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Cordelia Running
crunning@purdue.edu

Richard D. Mattes

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Humans are more sensitive to the taste of linoleic and α -linolenic than oleic acid

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1. Abstract

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 α -linolenic acids. The hypothesis tested was that oral sensitivity to non-esterified fatty acids (NEFA) 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 3-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 α -linolenic acids than to oleic acid. At higher shear rates, 5% oleic and linoleic acid were more viscous compared to other samples. More dilute emulsions showed no significant differences in viscosity. Particle sizes of the emulsions increased very slightly with increasing unsaturation. Considering the emulsion characteristics and the oral sensitivity data together, a taste mechanism for NEFA detection is supported.

2. Introduction

A major contributor to cardiovascular disease (CVD) is a diet high in saturated fatty acids (SFA) (33). Replacing SFA with mono- or polyunsaturated fatty acids (MUFA or PUFA, respectively) may improve blood lipid profiles, decrease markers for CVD, and improve insulin responses in insulin resistant or type II diabetic patients (34, 35). Thus, the type of dietary fatty acids should be a critical consideration when evaluating the healthfulness of high fat foods.

Oleic acid, linoleic acid, and α -linolenic acid, are all unsaturated fatty acids with 1, 2, and 3 double bonds, respectively. Oleic acid and linoleic acid are common in liquid vegetable oils such as safflower, canola, and olive oils while α -linolenic acid is predominately found in fish oil. The PUFAs, linoleic and α -linolenic acids, are ω -6 and ω -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 120 (GPR120) (4, 10, 12). While dietary fat, primarily present as triacylglycerol, has traditionally been valued for textural contributions to food, evidence indicates that non-esterified fatty acids (NEFA) are effective taste stimuli in the oral cavity (11, 20, 32). Large variability has been observed in NEFA oral sensitivity and may be modifiable 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 acids (23) or no difference between these two fatty acids (5). Data from graphs in another report show oral fatty taste thresholds for α -linolenic acid lower than linoleic acid which is, in turn, lower than oleic acid, but do not report means and standard deviations in order to test for significant differences (10). Notably, none of these previous reports tested individuals multiple times with individual NEFA. Data published on oleic acid indicates that individuals may learn

the taste of oleic acid over multiple tests, leading to lower thresholds in later visits than observed in the first test (29, 31). While learning effects are not always observed or may be blunted by using non-naïve participants (19), multiple visits should still 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 1) humans would be most sensitive to the taste of α -linolenic acid, followed by linoleic acid then by oleic acid and 2) learning effects would be observed over multiple tests, particularly in naïve participants. We expected these learning effects to attenuate over the course of the 21 visits conducted in the study (7 visits per NEFA). Due to ongoing concerns of controlling for emulsion texture in NEFA taste experiments, data was also collected and analyzed on particle size distributions and rheology of the samples. 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.

3. Methods

Participants

Participants were recruited through local advertisements. Eligibility criteria included: 18-60 years of age, in good health, normal taste and smell function, and available to complete 21 study visits within 3 months. 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 (NCT01996566). Participants were screened for their ability to detect an emulsion without added tastant (i.e., a mineral oil emulsion) compared to the blank solution (see (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 was measured at the first study visit, along with

age, sex, and self-reported ethnicity. All participants completed a validated food frequency questionnaire for habitual fat intake (2). Descriptive data about the participants is given in Table 5-1. Twenty-one (8 males and 13 females) participants enrolled in and completed the study. Their mean age was 28 (median 24, range 18-58) years, and mean BMI 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. Due to concerns of learning effects and high variability, (29, 31), participant thresholds were obtained 7 times for each of the 3 NEFA, for a total of 21 study visits per participant. The order of NEFA testing was randomized, but a restriction was used to ensure that each NEFA was tested first (visits 1-7), second (visits 8-14), or third (visits 15-21) an approximately equal number of times.

Samples

NEFA were obtained from commercial sources (Spectrum Chemicals O1914; Sigma Aldrich W338001, L2376). Carbohydrate gums were purchased from TIC Gums, Inc (gum arabic: Pre-Hydrated Gum Arabic Spray Dry FCC Powder; xanthan gum: TIC Gums Pre-Hydrated Ticaxan Rapid-3 Powder). Antioxidants were ordered from Spectrum Chemicals (disodium ethylenediaminetetraacetate (EDTA) E1001, *tert*-butylhydroquinone (TBHQ) T1073). "Blank" solutions were prepared by dissolving 10% (w/w) gum arabic, 0.05% xanthan gum, 0.01% TBHQ, and 0.01% EDTA into deionized water. This solution was allowed to rest for at least 45 minutes to allow the gums to fully hydrate. Next, the solution was mixed with T18 Ultra Turrax homogenizer with a S18N-19G dispersing element for 4 minutes at 14,000 rpm. NEFA were then 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 minutes. 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). To make the

blank, the solution of gums only was also homogenized for an additional 8 minutes (12 minutes total at 14,000 rpm for all samples). Maximum concentrations of all NEFA emulsions are given in Table 5-2. These concentrations were chosen based on pilot data indicating that higher concentrations of α -linolenic acid were more irritating and harder to clear from the oral cavity. All samples were tested at room temperature (approximately 21°C). Emulsions were made fresh each day and the same batch of blank that was used to prepare the emulsions (after only 4 minutes of homogenization, then with NEFA added for the additional 8 minutes) was used to dilute the NEFA and as the blank in threshold testing (with the additional 8 minutes of homogenization, no NEFA added). This was done to eliminate the effects of any small batch to batch variation in the gums (observable in Figure 5-2). NEFA dilutions were prepared in quarter-logarithmic (base 10) steps, which is the equivalent of dividing the concentration by 1.778. All samples had a pH of approximately 4.3, regardless of NEFA concentration or type. The maximum concentrations of α -linolenic acid is equal in percent weight to the fourth dilution step of the maximum oleic and linoleic acid concentrations.

Emulsion characteristics

Emulsions were characterized by particle size distributions and viscosity. For particle size data, a Mastersizer 2000 with a Hydro 2000MU dispersion unit was used. Deionized water was used as the dispersant. Optimal obscuration was between 10 and 15%, and refractive indices of 1.458 for oleic acid, 1.466 for linoleic acid, and 1.480 for α -linolenic acid, per the manufacturer (Sigma Aldrich), were used by the software to calculate the size of particles according to the Mie theory. Viscosity was measured using an ARG2 Rheometer (Texas Instruments, New Castle, DE) equipped with a 2° cone and plate geometry, a Peltier plate to maintain temperature, and a solvent trap with deionized water to minimize evaporation. Measurements were conducted as previously described (19); briefly, viscosity was measured from 1-300s⁻¹ (logarithmic scale) at 37°C with ten data points per decade. Duplicate measurements were made, and results were analyzed at each shear rate. Comparisons were made among the maximum concentrations of oleic, linoleic, and α -linolenic acid emulsions and

the blank, as well as among 0.89% concentrations of all NEFA compared to the blank (Bonferroni correction used for multiple comparisons).

Threshold testing

An ascending three-alternative forced choice (3AFC) method was used to determine taste thresholds. On the first testing day for each fat, the experiment began 18 dilution steps below the maximum concentration of each NEFA. The participants wore blindfolds and nose clips to minimize visual and olfactory cues. Participants were handed 3 samples, one NEFA dilution and two blanks, in random order. Participants were instructed to taste and expectorate each sample, rinsing with room temperature (approximately 21°C) water in between. 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 concentration 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 3 times at the same concentration. At this point, the test was again repeated with the next highest concentration of NEFA, as a double check, since the rate of false positives for just 3 correct answers in the ascending method is quite high (18). In our study, with an average run length (count of presentations of three 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 3 correct responses were given was deemed the threshold. If the participant was incorrect, the set of 3 correct responses was deemed a false positive, and the test continued until the participant could give 3 correct responses at one concentration followed by 1 correct response at the next higher concentration. Thus, a total of 4 sequential correct responses were required to finish the test. At visits 2-7 on each NEFA, the test began 4 dilution steps (1 logarithmic dilution) below the previous threshold. If a participant gave 4 correct responses at the very beginning of any test (3 at the first concentration, 1 at the next higher), the test was restarted 4 dilution steps below the original start point. Participants were not given any 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 4 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 ASTM E679 standard for conducting threshold tests (1). While actual thresholds could be higher than this value, or non-existent, 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. There were no restrictions placed on the time between visits, other than there was a maximum of one visit per day and participants had to be available to complete the study within 3 months.

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 (whether it was 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. BMI was also tested but 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 two participants had several visits with extremely low thresholds to linoleic and α -linolenic acids; these two participants will be 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 will be referred to as “non-performers.” All other participants had 6 or fewer no threshold visits, and will be referred to as “performers.” Consequently, data was analyzed with and without the two low outliers and the non-performers. 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, gender, total fat intake, saturated fat intake, and naivety. The low-outliers (BMI of 18.7 and 23.1, both non-naïve to fat taste testing) were removed for this analysis, as their mean thresholds were inordinately low and had extreme influence on the correlations. The non-performers 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 (BMIs of 24.0, 25.4, and 32.2; two naïve and one non-naïve to fat taste testing).

4. Results

Emulsion characteristics

Small increases in droplet diameter were observed with increasing degree of unsaturation (Figure 5-1). However, it should be noted that the blank solution gives an artifact in the 10-100 μ m range for more dilute samples. This can be seen in all NEFA emulsions at 0.89% (approximately, 33-34 mM). To confirm this was an artifact, emulsions were examined under light microscope. No large particles in the range of 10-100 μ m were observed. Further, the blank solution (no emulsified NEFA) was run through the Mastersizer to confirm a small, artificial peak in this range. This took a large amount of the blank solution (over 5mL, as opposed to 0.5-1mL for the emulsions), and we were unable to reach optimal obscuration (maximum obscuration was around 7%). However, the same peak in the 10-100 μ m range could be observed for the blank solution alone, again confirming that this is an artifact caused by the gum solution, rather than flocculation or coalescence of lipid droplets. Ideally, the blank solution would be used as the dispersant, but due to concerns about the more viscous nature of the gums compared to water (the dispersant we used) and concerns regarding being able to adequately clean the gum solution out of the Mastersizer after testing, this was not possible. More sample was required to reach optimal obscuration for the more dilute emulsions at 0.89%, so the small artificial peak was more apparent in these readings (i.e., there was more blank relative to the lipid droplets in the more dilute samples, enlarging the percentages in the 10-100 μ m range caused by the artificial peak of the blank).

As seen before (19), the highest concentrations of linoleic and oleic acids (5%, approximately 186mM) 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 (Figure 5-2). However, while significant, these differences were very small in magnitude (less than 10 mPa.s in this study), and quite likely undetectable by many participants (22). At 0.89% (approximately 33mM) NEFA, no significant differences were observable among the 3 different NEFA and the blank. Data are presented in Table 5-2 on viscosity at $50s^{-1}$, 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 were observable in the blank and the emulsions used to make the blank, due to natural variation in batches of the gums (as observed in Figure 5-2) so “batch” was used as a classification variable in all analyses. Thus, each day when emulsions were prepared, the same blank was used to prepare the emulsions and to conduct testing, to avoid and confounding influence of batch variation in the blank. Notably, in all rheological analysis, both for this study and previously, the only emulsions that were ever significantly thicker than the blank were those over 1.58% NEFA (about 59mM) (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 a logarithmic dilution and one quarter a logarithmic dilution, Table 5-3, Figure 5-3, which include data from all testing sessions). Analysis of the data without the two low outliers and the three non-performers gave the same results, with an added trend for lower thresholds to linoleic than to α -linolenic acid. The order of tasting was also significant, with participants performing better in session 2 and 3 than in session 1, and when the low outliers and non-performers were removed, better in session 3 than in session 2 (Figure 5-4). Over the 3 sessions, regardless of NEFA type, mean threshold concentrations decreased almost 10-fold (one logarithmic dilution). Figure 5-5 displays mean thresholds for NEFA type broken out by testing session; however, caution should be used when interpreting this chart, as this is between-subject data analyzed within each NEFA type.

Correlation analysis

No correlations were significant among the NEFA and age, sex, naivety, fat intake, or BMI, but a trend for higher thresholds for α -linolenic acid with higher BMI was observed ($p=0.07$, correlation coefficient of 0.43). Coefficients and p -values can be seen in Table 5-5. Low-outliers were removed from this analysis, as these two participants had an extreme influence on the outcomes. The non-performers 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 non-performers. Table 5-4 and Figure 5-6 show summaries of these data. 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 non-performers were removed, the total number of no threshold visits for α -linolenic acid had fewer no threshold visits than oleic acid. Naïve participants had more visits with no threshold than non-naïve participants, and the total number of no threshold visits decreased over time.

5. 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 observed in viscosity and particle size, as well as the additional evidence of learning 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 approximately 2.5-5.6 times more sensitive to the PUFAs than to the MUFA. Learning effects were marked as thresholds decreased by almost 10-fold from the first to the third testing session, and there was a reduction of no threshold visits over the course of the experiment. There is mixed evidence on

whether lean and obese individuals detect NEFA differently (20). In the current study this was only observed as a trend and only for α -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 observed differences in sensitivity to the NEFA. These findings are discussed in detail below.

Emulsion characteristics do not support textural detection

As the highest concentrations of oleic and linoleic acids were more viscous than the blank or than α -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 of NEFA were lower than the concentration at which viscosity differences were observed, it is unlikely that texture is the primary mechanism for detection. Further, the observed 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 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 discrimination ability of humans in this range of particle sizes are unavailable.

Different sensitivities to unsaturated NEFA

Observed thresholds in this study, 1-20mM, are in line with thresholds observed 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 inter-individual variability could contribute to the differences between laboratories (20). However, the pattern of lower thresholds to PUFAs compared to 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 acid compared to oleic acid has been observed before (23), and while *p*-values are not given, lower thresholds are also seen for α -linolenic acid compared to linoleic and oleic acids (10). Changes in the shape of the alkyl chain of the fatty acids could explain the increased sensitivity to linoleic and α -linolenic acids compared to oleic acid. The double bonds in the chains will make “kinks” in the chain, resulting in a more curvilinear structure for the PUFAs compared to the 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, this receptor 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 acid compared to α -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 compared to 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. Obese rats, with obesity induced by a high fat diet, have lower overall expression of CD36 in circumvallate papillae than normal weight rats on a control diet (37). In mice, obese and normal animals showed similar expression of CD36, but lower expression of CD36 is observed after a meal in normal mice and no change in expression in obese mice (8). Still other studies in mice show CD36 expression is correlated with oral fat exposure both from acute diet and from direct exposure to oil on the tongue (16). Further, 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 hour 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 has shown that lean humans on a high fat diet had decreased sensitivity compared to when they were on a low-fat diet (24). Potentially, this could be mediated through changes in CD36 expression. However, data in humans on acute regulation of CD36 in taste cells is not available.

The PUFAs used in this study are also much more susceptible to oxidation than oleic acid, with linoleic acid oxidizing about 3-10 times and α -linolenic acid at about 15-100 times the rate of oleic acid (9, 13). Potentially, the detection of these compounds could be affected by the anti-oxidant status of an individual's saliva. Available data indicate no differences in salivary antioxidant status among individuals hyper- or hypo-sensitive to oral sensation of oleic acid, but data are unavailable comparing salivary anti-oxidant status and oral sensitivity to PUFAs (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 the second and third sessions of NEFA tasting than in the first session. 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 naïve compared to non-naïve participants, is another indication that humans learn the taste of the NEFA with repeated exposure. While no overall effect of visit number was observed on threshold concentrations, participants did have lower thresholds in the second and third sessions than in the first session 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 observed 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 participants

actually understand the sensation they are attempting to detect, and training participants to be familiar with the stimulus and procedure may be required to ensure participants are performing optimally and consistently. Using 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 attempting to correlate thresholds with other variables.

6. Conclusions

Increased sensitivity to linoleic and α -linolenic compared to 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 seen in previous work (19). However, the affective response to very low, peri-threshold 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 hours), whereas triglycerides high in MUFA and saturated fatty acids lead to higher serum triglycerides after 8 hours (21, 28, 32, 36). This could be due to oral taste cues, given the greater sensitivity to the taste of PUFAs compared to MUFAs observed in the current study. Potentially, the different potencies of the various dietary fatty acids could be exploited to optimize both sensory properties and physiological effects while minimizing concentrations of triglycerides in a food.

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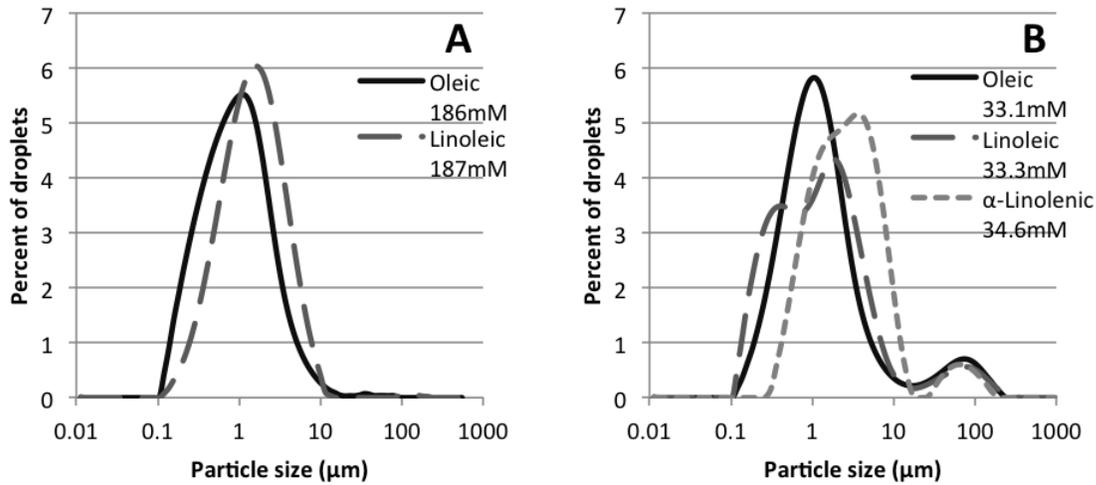


Figure 1: Particle size distributions for emulsions of A: 186mM oleic and 187mM linoleic, and B: 33.1mM oleic, 33.3mM linoleic, and 34.6mM α -linolenic acids. As seen in B, the blank solution creates an artificial peak at 30-110 μ m.

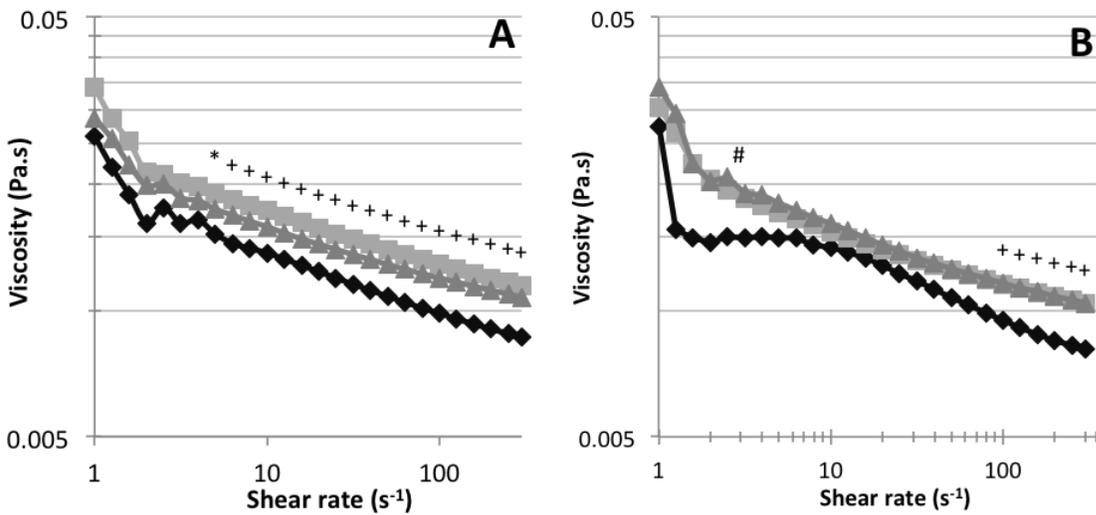


Figure 2: Mean viscosities from 1-300 s^{-1} for blank (diamonds), 5% linoleic acid emulsion (triangles), and 5% oleic acid emulsion (squares). * Indicates oleic acid emulsion, # indicates linoleic acid emulsion, and + indicates both oleic and linoleic acid emulsions are significantly more viscous than the blank ($p < 0.05$). A and B are from different days of testing, with different batches of xanthan gum.

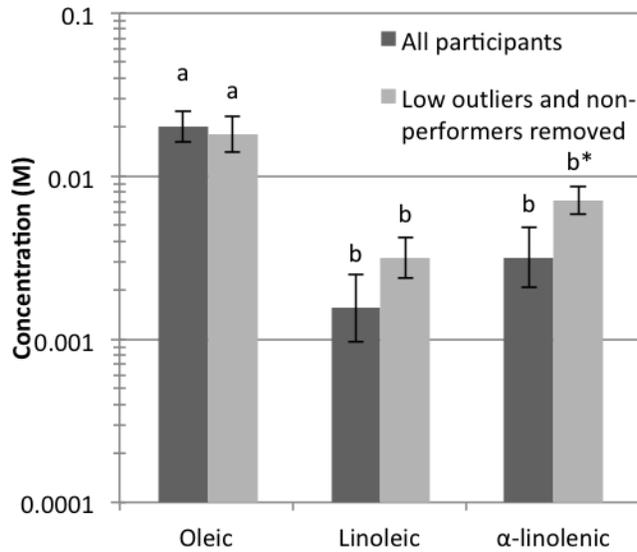


Figure 3: Mean thresholds by NEFA (includes all sessions), all participants N=21, low outliers and non-performers removed N=16; bars with different letters are significantly different within the groups, $p < 0.05$ (* p -value for difference between linoleic and α-linolenic acids in low outliers and non-performers removed group was 0.08)

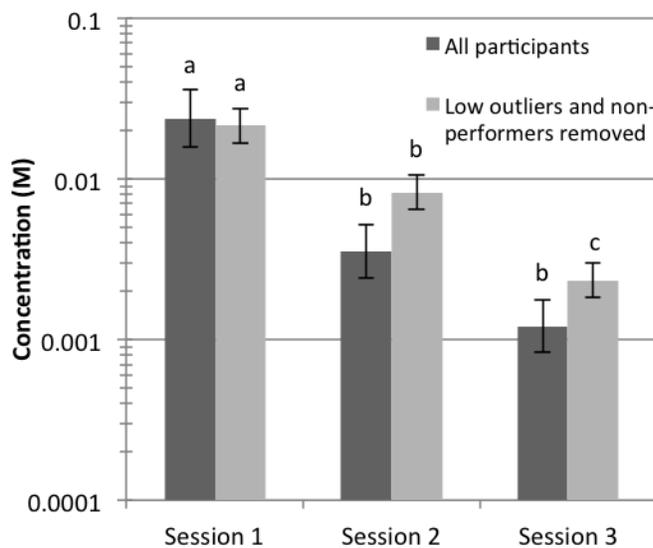


Figure 4: Mean thresholds by testing session (first 7 visits, second 7 visits, third 7 visits, includes all NEFA all participants N=21, low outliers and non-performers removed N=16; bars with different letters are significantly different within the groups, $p < 0.05$

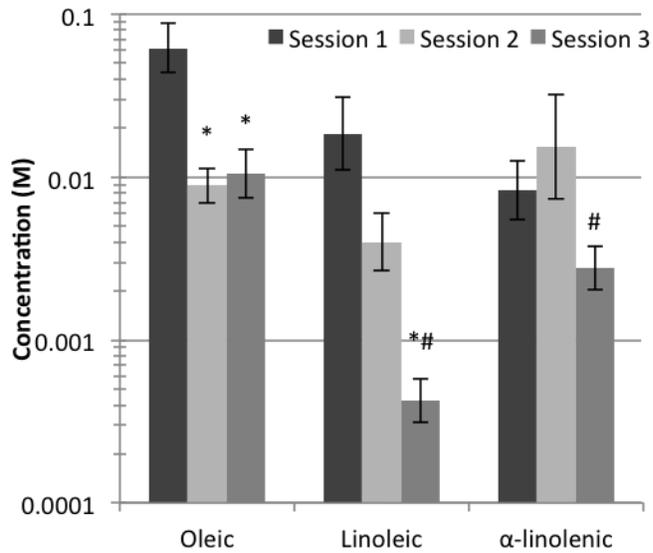


Figure 5: Mean thresholds for NEFA type by testing session (low-outliers and non-performers removed, N=16). Note this chart displays between-subject data. * Indicates significant difference from session 1, # indicates significant difference from session 2 (within NEFA type)

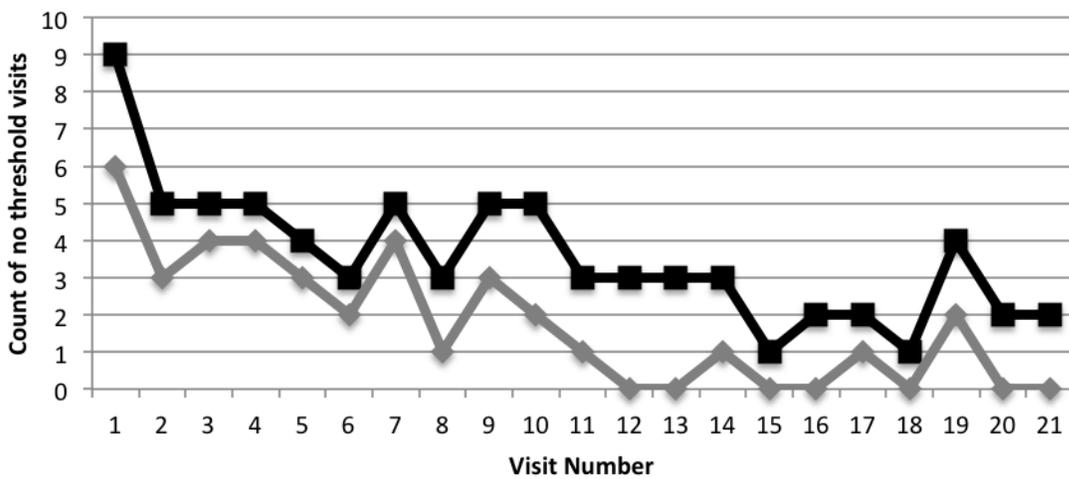


Figure 6: No threshold visits by visit number for all participants (black, N=21) and performers only (grey, N=18)

Table 1: Participant characteristics

	Males	Females
Total	8	13
Naïve	5	4
Low-outliers	0	2
Non-performers	2	1
Age in years	Mean: 26 Median: 24 Range: 18-33	Mean: 30 Median: 27.5 Range: 18-58
BMI in kg/m ²	Mean: 27.8 Median: 28.5 Range: 22.5-32.2	Mean: 23.7 Median: 23.1 Range: 17.4-40.6

Table 2: NEFA emulsion characteristics

	Percent weight	Molar*	Mean droplet diameter (\pm SD)		Viscosity at 50.1 s ⁻¹
			Surface D[3,2]	Volume D[4,3]	
Oleic acid	5.00%	186mM	0.58 \pm 0.11 μ m	1.49 \pm 0.28 μ m	12.5 \pm 0.5 Pa.s
	0.89%	33.1mM	0.75 \pm 0.09 μ m	6.98 \pm 0.57 μ m	8.9 \pm 0.3 Pa.s
Linoleic acid	5.00%	187mM	0.57 \pm 0.02 μ m	1.56 \pm 0.03 μ m	12.6 \pm 0.4 Pa.s
	0.89%	33.3mM	0.61 \pm 0.001 μ m	6.58 \pm 0.79 μ m	9.2 \pm 0.04 Pa.s
α -linolenic acid	0.89%	34.6mM	0.84 \pm 0.01 μ m	5.85 \pm 0.08 μ m	9.1 \pm 0.6 Pa.s
Blank	NA	NA	NA	NA	10.7 \pm 0.7 Pa.s

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

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 the viscosity of the NEFA emulsions and the blank (data from the same day as Figure 5-2B).

Table 3: Mean threshold values by NEFA type (mean \pm SE)

NEFA type	All participants	Performers, with low outliers removed
Oleic acid	19.9mM (-1.70 \pm 0.09 logM) ^a	17.9mM (-1.75 \pm 0.11 logM) ^a
Linoleic acid	1.55mM (-2.81 \pm 0.21 logM) ^b	3.15mM (-2.50 \pm 0.12 logM) ^b
α -linolenic acid	3.15mM (-2.50 \pm 0.18 logM) ^b	7.06mM (-2.15 \pm 0.08 logM) ^{b*}

Within each column, different letter superscripts indicate $p < 0.05$

* p - value for linoleic to α -linolenic acid comparison for performers, low outliers removed was 0.08

Table 4: No Threshold visits by NEFA type, testing order, and naïve status

NEFA type	All participants		Performers	
Oleic acid	28/147	19.0%	22/126	17.4%
Linoleic acid	18/147	12.2%	5/126	3.9%
α -linolenic acid	29/147	19.7%	10/126	7.9%
Testing order				
First 7 visits	36/147	24.4%	26/126	20.6%
Second 7 visits	25/147	17.0%	8/126	6.3%
Third 7 visits	14/147	9.5%	3/126	2.4%
Naivety				
Naïve	50/252	19.8%	25/231	10.8%
Non-naïve	25/189	13.2%	12/147	8.1%

Table 5: Spearman correlations of subject parameters and NEFA logM thresholds (p -values in parentheses); low outliers removed

	Oleic	Linoleic	α -linolenic
Age	-0.11(0.65)	-0.04(0.88)	0.24 (0.32)
Sex*	-0.21(0.38)	-0.29(0.22)	-0.09(0.72)
Naïve*	0.21(0.38)	0.35(0.15)	0.35(0.15)
Fat intake:			
-Total	0.22(0.35)	0.17(0.49)	0.17(0.49)
-Saturated	0.29(0.23)	0.32(0.19)	0.19(0.44)
BMI	0.03(0.91)	-0.05(0.82)	0.43(0.07)

*Male=0, Female=1; Non-naïve=0, naïve=1