Humans are more sensitive to the taste of linoleic and \(\alpha\)-linolenic than oleic acid

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Submitted 31 October 2014; accepted in final form 22 December 2014

Running CA, Mattes RD. Humans are more sensitive to the taste of linoleic and \(\alpha\)-linolenic than oleic acid. Am J Physiol Gastrointest Liver Physiol 308: G442–G449, 2015. First published December 24, 2014; doi:10.1152/ajpgi.00394.2014.—Health concerns have led to recommendations to replace saturated fats with unsaturated fats. However, addition of unsaturated fatty acids may lead to changes in the way foods are perceived in the oral cavity. This study tested the taste sensitivity to and emulsion characteristics of oleic, linoleic, and \(\alpha\)-linolenic acids. The hypothesis tested was that oral sensitivity to nonesterified fatty acids would increase with degree of unsaturation but that in vitro viscosities and particle sizes of these emulsions would not differ. Oral taste thresholds were obtained using the three-alternative, forced-choice, ascending method. Each participant was tested on each fat 7 times, for a total of 21 study visits, to account for learning effects. Viscosities were obtained for the blank solutions and all three emulsions. Results indicate lower oral thresholds to linoleic and \(\alpha\)-linolenic than oleic acid. At higher shear rates, 5% oleic and linoleic acid were more viscous than other samples. More-dilute emulsions showed no significant differences in viscosity. Particle sizes of the emulsions increased very slightly with increasing unsaturation. Together, the emulsion characteristics and oral sensitivity data support a taste mechanism for nonesterified fatty acid detection.

A MAJOR CONTRIBUTOR TO CARDIOVASCULAR disease is a diet high in saturated fatty acids (33). Replacement of saturated fatty acid with mono- or polyunsaturated fatty acids (MUFA or PUFA, respectively) may improve blood lipid profiles, decrease markers for cardiovascular disease, and improve insulin responses in insulin-resistant or type 2 diabetic patients (34, 35). Thus the type of dietary fatty acids should be a critical consideration in evaluation of the healthfulness of high-fat foods.

Oleic, linoleic, and \(\alpha\)-linolenic acids are unsaturated fatty acids with one, two, and three double bonds, respectively. Oleic and linoleic acids are common in liquid vegetable oils, such as safflower, canola, and olive oils, while \(\alpha\)-linolenic acid is predominantly found in fish oil. The PUFA, linoleic and \(\alpha\)-linolenic acids, are \(1\), \(2\), and \(3\) fatty acids, respectively, and humans lack endogenous desaturases to create the double bonds at these positions of the alkyl chain. Thus these fatty acids are considered essential fatty acids and must be obtained from the diet.

As different molecular structures of fatty acids influence health outcomes, structural differences could also influence affinity for various receptors, including proposed fatty acid taste receptors in the human mouth, as demonstrated for G protein-coupled receptor (GPR) 120 (4, 10, 12). While dietary fat, primarily present as triacylglycerol, has traditionally been valued for textual contributions to food, evidence indicates that nonesterified fatty acids (NEFA) are effective taste stimuli in the oral cavity (11, 20, 32). Large variability has been observed in NEFA oral sensitivity, which can be modified by dietary fat intake or by weight status (20, 23–26). However, most of the human work has tested only oleic acid. New data obtained through improved techniques and multiple tests per NEFA indicate that human oral sensitivity to varying NEFA differs according to properties of the alkyl chain (19). The current study is designed to evaluate differences in human sensitivity to NEFA that vary in degree of unsaturation, but not chain length. Previous studies have observed lower oral thresholds for linoleic than oleic acid (23) or no difference between these two fatty acids (5). Data from another report show a lower oral fatty taste threshold for \(\alpha\)-linolenic than linoleic acid, which is, in turn, lower than that for oleic acid, but means and standard deviations to test for significant differences were not reported (10). Notably, none of these previous reports tested individuals multiple times with individual NEFA. Data published on oleic acid indicate that individuals may learn the taste of oleic acid over multiple tests, leading to lower thresholds in later visits than in the first test (29, 31). While learning effects are not always observed or may be blunted by using nonnaive participants (19), multiple visits should be conducted because of the high variability of sensory threshold data and the high occurrence of false positives, which would artificially lower threshold values (18). Thus the present study was designed to observe not only whether oral sensitivity to NEFA increases with greater unsaturation of the alkyl chain, but also whether multiple tests would give more consistent data on this relationship.

Our hypotheses were as follows: 1) humans would be most sensitive to the taste of \(\alpha\)-linolenic acid, followed by linoleic acid and then oleic acid, and 2) learning effects would be observed over multiple tests, particularly in naive participants. We expected these learning effects to attenuate over the course of the 21 visits (7 visits per NEFA) conducted in the study. Because of ongoing concerns of controlling for emulsion texture in NEFA taste experiments, data on particle size distributions and rheology of the samples were also collected and analyzed. We hypothesized that there would be no difference in particle size among the emulsions of different NEFA and that viscosity would be similar among the emulsions and the blank solutions.

METHODS

Participants. Participants were recruited through local advertisements. Eligibility criteria for the subjects were as follows: 18–60 yr
of age, good health, normal taste and smell function, and available to complete 21 study visits within 3 mo. Participants provided written informed consent, the protocol was approved by Purdue University’s Human Subjects Institutional Review Board, and the study was registered at www.clinicaltrials.gov (registration no. NCT01996566). Participants were screened for their ability to detect an emulsion without added tastant (i.e., a mineral oil emulsion) compared with the blank solution (see Ref. 19 for details); however, no participants screened in the current study were able to detect the mineral oil emulsion, so all participants were eligible and completed the study. Height and weight of each participant, along with age, sex, and self-reported ethnicity, were recorded at the first study visit. All participants completed a validated food frequency questionnaire for habitual fat intake (2). Descriptive data about the participants are given in Table 1. Twenty-one (8 male and 13 female) participants enrolled in and completed the study. Their mean age was 28 (median 24, range 18–58) yr, and their mean body mass index was 25.3 (median 24.0, range 17.4–40.6) kg/m². Of the 21 participants, 8 had also completed a previous study on taste of oleic, caproic, and lauric acids (19) and 4 had participated in at least one study on the taste of oleic acid.

**Study design.** A randomized, crossover design was used. Because of concerns of learning effects and high variability (29, 31), participants were handed three samples, one NEFA dilution and two blanks, in a random order. For the first fat, the experiment began 18 dilution steps below the maximum concentration of each NEFA. The participants were blindfolded and noseclips to minimize visual and olfactory cues. Participants were instructed to taste and expectorate each sample, rinsing with room temperature (~21°C) water between samples. After the third sample, the participant indicated which sample seemed different and, thus, should contain NEFA. If the participant was correct, the procedure was repeated with the same sample.

**Samples.** NEFA were obtained from commercial sources (catalog no. O1914, Spectrum Chemicals; catalog nos. W338001 and L2376, Sigma Aldrich). Carbohydrate gums [Pre-Hydrated gum arabic spray dry FCC powder (gum arabic) and Pre-Hydrated Ticaxan Rapid-3 powder (xanthan gum)] were purchased from TIC Gums. Antioxidants [disodium EDTA (catalog no. E1001) and tert-butylhydroquinone (catalog no. T1073)] were obtained from Spectrum Chemicals. For preparation of “blank” solutions, 10% (wt/wt) gum arabic, 0.05% xanthan gum, 0.01% tert-butylhydroquinone, and 0.01% EDTA were dissolved in deionized water. This solution was allowed to rest for ≥45 min to allow the gums to fully hydrate. Next, the solution was mixed with a T 18 Ultra-Turrax homogenizer with a 518N-19G dispersing element for 4 min at 14,000 rpm. NEFA were added to this solution at appropriate concentrations, and the mixture was homogenized under nitrogen flow (to reduce oxidation of the PUFA) for an additional 8 min. Linoleic and α-linolenic acid emulsions were homogenized on an ice bath to further reduce potential oxidation (the ice bath solidified the oleic acid, so it was not homogenized on ice). The blank was made by homogenization of the solution of gums only for an additional 8 min (12 min total at 14,000 rpm for all samples). Maximum concentrations of all NEFA emulsions are given in Table 2.

<table>
<thead>
<tr>
<th>Table 1. Participant characteristics</th>
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<tr>
<td><strong>n</strong></td>
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<tr>
<td>Total</td>
</tr>
<tr>
<td>Naïve</td>
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<tr>
<td>Low outliers</td>
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<td>Nonperformers</td>
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<td>Mean</td>
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<td>Median</td>
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<tr>
<td>BMI, kg/m²</td>
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<tr>
<td>Mean</td>
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<tr>
<td>Median</td>
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<td>Range</td>
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BMI, body mass index.

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<tr>
<th>Table 2. NEFA emulsion characteristics</th>
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<tr>
<td>NEFA</td>
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<td></td>
</tr>
<tr>
<td>Oleic acid</td>
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<tr>
<td>Linoleic acid</td>
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<tr>
<td>α-Linolenic acid</td>
</tr>
<tr>
<td>Blank</td>
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</tbody>
</table>

Droplet diameter values are means ± SD. *Density of all solutions and emulsions was measured at 1.05 g/ml; this was accounted for in conversion of percent weight to molarity. There were no significant differences in surface- or volume-weighted droplet diameters when tested at the same concentrations. No day-to-day variation was observed in particle size measurements. At 50.1 s⁻¹, no significant differences were observed between viscosity of nonesterified fatty acid (NEFA) emulsions and blank (data from the same day as Fig. 2B).
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collection of NEFA. If the participant was incorrect, the next-higher concentration of NEFA was used. The test continued until the participant selected the NEFA correctly three times at the same concentration. At this point, the test was repeated with the next-higher concentration of NEFA, as a double check, since the rate of false positives for just three correct answers in the ascending method is quite high (18). In our study, with an average run length (count of presentations of 3 samples before the test ended) of 12, a false-positive rate of 22.7% would be expected if only 3 correct responses were required. For 4 correct responses, the false-positive rate drops to 7.5% (18). Thus, if the participant was correct at this next-higher concentration, the test was complete, and the concentration at which three correct responses were given was deemed the threshold. If the participant was incorrect, the set of three correct responses was deemed a false positive, and the test continued until the participant could give three correct responses at one concentration followed by one correct response at the next-higher concentration. Thus a total of four sequential correct responses were required to finish the test. At visits 2–7 on each NEFA, the test began four dilution steps (1 logarithmic dilution) below the previous threshold. If a participant gave four correct responses at the very beginning of any test (3 at the first concentration and 1 at the next-higher concentration), the test was restarted four dilution steps below the original start point. Participants were not given feedback during testing to indicate whether their responses were correct or incorrect but were informed at the end of each visit at what concentration step they finished the test. If a participant proceeded all the way to the maximum concentration of any NEFA and still did not give four correct responses, the visit was deemed a “no-threshold” visit. Thresholds of 0.333 M (one-quarter logarithmic dilution above the maximum concentration for any NEFA) were assigned to these visits for the data analysis, as suggested in the American Society for Testing and Materials E679 standard for conducting threshold tests (1). While actual thresholds could be higher than this value, or nonexistent, using the same number for all three NEFA will actually decrease the power of finding a difference in threshold among the NEFA, so the bias by assigning this value to the no-threshold visits makes the analysis more conservative. No restrictions were placed on the time between visits, except as follows: a maximum of one visit per day and the requirement that participants be available to complete the study within 3 mo.

Statistics for threshold data. SAS 9.2 was used to analyze the data using repeated-measures ANOVA, and significance was set at \( P < 0.05 \). A mixed model was used, using NEFA type, subjects, and session of testing (the first set of 7 visits, second 7 visits, or third 7 visits) as classification variables and subjects as a repeated measure. Visit number was tested as a quantitative variable, in place of using session as a classification variable, but the effect of visit number was not statistically significant, and the model with visit number as a quantitative variable had a poorer fit for the data than the model with session of testing as a classification variable. Body mass index (BMI) was also tested but was not statistically significant in the model, and there were no interactions of BMI with NEFA thresholds or visit number; the model fit was improved with removal of BMI. Analysis of residuals indicated that two participants had several visits with extremely low thresholds to linoleic and \( \alpha \)-linolenic acids; these two participants are referred to as the “low outliers.” Additionally, three participants had no-threshold visits on more than half of the total visits (12, 13, and 13 total no-threshold visits) and are referred to as “nonperformers.” All other participants had six or fewer no-threshold visits and are referred to as “performers.” Consequently, data were analyzed with and without the two low outliers and the nonperformers. Removal of these groups resulted in a normal distribution of residuals and did not affect the balance of the NEFA testing order. Spearman’s rank correlations were tested between individuals’ mean thresholds (logged value for each NEFA type) and BMI, sex, total fat intake, saturated fat intake, and naivety. The low outliers (BMI = 18.7 and 23.1, both nonnaive to fat taste testing) were removed for this analysis, as their mean thresholds were inordinately low and had extreme influence on the correlations. The nonperformers were left in for the correlation analysis, as their mean thresholds (calculated as described above to include no-threshold visits) did not substantively influence the correlations (BMI = 24.0, 25.4, and 32.2; 2 naive and 1 nonnaive to fat taste testing).

RESULTS

Emulsion characteristics. Small increases in droplet diameter were observed with increasing degree of unsaturation (Fig. 1). However, the blank solution gives an artifact in the 10- to 100-\( \mu \)m range for more-dilute samples. This can be seen in all NEFA emulsions at 0.89% (\( \sim 33–34 \) mM). To confirm that this was an artifact, emulsions were examined under a light microscope. No large (10- to 100-\( \mu \)m) particles were observed. Furthermore, the blank solution (no emulsified NEFA) was run through the Mastersizer to confirm a small, artificial peak in this range. This required a large amount of the blank solution (\( \geq 5 \) ml, as opposed to 0.5–1 ml for the emulsions), and we were unable to reach optimal obscurancy (maximum obscurancy was \( \sim 7\% \)). However, the same peak in the 10- to 100-\( \mu \)m range could be observed for the blank solution alone, again confirming that this is an artifact caused by the gum solution.

% of droplets

\begin{array}{c}
\text{Particle size (µm)} \\
0.01 0.1 1 10 100 1000
\end{array}

A

\begin{array}{c}
\text{Percent of droplets} \\
0 1 2 3 4 5 6 7
\end{array}

\begin{array}{c}
\text{Oleic} \\
186\text{mM}
\end{array}

\begin{array}{c}
\text{Linoleic} \\
187\text{mM}
\end{array}

B

\begin{array}{c}
\text{Percent of droplets} \\
0 1 2 3 4 5 6 7
\end{array}

\begin{array}{c}
\text{Oleic} \\
33.1\text{mM}
\end{array}

\begin{array}{c}
\text{Linoleic} \\
33.3\text{mM}
\end{array}

\begin{array}{c}
\text{\( \alpha \)-Linolenic} \\
34.6\text{mM}
\end{array}

\begin{array}{c}
\text{Particle size (µm)} \\
0.01 0.1 1 10 100 1000
\end{array}

Fig. 1. Particle size distributions for emulsions of 186 mM oleic acid and 187 mM linoleic acid (A) and 33.1 mM oleic acid, 33.3 mM linoleic acid, and 34.6 mM \( \alpha \)-linolenic acid (B). Blank solution in B creates an artificial peak at 30–110 µm.
As reported previously (19), the highest concentrations of linoleic and α-linolenic acids (5%, ~186 mM) were more viscous than other concentrations of NEFA and the blank, especially at high shear rates and regardless of batch-to-batch variation in the gums (Fig. 2). However, while significant, these differences were very small in magnitude (<10 mPa·s in this study) and quite likely undetectable by many participants (22). At 0.89% (~33 mM) NEFA, no significant differences were observed among the three different NEFA and the blank. Data in Table 2 are shown for viscosity at 50 s⁻¹, as this shear rate has been correlated with oral perception of thickness (27); no differences were observed on this day of testing among any of the samples. Small day-to-day variations in the blank and the emulsions used to make the blank were observed due to natural variation in batches of the gums (Fig. 2), so “batch” was used as a classification variable in all analyses. Thus, on each day when emulsions were prepared, the same blank was used to prepare the emulsions and to conduct testing to avoid any confounding influence of batch variation in the blank. In all rheological analysis, for this study and previous studies, only the >1.58% NEFA (~59 mM) emulsions were significantly thicker than the blank (19).

**Differences in oral thresholds.** The mean thresholds for linoleic and α-linolenic acids were 5.6 and 2.5 times lower than for oleic acid (about one-half of a logarithmic dilution and one-quarter of a logarithmic dilution; Table 3 and Fig. 3, which include data from all testing sessions). Analysis of the data without the two low outliers and the three nonperformers gave the same results, with an added trend for lower thresholds to

<table>
<thead>
<tr>
<th>NEFA</th>
<th>All Participants</th>
<th>Performers, With Low Outliers Removed</th>
</tr>
</thead>
<tbody>
<tr>
<td>NEFA concentration, mM</td>
<td>Threshold value, logM</td>
<td>NEFA concentration, mM</td>
</tr>
<tr>
<td>Oleic acid</td>
<td>19.9</td>
<td>17.9</td>
</tr>
<tr>
<td>Linoleic acid</td>
<td>1.55</td>
<td>3.15</td>
</tr>
<tr>
<td>α-Linolenic acid</td>
<td>3.15</td>
<td>7.06</td>
</tr>
</tbody>
</table>

Values are means ± SE. Within each column, different letter superscripts indicate *P < 0.05. *P = 0.08 vs. linoleic acid for performers, with low outliers removed.

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Fig. 2. Mean viscosities at 1–300 s⁻¹ for blank (diamonds), 5% linoleic acid emulsion (triangles), and 5% oleic acid emulsion (squares). *P < 0.05, oleic acid emulsion vs. blank, #P < 0.05, linoleic acid emulsion vs. blank, +P < 0.05, oleic and linoleic acid emulsions vs. blank. Results in A and B are from different days of testing, with different batches of xanthan gum.

Fig. 3. Mean thresholds by nonesterified fatty acid (NEFA) (includes all sessions) for all participants (n = 21) and with low outliers and nonperformers removed (n = 16). Bars with different letters are significantly different within groups (P < 0.05). *P = 0.08, linoleic vs. α-linolenic acid in group with low outliers and nonperformers removed.
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Linoleic than α-linolenic acid. The order of tasting was also significant, with participants performing better in sessions 2 and 3 than in session 1 and, when the low outliers and nonperformers were removed, better in session 3 than in session 2 (Fig. 4). Over the three sessions, regardless of NEFA type, mean threshold concentrations decreased ~10-fold (1 logarithmic dilution). Figure 5 displays mean thresholds for NEFA type broken out by testing session; however, caution should be used in interpretation of these findings, which are between-subject data analyzed within each NEFA type.

Correlation analysis. No correlations were significant among the NEFA and age, sex, naïveté, fat intake, or BMI, but a trend for higher thresholds for α-linolenic acid with higher BMI was observed ($P = 0.07$, correlation coefficient = 0.43). Coefficients and $P$ values are shown in Table 4. Low outliers were removed from this analysis, as these two participants had an extreme influence on the outcomes. The nonperformers were left in the analysis, as the results did not change when these participants were included.

No-threshold visits. Analyses were conducted on the total number of no-threshold visits by NEFA type, participant, testing order, overall visit number, and whether the participants were naïve to NEFA tasting experiments. Analysis was also conducted with and without the three nonperformers. These data are summarized in Table 5 and Fig. 6. With all participants included, linoleic acid had the fewest no-threshold visits, with α-linolenic and oleic acids showing comparable counts of no-threshold visits. However, when the three nonperformers were removed, there were fewer total no-threshold visits for α-linolenic than oleic acid. The number of no-threshold visits was greater for naïve than nonnaïve participants, and the total number of no-threshold visits decreased over time.

**DISCUSSION**

The major findings in this study are the differences in sensitivity to NEFA of varying degrees of unsaturation, which are not explained by the differences in viscosity and particle size, as well as the additional evidence of effects on NEFA taste responses with numerous testing visits. Our hypothesis that humans would be most sensitive to α-linolenic acid was not supported by the data; however, humans were ~2.5–5.6 times more sensitive to the PUFA than MUFA. Learning effects were marked, as thresholds decreased by ~10-fold from testing session 1 to 3, and the number of no-threshold visits was reduced over the course of the experiment. There is mixed evidence on whether lean and obese individuals detect

### Table 4. Spearman correlations of subject parameters and NEFA logM thresholds, with low outliers removed

<table>
<thead>
<tr>
<th>NEFA</th>
<th>Oleic</th>
<th>Linoleic</th>
<th>α-Linolenic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age $r$</td>
<td>-0.11</td>
<td>-0.04</td>
<td>0.24</td>
</tr>
<tr>
<td>Sex $P$</td>
<td>0.65</td>
<td>0.88</td>
<td>0.32</td>
</tr>
<tr>
<td>Naïve $P$</td>
<td>-0.21</td>
<td>-0.29</td>
<td>-0.09</td>
</tr>
<tr>
<td>Fat intake</td>
<td>0.21</td>
<td>0.35</td>
<td>0.35</td>
</tr>
<tr>
<td>Total</td>
<td>0.22</td>
<td>0.17</td>
<td>0.17</td>
</tr>
<tr>
<td>Saturated</td>
<td>0.29</td>
<td>0.32</td>
<td>0.19</td>
</tr>
<tr>
<td>BMI</td>
<td>0.03</td>
<td>0.05</td>
<td>0.43</td>
</tr>
</tbody>
</table>

*Male = 0, female = 1; nonnaïve = 0, naïve = 1.

### Table 5. No-threshold visits by NEFA type, testing order, and naïve status

<table>
<thead>
<tr>
<th>NEFA type</th>
<th>All Participants</th>
<th>Performers</th>
</tr>
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<tbody>
<tr>
<td>Oleic acid</td>
<td>28/147 (19.0%)</td>
<td>22/126 (17.4%)</td>
</tr>
<tr>
<td>Linoleic acid</td>
<td>18/147 (12.2%)</td>
<td>5/126 (3.9%)</td>
</tr>
<tr>
<td>α-Linolenic acid</td>
<td>29/147 (19.7%)</td>
<td>10/126 (7.9%)</td>
</tr>
<tr>
<td>Testing order</td>
<td></td>
<td></td>
</tr>
<tr>
<td>First 7 visits</td>
<td>36/147 (24.4%)</td>
<td>26/126 (20.6%)</td>
</tr>
<tr>
<td>Second 7 visits</td>
<td>25/147 (17.0%)</td>
<td>8/126 (6.3%)</td>
</tr>
<tr>
<td>Third 7 visits</td>
<td>14/147 (9.5%)</td>
<td>3/126 (2.4%)</td>
</tr>
<tr>
<td>Naïve status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Naïve</td>
<td>50/252 (19.8%)</td>
<td>25/231 (10.8%)</td>
</tr>
<tr>
<td>Nonnaïve</td>
<td>25/189 (13.2%)</td>
<td>12/147 (8.1%)</td>
</tr>
</tbody>
</table>

Fig. 4. Mean thresholds by testing session [first 7 visits (session 1), second 7 visits (session 2), and third 7 visits (session 3)], including all NEFAs, all participants ($n = 21$), and group with low outliers and nonperformers removed ($n = 16$). Bars with different letters are significantly different within groups ($P < 0.05$).

Fig. 5. Mean thresholds for NEFA type by testing session in group with low outliers and nonperformers removed ($n = 16$). Between-subject data are shown. *$P < 0.05$ vs. session 1; **$P < 0.05$ vs. session 2 (within NEFA type).
NEFA differently (20). In the current study this was only observed as a trend and only for \(\alpha\)-linolenic acid. Thus the influence of body mass or weight status on NEFA taste sensitivity remains unresolved. Small differences were observed in the physical characteristics of the NEFA emulsions and blank, but these differences are unlikely to have caused the differences in sensitivity to the NEFA (see below).

_Emulsion characteristics do not support textural detection_. As the highest concentrations of oleic and linoleic acids were more viscous than the blank or \(\alpha\)-linolenic acid emulsions, some of the less-sensitive participants may have been detecting these two NEFA through tactile means. However, as the overall mean thresholds for all NEFA were lower than the concentration at which viscosity differences were observed, it is unlikely that texture is the primary mechanism for detection. Furthermore, the viscosity differences would not explain a difference in sensitivity between oleic and linoleic acids, as these had similar viscosities across all concentrations but different oral thresholds. Additionally, while the differences in viscosity are significant, they are still small. Available data indicate that most people are not adept at discriminating viscosities in this range (22). Particle size differences among the emulsions were also small, and it is again unlikely that such small differences could explain the differences in oral sensitivity to the NEFA. However, specific data on the ability of humans to discriminate in this range of particle sizes are unavailable.

_Different sensitivities to unsaturated NEFA_. Thresholds observed in this study, 1–20 mM, are in line with those in other recent studies from the authors’ laboratory (19, 29–31), but the threshold for oleic acid in the current study is higher than thresholds observed by other research groups, who report thresholds for this NEFA in the lower millimolar range (6, 10, 23–26). The carbohydrate gums or emulsifiers used, methods of emulsification, and actual interindividual variability could contribute to the differences between laboratories (20). However, the pattern of lower thresholds to PUFAs than MUFA is consistent with the limited data that are available. The differences in oral sensitivity are likely due to the different chemical properties of the unsaturated fatty acids. Greater sensitivity to linoleic than oleic acid has been observed (23), and while \(P\) values are not given, lower thresholds were also seen for \(\alpha\)-linolenic than linoleic and oleic acid (10). Changes in the shape of the alkyl chain of the fatty acids could explain the increased sensitivity to linoleic and \(\alpha\)-linolenic acids compared with oleic acid. The double bonds in the chains will make “kinks” in the chain, resulting in a more curvilinear structure for the PUFAs than MUFA. More unsaturation leads to slightly increased solubility and higher diffusion rates across cell membranes (14). The different shapes also change affinity for receptors, including GPR120, a proposed human NEFA taste receptor, generally showing greater affinity for longer-chain, more-unsaturated NEFA (4, 10, 12). GPR40, a proposed NEFA taste receptor in mice, does not demonstrate differences in affinity based on degree of unsaturation (3) but has not been identified in human taste tissue (10). However, it is interesting that linoleic acid has the lowest threshold, with a trend for lower thresholds for linoleic than \(\alpha\)-linolenic acid in the performers of this study. The available human data do not repeat this pattern (10). As the current study tested participants multiple times for all NEFA, variability of the data is lower and gives increased confidence in the results, showing similar thresholds for the two PUFAs and lower thresholds for the two PUFAs than the MUFA.

_Rodent studies indicate that expression of the proposed NEFA taste receptor CD36 is altered by exposure to fat, either as fatty acids or as part of a high-fat diet_. Overall expression of CD36 in circumvallate papillae was lower in rats in which obesity was induced by a high-fat diet than in normal-weight rats fed a control diet (37). In mice, obese and normal animals showed similar expression of CD36, but expression of CD36 was lower after a meal in normal mice and did not change in obese mice (8). Still other studies in mice show that CD36 expression is correlated with oral fat exposure from an acute diet and from direct exposure to oil on the tongue (16). Furthermore, decreasing CD36 expression in rodent taste buds using small interfering RNA leads to decreased preference for NEFA (7). If such mechanisms are reflected in human regulation of CD36 expression in the taste buds, this could potentially confound results of taste threshold studies. In the current study, participants were asked not to eat or drink for 1 h prior to the visit; however, exactly what or when they ate was not controlled. Depending on the time course of the changes in CD36 expression, the initial exposure to NEFA at the beginning of a testing session could even influence taste perception by the end of the session. One study showed decreased sensitivity in lean
humans on a high-fat diet compared with lean humans on a low-fat diet (24). Potentially, this could be mediated through changes in CD36 expression. However, data on acute regulation of CD36 in taste cells in humans are not available.

The PUFAs used in this study are also much more susceptible to oxidation than oleic acid, with linoleic acid oxidizing at ~3–10 times and α-linolenic acid at ~15–100 times the rate of oleic acid (9, 13). Potentially, detection of these compounds could be affected by the antioxidant status of an individual’s saliva. Available data indicate no differences in salivary antioxidant status among individuals hyper- or hyposensitive to oral sensation of oleic acid, but data comparing salivary antioxidant status and oral sensitivity to PUFAs are unavailable (17). Whether the mechanisms in saliva to protect against oxidation interact with taste systems is unknown, but sensing of oxidation is another potential route of action for the transport or binding of NEFA and stimulation of an oral sensation.

Learning NEFA taste. Fewer no-threshold visits were observed in NEFA tasting sessions 2 and 3 than session 1. A general downward trend was also observed in no-threshold visits with overall visit number. These data, combined with the observation of more no-threshold visits for naive than nonnaive participants, are another indication that humans learn the taste of the NEFA with repeated exposure. While no overall effect of visit number on threshold concentrations was observed, thresholds were lower in sessions 2 and 3 than session 1 of NEFA testing. Again, this gives further evidence of learning, which has been noted in NEFA taste testing previously (29, 31) and is a commonly accepted phenomenon in threshold testing (1, 15). The learning effects should be considered by researchers in future studies. Given the dramatic decrease in no-threshold visits from session 1 to sessions 2 and 3, care should be taken to ensure that participants actually understand the sensation they are attempting to detect, and familiarization with the stimulus and procedure may be required to ensure that the participants are performing optimally and consistently. Use of a single-point observation, in which a participant could easily fail to detect the sensation even at the maximum concentration, may lead to inaccurate conclusions when an attempt is made to correlate thresholds with other variables.

Conclusions. Increased sensitivity to linoleic and α-linolenic acids compared with oleic acid could potentially complicate current health recommendations to increase PUFAs in the food supply, as the sensation from these compounds is generally perceived as unpleasant, from the verbal descriptions we received from participants and as reported elsewhere (19). However, the affective response to very low, perithreshold concentrations of these NEFA in actual foods remains untested. Previous work with modified sham feeding (where the stimulus is chewed but not swallowed) has demonstrated that triglycerides high in PUFAs result in greater initial serum triglyceride peaks and area under the curve (first 4 h), whereas triglycerides high in MUFA and saturated fatty acids lead to higher serum triglycerides after 8 h (21, 28, 32, 36). This could be due to oral taste cues, given the greater sensitivity to the taste of PUFAs than the MUFA in the current study. Potentially, the different potencies of the various dietary fatty acids could be exploited to optimize sensory properties and physiological effects while minimizing concentrations of triglycerides in a food.

ACKNOWLEDGMENTS

The authors thank Dr. Osvaldo Campanella for use of the rheometer, Dr. Ganesan Narsimhan and Laura Zimmerman for training on and use of the Mastersizer, and Dr. Bruce Craig for assistance with statistical analysis.

GRANTS

This work was supported by US Department of Agriculture Hatch Grant Accession Number 208684.

DISCLOSURES

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

AUTHOR CONTRIBUTIONS

C.A.R. and R.D.M. developed the concept and designed the research; C.A.R. performed the experiments; C.A.R. analyzed the data; C.A.R. and R.D.M. interpreted the results of the experiments; C.A.R. prepared the figures; C.A.R. drafted the manuscript; C.A.R. and R.D.M. edited and revised the manuscript; C.A.R. and R.D.M. approved the final version of the manuscript.

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