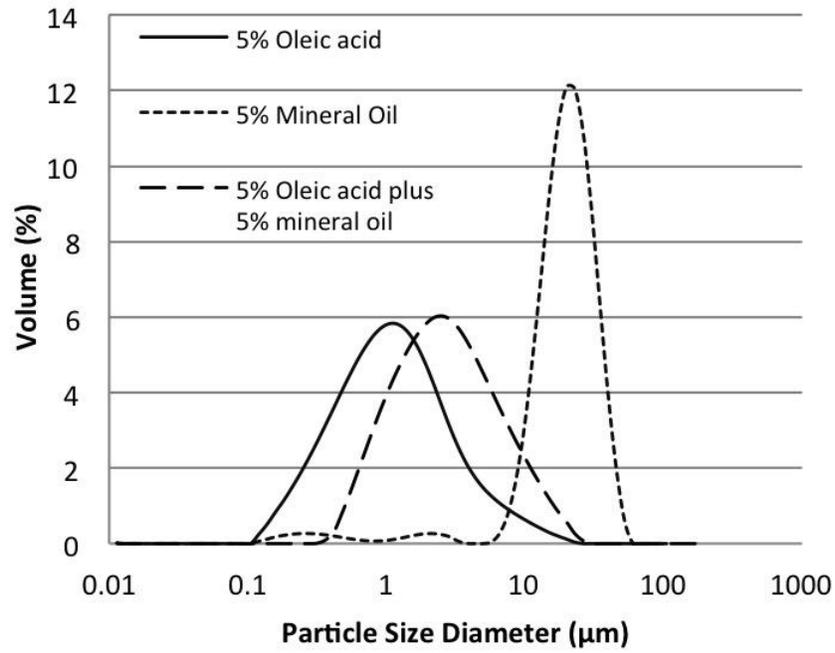
Figure 1: Particle size distributions of 5% (186 mM) (wt/wt) oleic acid emulsion, 5% (wt/wt) mineral oil plus 5% (186 mM) oleic acid emulsion, and 5% mineral oil emulsion.
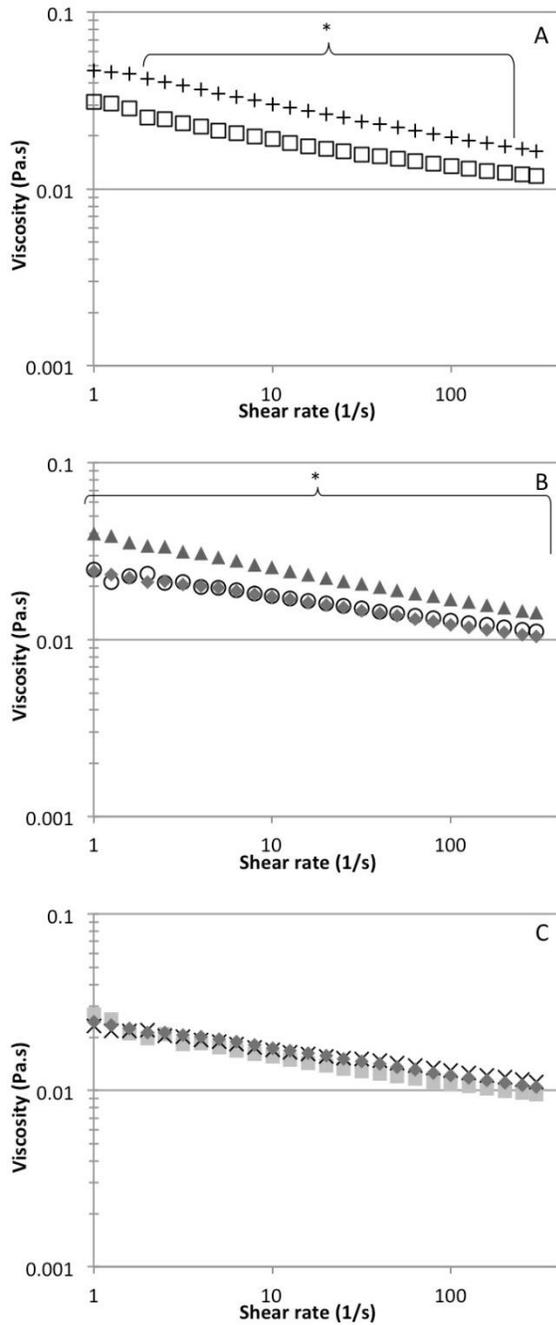
Figure 2: A) viscosities for 5% (186 mM) oleic acid plus 5% mineral oil (squares) and 5% mineral oil (+). *P < 0.05. B) viscosities for 5% (186 mM) oleic acid (triangles), 1.58% (58 mM) oleic acid (circles), and the blank (diamonds). *P < 0.05 for 5% oleic acid compared with the blank. C) viscosities for 0.137% (34 mM) caproic acid (squares), 0.708% (59 mM) lauric acid (X), and the blank (diamonds).
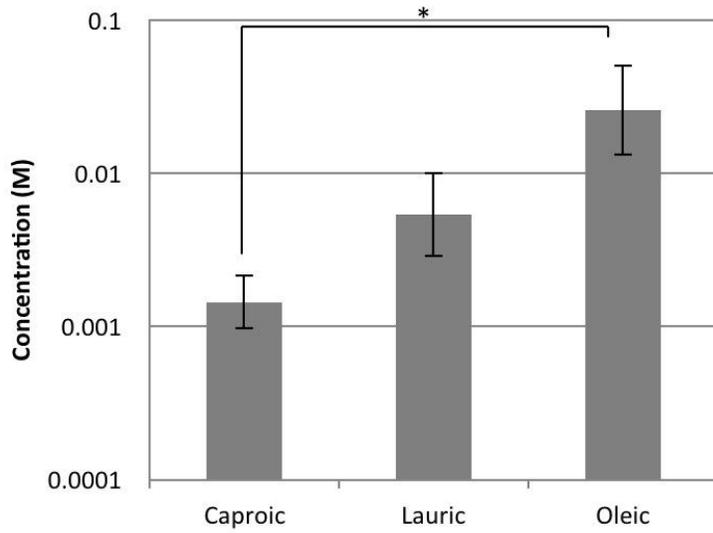
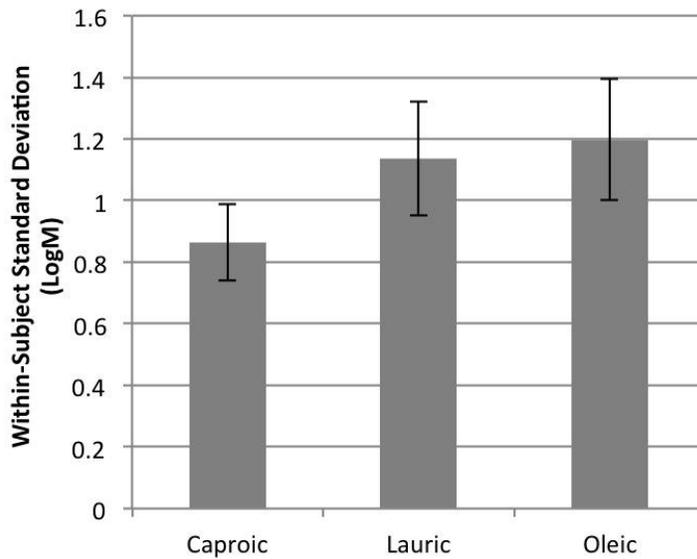Figure 3: Mean oral thresholds for caproic, lauric, and oleic acids. *Significantly different (P = 0.002).

Figure 4: Mean within-subject SDs in threshold by non-esterified fatty acids type (i.e., SD of the threshold over the 7 visits). Bars indicate the SE of mean SDs (within subjects).

Table 1: Concentrations of NEFA and mineral oil

	Percent weight	Molar*
Oleic acid	5.000%	0.186 M
Lauric acid	0.708%	0.059 M
Caproic acid	0.137%	0.034 M
Mineral oil	5.000%	NA
Mineral oil plus oleic acid	5.000% each, total 10.000% lipid	NA

* Density of all solutions and emulsions was measured at 1.05g/mL. This was accounted for in the conversion of percent weight to molarity.

Table 2: Total "No Threshold" Visits by participant and NEFA type

Participant	Caproic	Lauric	Oleic	Total
1	0	0	0	0
2	1	5	4	10
3	0	0	1	1
4	0	0	0	0
5	1	6	0	7
6	0	0	0	0
7	0	0	0	0
8	0	0	1	1
9	1	6	6	13
10	0	0	0	0
11	0	6	1	7
12	0	0	2	2
13	0	0	3	3
14	0	2	1	3

15	0	3	3	6
16	0	0	0	0
17	1	2	5	8
Total	4	30	27	61

Table 3: Dominant sensations at threshold concentration

	Irritant	Bitter	More Sour (than blank)	Less Sour (than blank)	Textural
Caproic	32	25	11	10	6
Lauric	50	16	4	14	10
Oleic	10	28	6	17	10