Video Article

Measuring Oral Fatty Acid Thresholds, Fat Perception, Fatty Food Liking, and Papillae Density in Humans

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Abstract

Emerging evidence from a number of laboratories indicates that humans have the ability to identify fatty acids in the oral cavity, presumably via fatty acid receptors housed on taste cells. Previous research has shown that an individual's oral sensitivity to fatty acid, specifically oleic acid (C18:1) is associated with body mass index (BMI), dietary fat consumption, and the ability to identify fat in foods. We have developed a reliable and reproducible method to assess oral chemoreception of fatty acids, using a milk and C18:1 emulsion, together with an ascending forced choice triangle procedure. In parallel, a food matrix has been developed to assess an individual's ability to perceive fat, in addition to a simple method to assess fatty food liking. As an added measure tongue photography is used to assess papillae density, with higher density often being associated with increased taste sensitivity.

Video Link

The video component of this article can be found at https://www.jove.com/video/51236/

Introduction

Excessive dietary fat consumption is a potential contributor to weight gain[1-3] and obesity has become a modern day global epidemic. Research suggests higher levels of fat intake, particularly as part of an *ad libitum* diet, may be associated with a higher BMI[2,3], however the factors influencing dietary fat consumption and preference are far from clear. Searching for the mechanisms which underlie fat consumption is therefore an obvious goal and of particular interest is an oral mechanism responsible for fat detection, commonly termed 'fatty acid taste'[2].

From an evolutionary standpoint, the taste system presumably served as a gatekeeper of the digestive system, guiding the consumption of energy dense nutrients and expulsion of potentially toxic compounds[4]. The sense of taste is elicited through specialized taste receptor cells which are distributed within three types of tongue papillae; fungiform, foliate, and circumvallate papillae, which can each contain up to several hundred taste buds[5]. In addition to the widely accepted five prototypical tastes (sweet, salty, sour, bitter, and umami), it is not entirely surprising that there has been suggestion of an oral mechanism for detecting fat, or more likely their breakdown products fatty acids[6].

Previous research has consistently shown fatty acids can be detected in the oral cavity over a range of concentrations[7-11], despite the fact that it is not a 'taste' in the traditional sense, as it has no single discernible perceptual quality associated with it (*i.e.* sweet)[12]. Work from our laboratory has highlighted functional implications of impaired fatty acid chemoreception, namely on body weight and dietary fat consumption. Those who are less able to detect fatty acids (hyposensitive) appear to have a higher body mass index (BMI) and consume more energy[9], while a relationship between oral fatty acid sensitivity and dietary fat consumption has also been observed; that is, fatty acid hyposensitive individuals have been shown to consume more animal fats, including, meat, high fat dairy, and fatty spreads all of which are been implicated as contributors to weight gain[13]. Additionally, individuals who are more sensitive to fatty acids appear to be better equipped at differentiating between samples with varying fat contents[9]. While other research groups have failed to find similar associations[10,14,15], this growing area of research remains intriguing.

These individual differences in fatty acid chemoreception appear to be somewhat modulated by environmental factors, including diet. Habitual fat intake has been associated with impaired oral fatty acid chemoreception and consequently, a heightened preference for, and increased consumption of dietary fat[16]. In addition to gustatory adaptation, the gastrointestinal tract (GI) also appears responsive to such changes in fat intake[17] and impaired GI fatty acid sensitivity may be implicated in the inability to generate appropriate satiety signaling responses which discourage excess energy consumption[18].

In addition to environmental factors, fatty acid chemoreception may also be dictated by genetic or physiological differences between individuals, including the concentration of fungiform papillae density (and presumably taste receptors) on an individual's tongue[19]. Higher density of fungiform tongue papillae has been linked to heightened oral sensitivity for numerous orally detected compounds including 6-*n*-propylthiouracil (PROP)[20], sugar[21], and salt[22], while others have also noted an association with creamy perception[23]. PROP supertasters (who presumably have a higher number of fungiform papillae) are able to distinguish high fat from low fat salad dressings[24] and are able to discriminate the fat content and

creaminess of dairy foods more accurately than non-tasters[23,25]. At this stage however, the relationship between fungiform papillae density and oral fatty acid 'taste' detection is unknown.

At the basis of human oral fatty acid chemoreception research is the application of various sensory techniques. Identifying individual variability in oral fatty acid detection is a major focus and largely depends on the determination of fatty acid detection thresholds, that is, the lowest point at which fatty acid is able to be detected in solution[9]. While the specific testing method and stimulus vehicle used varies across the literature and between research groups, the typical procedure involves presenting a participant with a set of emulsified fatty acid and control (no fatty acid) solutions and identifying which is the 'odd' sample. Here we present an established, reliable and reproducible method for threshold determination[10] using emulsified milk solutions and an ascending forced choice triangle procedure.

The extent to which oral fatty acid sensitivity influences diet, namely consumption of fatty foods, and the ability to perceive fat in foods is also of interest and here we also report on two additional established techniques to further extend our understanding of fatty acid chemoreception. Fatty food liking can be identified by providing individuals with samples of commercially available foods, with both a regular and a low-fat option who are asked to indicate liking of each[16]. In regards to fat perception, a fat ranking task has been developed by our laboratory, designed to evaluate an individual's ability to detect fat in custard, a typical food matrix[16]. To evaluate genetic or physiological differences between individuals, a commonly used tongue photography method involves staining, photographing and quantifying fungiform papillae[26]. While using this technique in fatty acid research is in its infancy, increasing application, especially in both lean and overweight/obese population groups may help to identify inherent causes of excess fat consumption.

Protocol

The following techniques have been approved for use by the Deakin University Human Research Ethics Committee.

1. Demographics and Anthropometry

1. Record demographic information from participants, including date of birth and gender.
2. At baseline (and other study points if temporal study design) take height and weight measurements. Ensure that participants have taken off shoes, heavy jackets or other clothing items, and have removed any heavy items from their pockets.
    1. Measure participant's height using a stadiometer. Record measurements to the nearest cm.
    2. Weigh participants using dedicated scales. Record weight to the nearest g.
    3. Calculate BMI using the equation: weight (kg)/height^2 (m^2). From this, participants are categorized according to standard BMI definition values; healthy 18.5-25 kg/m^2, overweight 25-30 kg/m^2 or obese >30 kg/m^2 [27].

2. Producing Samples for Oral Fatty Acid Threshold Assessment

1. Use non-fat UHT milk as the base for fatty acid taste threshold assessment. Product can be purchased and stored in bulk if necessary, and will keep unopened for up to 6 months or until the product has reached its expiry. Prepare 2 types of vehicles: vehicle with added fatty acid and a control vehicle. The volume of solutions prepared for testing will depend on participant number. The following protocol provides typical amounts for 2 participants.
2. Prepare a base milk solution to use for both the control and fatty acid vehicle by placing 5% w:v food grade gum arabic (*e.g.*, 100 g per 2 L of milk) into a 3 L glass beaker. If required, hydrate gum prior to use (this will vary depending on gum manufacturer).
3. Add 0.01% w:v EDTA to the gum to prevent oxidation (*e.g.*, 200 mg per 2 L of milk).
4. Allocate approximately 1 L of non-fat milk per participant (*e.g.*, for 2 participants, use 2 L milk) and pour into beaker.
5. Using a laboratory grade mixer with emulsor screen, homogenize the solution at 12,000 rpm for 2 min. Set solution aside.
6. Prepare the fatty acid solutions using food grade C18:1. Oxidation can be assessed through gas chromatography if necessary.
7. Prepare a series of 13 variants of the fatty acid vehicle (UHT non-fat milk) with increasing concentrations of C18:1 (0.02, 0.06, 1, 1.4, 2, 2.8, 3.8, 5, 6.4, 8, 9.8, 12, and 20 mM/L). To do this, label 250-ml glass beakers with each concentration.
8. Add 5% liquid paraffin to each beaker (*e.g.*, 5 ml paraffin per 100 ml of milk solution).
9. Based on C18:1 concentration, add the appropriate amount of C18:1 to each beaker (see **Table 1**).

C18:1 concentration (mM)	µl/100 ml
0.02	0.56
0.06	1.9
1	31.5
1.4	44.1
2	63.1
2.8	88.4
3.8	119.9
5	157.8
6.4	202
8	250

9.8	309
12	380
20	631.2

Table 1. Example C18:1 concentration per 100 ml solution. Increasing concentrations (µl/L) of C18:1 are used to prepare the series of 13 emulsions for oral fatty acid threshold testing.

10. Following use, fill the C18:1 container with N_2 to minimize oxidation and store below 4 °C.
11. Add the base milk solution to each fatty acid beaker to a total volume of 100 ml. Set aside.
12. Using the remaining base solution, prepare the control vehicle. In a 2 L glass beaker, add 5% of the remaining volume in liquid paraffin (*e.g.*, 35 ml liquid paraffin in a final volume of 750 ml) together with the remaining base solution and homogenize for 30 sec per 100 ml of liquid.
13. Homogenize the control vehicle for 30 sec per 100 ml. This step is conducted prior to the fatty acid solutions to prevent contamination with C18:1.
14. Homogenize each fatty acid vehicle, beginning with the lowest concentration for 30 sec per 100 ml.
15. Sanitize the homogenizer both prior to and following testing.
16. As the homogenization process can raise the temperature of the solutions, check temperature of control and C18:1 samples with a thermometer. Serve all samples at RT (20 °C).
17. Milk samples must be freshly prepared on the same day as testing. Taste each solution prior to testing to assess freshness and suitability. Note: Depending on volume required, solution preparation will take a minimum of 60 min.

3. Oral Fatty Acid Threshold Testing

1. Ensure that participants have refrained from eating or drinking (including coffee, gum, mouthwash, etc.) for at least 1 hr prior to testing.
2. Minimize non-taste cues by conducting testing under red lighting with participants wearing nose clips.
3. Use the ascending forced choice triangle procedure to determine oral fatty acid thresholds. Label 30-ml plastic portion cups with a three-digit identification number. Provide each participant with a set of three 20-ml solutions in random order; two control vehicles and one fatty acid vehicle with the lowest concentration of C18:1 (0.02 mM).
4. To determine a participant's oral fatty acid threshold, instruct the participant to taste each solution from left to right and expectorate into a sink. Ask participants to not swallow the samples.
5. Ask the participant to identify which of the 3 samples is 'odd' or 'different' and if they are unsure, they must guess (forced choice).
6. Have participants rinse their mouth with deionized water after each set of samples.
7. If correctly identified, provide the participant with a second set of 3 solutions (2 control and 1 fatty acid solution in random order) with the same fatty acid concentration. If incorrectly identified, provide the participant with a second set of 3 solutions, but with the next highest concentration of C18:1 (0.06 mM).
8. Continue this procedure until the participant is able to correctly identify the 'odd' sample 3x in a row at the same concentration. The concentration at which they are able to correctly identify the 'odd' sample is recorded as the participants C18:1 detection threshold. See **Figure 1** for a graphical representation of this process.
9. Based on detection threshold, characterize participants as hypersensitive, or hyposensitive to C18:1. In line with previous literature, hypersensitive individuals can detect C18:1 at concentrations <3.8 mM, while hyposensitive individuals require concentrations >3.8 mM. Note: Depending on the number of incorrect answers, the testing procedure can take between 10-30 min to complete.

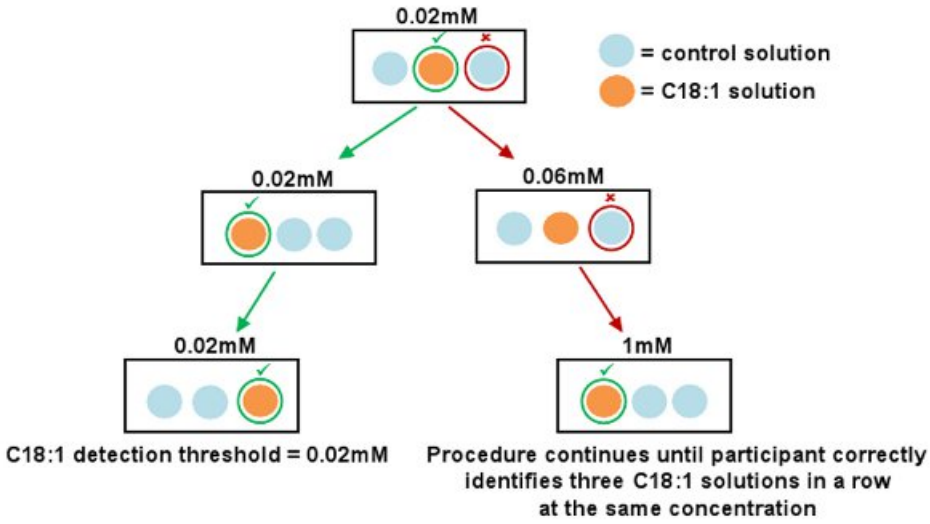

Figure 1. Ascending forced choice triangle procedure used for determining fatty acid detection thresholds. Participants are provided with three solutions (two control solutions and one C18:1 solution at a given concentration) and asked to identify the 'odd sample'. If correct, participants are given a second set of samples with the same C18:1 concentration. If incorrect, the participant is provided with another set of samples with a higher concentration of C18:1. This procedure continues until three 'odd' solutions are correctly identified at a given concentration. This point is deemed the individuals 'fatty acid detection threshold'.

4. Fat Ranking Task

This task involves participants tasting four samples of instant custard, each with different fat contents (0, 2, 6, and 10%) and ranking them in order of perceived ascending fat concentration.

1. Prepare 1 batch of custard using non-fat instant vanilla custard powder according to packet instructions. Mix 2 tablespoons custard powder, 1 tablespoon sugar, and 2 cups non-fat milk in a microwave safe bowl. If the suggested product is unavailable, this may be substituted with a similar non-fat instant product (*e.g.*, cook and serve custard).
2. Using high power, heat the mixture using a 1,400 W microwave in 30 sec intervals for a total of approximately 5 min, or until thick. This may vary depending on the brand of custard and the wattage of the microwave used. Allow custard to cool.
3. Label four 500-ml kitchen bowls (or similar) with fat percentages.
4. Divide the custard into 4 separate 100-g batches.
5. Add 0, 2, 6, and 10% vegetable oil to each bowl to achieve the desired fat content (*e.g.*, in a 100 g batch, add 0 ml, 2 ml, 6 ml, and 10 ml vegetable oil to achieve respective fat percentages) and combine. Stir each sample well to ensure all ingredients are completely amalgamated.
6. Label four 30-ml plastic portion cups with randomized three-digit numbers. Fill the portion cups with 20 g of each custard (1 type of custard per cup).
7. Refrigerate samples prior to testing and serve cold (4 °C).
8. Carry testing out under red lights to minimize visual cues.
9. Have participants taste, swallow and rank the 4 custards from perceived lowest to highest fat content and receive a score out of 5 depending on their responses.
10. Scoring for this task is shown in **Table 2**.
    Note: Approximate preparation time for the custard samples is 30 min. The fat ranking task should take no longer than 10 min to complete.

Ranking order	Score
0, 2, 6, 10	5
2, 0, 6, 10	4
0, 2, 10, 6	3
0, 6, 2, 10	2
1, 6, 10, 2	1
6, 0, 2, 10	1
2, 10, 6, 0	1

2, 6, 0, 10	0
6, 2, 10, 0	0
0, 10, 2, 6	0

Table 2. Fat ranking task scoring. Participants are given 4 samples of custard with 0, 2, 6, or 10% fat added. Participants are asked to rank samples from lowest to highest fat content and score 0 to 5 points (5 being the maximum).

5. Fatty Food Liking

1. Prepare small samples (5-20 g) of both regular and low-fat options of commercially available foods. Foods include regular and low-fat versions of: cream cheese (served on a cracker), chocolate mousse, cheese, dry biscuits, peanut butter dip served on a piece of carrot, mayonnaise, salad dressing (served on a slice of cucumber), and yogurt.
2. Label each sample with a random three-digit number for identification.
3. Present samples in a random order to prevent order effects.
4. Instruct participants to taste each sample individually. Food items are ingested, but participants can eat as much or as little of each sample as they desire.
5. Have participants rate how much they like or dislike each sample. Measure liking using a 100-mm hedonic generalized magnitude scale (gLMS; see **Figure 2**) ranging from strongest imaginable dislike to strongest imaginable like. Record liking by placing a vertical line at the point which represents the participants like or dislike of the food.

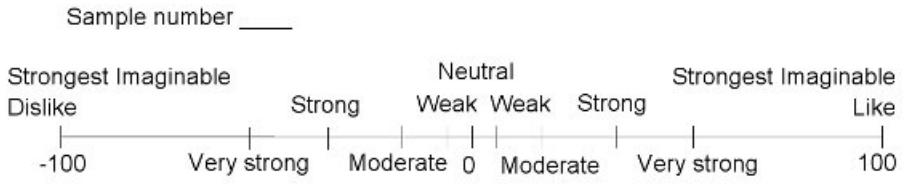

Figure 2. Hedonic gLMS. The hedonic gLMS[30,31] used to assess liking of both regular and low fat commercially available foods. Participants taste and rate each sample and place a vertical line at the point which best represents their like, or dislike of the sample.

6. Non-taste inputs are not minimized for this task, so carry this task out under normal light and do not have participants wear nose clips.

6. Tongue Photography

1. Set up a camera and tripod for photography. Regular indoor lighting is sufficient.
2. Set the camera to macro mode (or similar) for close up photography.
3. Use a hole punch to create a 6-mm diameter circle on a 1.5 cm x 1.5 cm (or similar) square of filter paper. Label the paper with the participant's identification number.
4. In a 50-ml beaker, combine blue food coloring with deionized water at a 1:20 ratio. A small amount is required per participant.
5. Pour 30 ml food grade ethanol into a 50-ml beaker for tweezer sterilization.
6. Using masking tape, mark a 20 cm x 30 cm rectangle on the side of the testing table (this should be regular desk height), as shown in **Figure 3a**.

Figure 3. A) Tongue photography setup. Demonstration of the table setup required prior to tongue photography. **B)** Tongue photography. Demonstration of the tongue photography method

7. Have participants place their elbows on the marked corners of the rectangle, rest their chin in their palms and to comfortably protrude their tongue, using the lips to steady this position (**Figure 3b**). The participant must remain in this position for the duration of the testing.
8. Using a rectangular (1.5 cm x 3 cm) strip of filter paper, briefly dry the bottom portion of the tongue.
9. Dip a cotton bud into the food coloring/water solution and transfer a small amount of dye onto the anterior dorsal surface of tongue, immediately right of the midline point and close to the tip (see **Figure 4**). Dry the tongue for a second time with filter paper.
10. Dry ethanol sterilized tweezers with paper towel and using tweezers, place the pre-labeled 1.5 cm^2 filter paper onto the participant's tongue, with the 6-mm hole over the blue food coloring (see **Figure 4**).
11. Using flash, take three-digital photographs of the participant's tongue. For confidentiality, ensure only the participant's mouth and tongue are visible.
12. Remove the 1.5 cm^2 filter paper from the participant's tongue with tweezers that have again been sterilized in food grade ethanol. Upload photographs to a photo editing software and with the zoom function, count all visible fungiform papillae.
13. Differentiate fungiform papillae from other papillae as larger mushroom shaped, elevated structures. They do not take on the dye solution as strongly, and as such appear much lighter in color.
    Note: Tongue photography should take no longer than 10 min to complete.

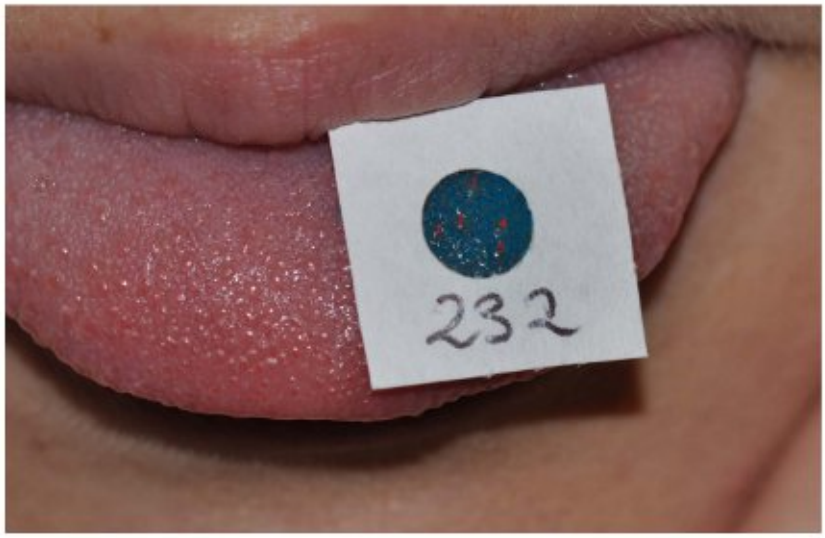

Figure 4. Quantifying fungiform papillae density. Location of the 6-mm area for fungiform papillae assessment. Using photography editing software, numerical figures indicate each fungiform papilla.

Representative Results

The methods detailed above are important as some emerging evidence has indicated that impaired fatty acid chemoreception in the oral cavity and GI tract may be associated with increases in BMI and the development of obesity[17]. Several studies have used the described protocols to investigate oral fatty acid detection and our recent publication has shown the method is both reliable and reproducible[10]. Studies using this methodology have been able to reliably determine individual's oral fatty acid detection thresholds by identifying the point at which participants are able to detect a difference between milk samples[9]. After three testing sessions utilizing this protocol, Stewart *et al.*[9] found that the mean detection threshold for C18:1 was 2.2 ± 0.1, with detection thresholds ranging from 1-6.4 mM (see **Figure 5**). More recently, we have established a C18:1 detection threshold range from 0.26-12 mM, (mean: 2.64 ± 0.7 mM)[10]. These results support the notion that fatty acids can be detected in the oral cavity, and that marked individual differences in sensitivity to C18:1 exist. Based on these results, we are able to classify individuals as hypersensitive or hyposensitive to C18:1. Hypersensitive individuals are able to correctly identify C18:1 <3.8 mM, while hyposensitive subjects require concentrations >3.8 mM. Research from our laboratory has found oral sensitivity to C18:1 is associated with dietary fat consumption and BMI (**Figure 6**), where C18:1 hyposensitive individuals consume more saturated and animal fats and have a higher BMI[13]. Interestingly, in a study conducted by Stewart and Keast[16], it was found that consuming a low fat diet resulted in increased sensitivity to C18:1 for both lean and overweight participants (**Figure 7**). However, this study also found that when participants consumed a high fat diet, lean individuals had reduced sensitivity to C18:1, while overweight individuals had no change in taste sensitivity (**Figure 8**). This suggests that habitually consuming a high fat diet, which is more likely for overweight individuals, may result in attenuated fatty acid chemoreception[28]. However, as there were no differences in baseline sensitivity between lean and overweight participants, these results may indicate that lean individuals are simply more susceptible to dietary changes regarding fat intake. This may also suggest that it was the presence of the specific intervention (high- vs low-fat) that may have influenced outcomes, rather than habitual diets, which may or may not have been different to the intervention diet. Despite this, this study indicates that there are some fundamental differences between lean and overweight individuals regarding fatty acid taste sensitivity, which requires further investigation.

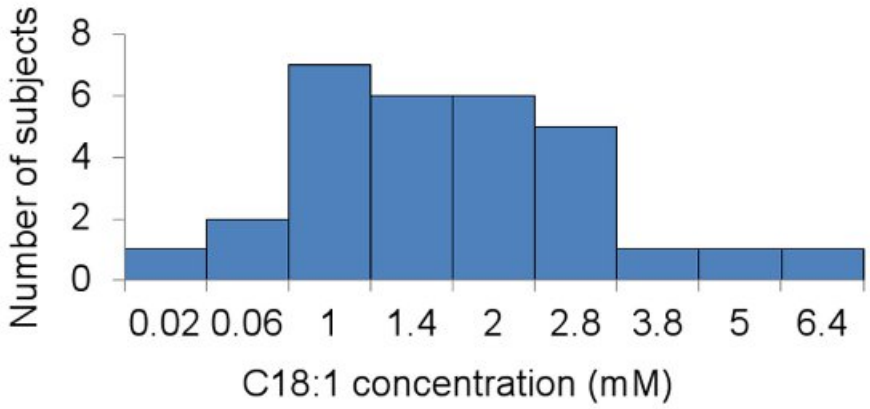

Figure 5. C18:1 taste detection thresholds. Marked variability has been shown in sensitivity to C18:1 with participants able to detect C18:1 across a range of concentrations (1 mM-6.4 mM).

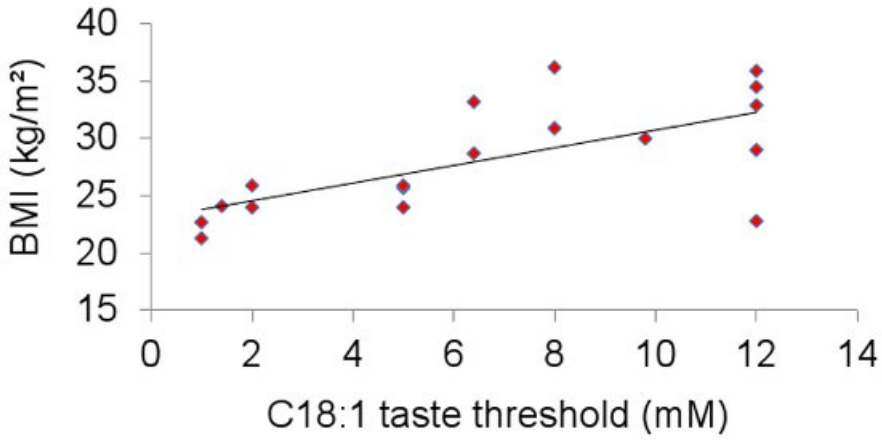

Figure 6. C18:1 taste detection thresholds and association with BMI. An association between the ability to detect C18:1 and body composition has been shown, whereby those with higher detection thresholds (hyposensitive individuals) have significantly higher BMI values ($P = 0.002$, $r^2 = 0.467$).

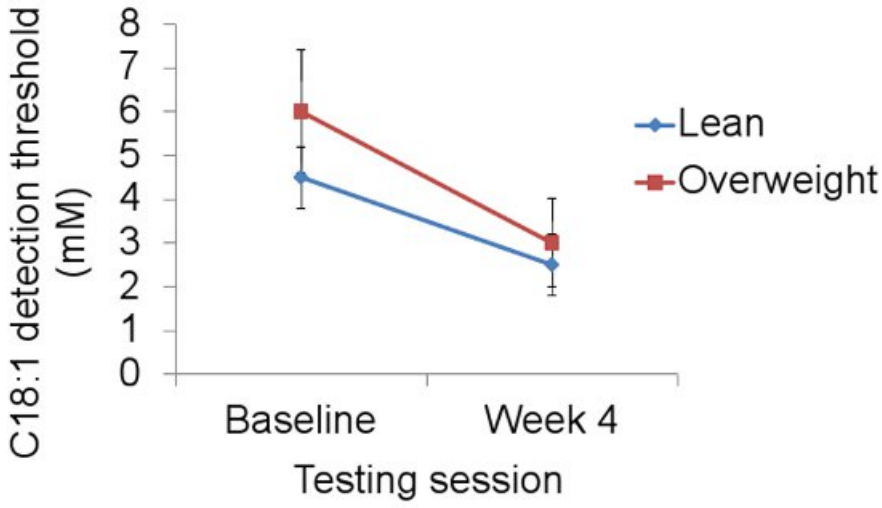

Figure 7. C18:1 detection thresholds following a low fat diet. Following 4 week consumption of a low fat diet, C18:1 detection thresholds increased for both lean and obese individuals.

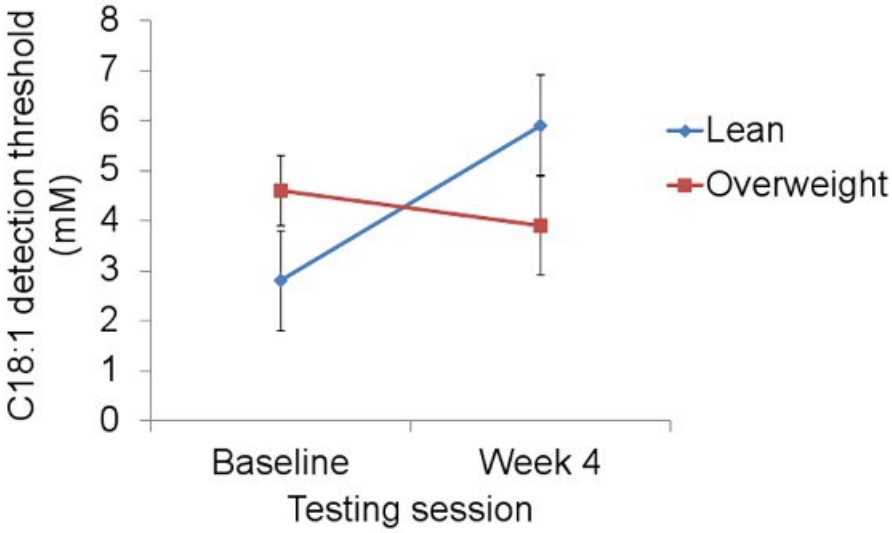

Figure 8. C18:1 detection thresholds following a high fat diet. Following 4 week consumption of a high fat diet, lean individuals displayed reduced sensitivity to C18:1 (P = 0.006), while overweight individuals demonstrated no change (P= 0.609).

Similarly to the impact of diet on detection thresholds for fatty acids, there is research to suggest that food liking may be plastic and altered by exposure. For example, it appears that a high fat diet increases preference for a higher fat product, with the opposite occurring following consumption of a low fat diet[16]. However, these changes have not been consistent in the literature. It appears that changes to preferences are mediated by the length of time the individual has been adhering to a high or low-fat diet. Specifically, Mattes[29] found significant changes in participant food preferences after 12 weeks on a reduced fat diet, while Stewart and Keast[16] found only sporadic and marginal changes after four weeks on a similar diet. Consuming a high fat diet altered participant preferences for yogurt, with preferences for low-fat yogurt increasing, converse to expected results (Baseline (BL): 19.44 ± 5.73, Week 4 (WK4): 21.94 ± 5.21, P = 0.046). Further, after four weeks on a low-fat diet, preferences for low-fat butter increased in all participants (BL: 6.23 ± 4.26, WK4: 7.32 ± 3.04, P = 0.046). Preferences for low-fat yogurt increased for lean participants only (BL: 2.51 ± 3.26, WK4: 3.68 ± 4.94, P = 0.07) while preferences for low-fat mousse decreased for all participants (P = 0.01).

The ability to detect fat in foods is assessed by asking participants to taste and rank a series of custards with differing fat contents. Fat perception is identified based on how well participants were able to rank the samples. Fat perception has been known to change with diet, for

example, following a low fat diet has resulted in improvements to participant's performance in correctly identifying and ranking the degree of fat in each custard sample[11]. Furthermore, it appears that there is an association between sensitivity to C18:1 and identification and ranking of fat content[4]. Indeed, individuals who were hypersensitive to C18:1 performed significantly better on the fat ranking task (4.3 ± 0.6) compared to hyposensitive individuals (2.3 ± 0.1, P = 0.02) (scores are out of a maximum of five)[9]. This indicates that individuals who are more sensitive to fatty acids were also better at differentiating between the four varying fat concentrations in custard. While there was a trend for performance to improve after consuming the low-fat diet for four weeks, this was not a significant change (BL: 1.3 ± 0.3, WK4: 2 ± 0.3, P = 0.077)[16].

Papillae density *i.e.* the number of papillae (and hence taste buds) present on the tongue varies between individuals and is indicative of taste function. Higher fungiform papillae density has been linked to heightened taste sensitivity, for compounds including sucrose[21] and the bitter substance PROP[20]. Tongue papillae density, as determined by tongue photography, varies considerably between subjects. For example, Zhang *et al.*[21] found that there were significant individual differences between participants, ranging from a concentration of 7.07 ± 0.35/cm^2 to 233.43 ± 0.00/cm^2 (data was for a single participant), whereas others[26] have found a mean fungiform papillae concentration to be 156.00 ± 5.86/cm^2. Further, it has been found that papillae can appear significantly differently in structure between individuals, with variation in height, width, and shape,[21] though limited evidence regarding the possible implications of these differences. Given previous findings linking papillae number with taste sensitivity, it is plausible that a similar relationship may also exist for oral fatty acid sensitivity, whereby those who are more orally sensitive to fatty acids may have a higher density of taste papillae and therefore a higher number of oral fatty acid receptors. While this association is yet to be established, it presents a novel area of research may help implicate the underlying mechanisms guiding the over consumption of fat.

Discussion

The techniques described for determination of oral fatty acid thresholds, fatty food liking, and tongue papillae density have been validated and used in a number of published works in recent years and we suggest oral fatty acid threshold assessment, the fat ranking task and fatty food liking be performed in duplicate at each relevant time point in a study. There has been some discussion regarding the optimum method for assessing detection thresholds[32]. In particular, the composition of solutions used varies between laboratories, as does the methodology itself. Specifically, the fatty acid used in this protocol, C18:1, we believe is a generally representative and easy to use fatty acid, as opposed to other fatty acids, including linoleic acid (C18:2) and lauric acid (C12:0), which have been used previously[9]. C18:1 is commonly found in the food supply and unlike C12:0 is liquid at RT, and is more resistant to oxidation than C18:2[9]. C18:1 has also been shown to provide reliable data across multiple testing sessions, and is highly correlated with C18:2 and C12:0[10]. Furthermore, C18:1 has been examined extensively throughout relevant literature, and is thus more helpful for comparisons.

A major point of difference between the protocol outlined within the present paper and other procedures used in other laboratories are the vehicles used for presenting fatty acid stimuli and the systematic approach by which detection thresholds are determined. Two major fatty acid vehicles used within the literature are non-fat milk[10,17] and water emulsions[6]. While both have demonstrated efficacy for fatty acid threshold determination, participants may be more likely to identify the taste of fat within milk, that is, it is unusual to taste fatty acids in water, which may result in lower levels of external validity for studies utilizing a water base. Non-fat milk provides a vehicle for fatty acid chemoreception, without compromising validity. Although these two methods are yet to be directly compared in the literature, it is known that fatty acids are poorly soluble in water[33]. As a result of fatty acid solubility in milk-based solutions, this emulsion could both be kept longer and be more homogenous than water-based solutions, though this is yet to be confirmed. When implementing this methodology, it is important to note free fatty acids may be naturally present in milk[34] and consequently, the product should be used well within its expiry to prevent the increase of free fatty acids (which develop with age) and potential interference with taste threshold performance. Successful preparation of the solutions depends on numerous factors. Firstly, the order in which the 'ingredients' are added is imperative. Vehicle preparation steps should carefully follow those outlined earlier to ensure proper vehicle composition and a stable emulsion. Secondly, temperature must be controlled for. Each sample must be presented to participants at RT to ensure participants do not detect the 'odd sample' due to factors other than 'taste'. Finally, all samples must be correctly homogenized for the suggested period of time. While the emulsion of fatty acids and no-fat milk is more effective than if water were to be used, there is still a chance of emulsion separation within the sample.

The specific testing method used in oral fatty acid threshold determination must also be considered. Two sensory-based methods have been commonly described in the literature; one being the ascending forced choice triangle procedure and the alternate, the staircase method[35]. The ascending forced choice triangle methodology is an established method for taste threshold determination and can be considered useful for several reasons, including the fact that, unlike the staircase method, the ascending method begins with the lowest concentration of C18:1 (0.02 mM) and increases until the participant is able to detect the presence of fatty acid in solution[9]. Conversely, the staircase method involves increasing or decreasing the fatty acid concentration from a predetermined midpoint[11]. However, starting a threshold determination at a point above threshold may cause a desensitization of response impairing ones tasting ability. Further, the ascending method has a lower probability of random chance influencing results (3.7%) compared with the staircase method (11.1%)[11]. As such, we suggest the ascending forced choice triangle method, combined with non-fat milk as a vehicle for taste testing appears to be an effective means of accurately determining oral thresholds.

Food acceptance or liking testing is one of the more straightforward assessments performed within sensory research and as such there are few problems that tend to arise. However, the type of liking scale used is an important focus. In this case, a hedonic gLMS is the most effective, as it has good discriminatory power and is easy for participants to use[36]. The end points of the hedonic gLMS are labeled with the descriptors 'strongest imaginable dislike' and 'strongest imaginable like' and participants evaluate liking against all hedonic experiences, not solely foods[30,31]. This is effective in controlling for ceiling effects produced by standard 9-point scales, as all experiences are considered and compared. Further, the hedonic gLMS is more able to demonstrate greater individual variance, as the scale is broader[36]. Food acceptance testing itself may be limited by the foods presented, in that we only present two options per food type. Further research may include several more brands or types of each food, each with differing fat contents, or perhaps specifically made products where fat content can be controlled and is the only variable. It is important to note that the interpretation of all data must be done with caution. While a potential link between liking, preference and intake is plausible and intriguing, results are generated within a laboratory environment and there may be limits to the applicability of these findings to real-world situations.

Assessing papillae density through tongue photography is a more difficult process, with specific steps that must be taken in order to produce appropriate and applicable results. In particular, it is important to identify the correct papillae type. Three types of taste papillae are visible on the human tongue; fungiform, foliate and circumvallate[4]. Fungiform papillae can however be easily distinguished as mushroom-shaped structures[26], and are generally the papillae that are recorded during sensitivity assessments. Fungiform papillae tend to range in concentration from 5-60 per 6-mm area[37] (depending on sensitivity), though there have been studies indicating that some individuals may have upwards of 230 papillae the same area[21]. The type of camera used is fundamental to obtaining appropriate results and may account for this variability. Prior to the use of digital photography in this area, videomicroscopy was the gold standard for identifying and recording papillae density. However, it has been determined that the same level of identification is possible using an appropriate digital camera[26]. Further, digital photography takes only several minutes, where videomicroscopy may take up to an hour[26]. Not only this, but digital photography has the potential to be far less costly, and more portable, which may be helpful for use with various participant groups[26]. Finally, while we aim to measure fungiform papillae density for associations with oral fatty acid detection, we also suggest taste thresholds for the five prototypical tastes also be performed in parallel. Given previous linkage with papillae density and taste function, this could serve as an additional 'checking measure' which may add integrity to the data, especially given this is a new area of research.

The area of oral chemoreception research, particularly regarding fatty acids, is an emerging one, and as such it is important for all research to be performed to a high standard, preferably with the use of consistent protocols to allow for direct comparisons.

Disclosures

The authors have nothing to disclose.

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