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Original Article

Oil Perception—Detection Thresholds for Varying Fatty Stimuli and Inter-individual Differences

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Abstract

Multiple lines of research have demonstrated that humans can perceive fat in the form of free fatty acids (FFAs). However, the dietary concentration of FFAs is generally very low and fat is mainly consumed as triacylglycerol (TAG). The aim of this study was to examine the perception of different fatty stimuli and possible associations between them. Therefore, detection thresholds for 4 fatty stimuli (oleic acid [FFA], paraffin oil [mixture of hydrocarbon molecules], canola oil [TAG-rich], and canola oil spiked with oleic acid [rich in TAGs and FFAs]) were determined in 30 healthy participants. Additionally, inter-individual differences in fat perception were examined. It was observed that oleic acid was perceivable at significantly lower concentrations than all other stimuli ($P < 0.001$). Similarly, canola oil with oleic acid was detectable at lower concentrations than canola oil alone ($P < 0.001$). Moreover, canola oil detection thresholds were significantly lower than paraffin oil detection thresholds ($P = 0.017$). Participants who were sensitive for low concentrations for oleic acid showed lower detection thresholds for canola oil with and without oleic acid, compared with participants that were less sensitive for oleic acid. The results of this study demonstrate that the higher the concentrations of FFAs in the stimuli, the lower the individual fat detection threshold. Moreover, participants being sensitive for lower concentrations of FFAs are also more likely to detect low concentrations of TAG-rich fats as it is found in the human diet.

Key words: fat perception, fat taste, free fatty acids, triacylglycerol

Introduction

Fat is a source of high-dense calories, essential fatty acids and necessary for the digestion of fat-soluble vitamins. Moreover, fat can modify flavor release (de Roos 2005) and increase the palatability and consumption of a meal (Drewnowski 1997; Wolfram et al. 2015). The increased prevalence of global overweight and obesity (Ng et al. 2014) has seen an increased demand for fat-reduced

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products to combat the risk of fat and energy overconsumption. To effectively reduce the fat content in foods, the question of which dietary fat attribute might be dominant in perception has been investigated repeatedly. Depending on the product (e.g., solid or liquid) and its typical fat content, the senses of vision, olfaction, taste and somatosensation differ in their impact on fat discrimination. Sometimes the addition of flavors to compensate for the sensory gap caused by fat reduction (Heilig 2013; 2016) or to increase the perceived creaminess without changing the caloric content can mask differences in fat concentrations, resulting in preserved pleasantness and satiating effects (McCrickerd et al. 2014; Le Calve et al. 2015). Nonetheless, in some food products with even small differences in viscosity, variations in fat concentrations can be perceived which cannot be disguised by additive flavors (Le Calve et al. 2015).

Generally humans refer to the fat content of their foods via textural attributes (Tomaschunas et al. 2012; Sonne et al. 2014), such as the thickness of gravy or the creaminess of dairy products. Apart from these textural cues, there is increasing evidence that fat can also evoke taste sensations in the oral cavity (Mattes 2011; Keast 2015). Several studies have shown that humans can detect free fatty acids (FFAs) of different chain lengths when visual, olfactory and textural cues are masked (Mattes 2009a; Stewart et al. 2010; Newman 2013; Running and Mattes 2014). These FFAs are known to be the effective stimuli that interact with receptors located on taste buds in the oral cavity such as cluster of differentiation 36 (CD36) (Gaillard et al. 2008; Laugerette et al. 2005) and G-protein coupled receptor 120 (GPR120), also called free fatty acid 4 receptor (FFA4 receptor; Hirasawa et al. 2005; Cartoni et al. 2010; Oh et al. 2010). This stimulation of receptor cells on the tongue's surface can evoke sensory signals that are transmitted to the taste processing regions of the brain where they can trigger physiological responses, such as the release of gastric lipase (Wøjdemann et al. 1997).

The bitter and pungent taste sensation evoked by an elevated concentration of FFAs might have helped to prevent the consumption of rancid foods during evolution. However, in the human diet the amount of FFAs is low and even in fatty foods such as oil there are only about 1–2% of FFAs (Gunstone and Norris 1983; Koriyama et al. 2002). Dietary fat is mainly consumed in the form of triacylglycerols (TAGs) where fatty acids are bound to a glycerol structure that can be hydrolyzed by salivary, gastric, and pancreatic lipases during digestive processes. In emulsions, those TAGs are likely to be additionally coated by phospholipids, proteins, and emulsifiers. Although TAGs represent the main part of dietary fat, research on detection thresholds of TAGs in emulsions and their association with detection thresholds for FFAs is relatively scarce. By examining FFA concentrations in saliva following the mastication of TAG-rich high-fat foods, it was suggested that the observed amounts of FFAs were sufficient to initiate gustatory signals (Kulkarni and Mattes 2013). Nevertheless, in a second study it was observed that lingual lipase was not always active. Hence, the authors assumed that lingual lipase might play a subservient role in oral fat detection and might only be required when stronger oral processing of fatty food is necessary (Kulkarni and Mattes 2014). Studies examining detection thresholds for the FFA oleic acid and the TAG triolein reported that oleic acid could be detected at lower concentrations than triolein (Pepino et al. 2012; Voigt et al. 2014). Moreover, it was reported that participants with higher lingual lipase activity were more sensitive for triolein (Voigt et al. 2014).

The aim of this study was to examine detection thresholds for different fatty stimuli, associations between these fatty stimuli, and to increase our knowledge of whether texture or taste sensations are the major contributors in oral fat perception. Therefore, detection thresholds for oleic acid (a FFA), paraffin oil (hydrocarbon-mixture), canola oil (rich in TAGs), and canola oil spiked with oleic acid (rich in TAGs and FFAs) were determined in a repeated measurements design. We expected that with an increasing amount of FFAs, fat detection thresholds would decrease (and sensitivity would rise). Furthermore, we hypothesized that participants that were sensitive for low concentrations of oleic acid would also be more sensitive for lower concentrations of canola oil than participants that were less sensitive for oleic acid.

Material and methods

Participants

A total of 30 participants were enrolled in this study. Inclusion criteria comprised of written informed consent (as approved by the Deakin University Human Research Ethics Committee and in conformance with the Declaration of Helsinki), aged between 18–55 years, no lactose-intolerance, not being pregnant or lactating and not suffering from impaired taste or smell.

Study design

To examine detection thresholds for different fatty stimuli, we performed a prospective observatory study with a repeated measurements design at the Centre for Advanced Sensory Science, Deakin University, Burwood, Victoria, Australia. The study involved 4 stimuli—oleic acid, paraffin oil, canola oil, and canola oil spiked with oleic acid. The FFA oleic acid was chosen as a stimulus to control for a participants' sensitivity for FFAs based on an established procedure (Haryono et al. 2014). In the second stimulus, the fat-like oily hydrocarbon-mixture paraffin oil, no FFAs with a carboxyl-group at their apex are present, nor can they be hydrolyzed by human lipases. Therefore, it served as a textural control stimulus in this study. In contrast, the TAG-rich canola oil that was used as the third stimulus can be hydrolyzed by human lipases. Hence, canola oil could be perceived in 3 different ways: by the low concentration of FFAs that are already present in oil (1–2%) (Gunstone and Norris 1983; Koriyama et al. 2002), by an increasing amount of FFAs due to lingual lipase activity or by textural cues evoked by the high concentration of TAGs in this stimulus. Moreover, canola oil served as a reference for a dietary fat that is used in food preparation and consumed worldwide. To further examine the impact of taste and textural sensations on fat perception, canola oil spiked with oleic acid was chosen as the fourth stimulus. Here, increasing amounts of canola oil were spiked with a fixed amount of oleic acid. It was expected that participants would either detect this stimulus due to taste sensations, evoked by the added oleic acid, or due to textural attributes that should correlate with detection thresholds of canola oil on its own.

Each of the 4 stimuli was measured on 3 non-consecutive days in randomized order, summing up to 12 study appointments. Participants were instructed to fast overnight and to come to the sensory laboratory at the same time each morning for all 12 appointments. Anthropometric measurements (weight [Tanita Body Scan Composition Monitor Scales], height [Seca, MedShop Australia], waist and hip circumference [Seca, MedShop Australia], according to the methodology of the World Health Organization [WHO 2008]) were collected during the initial study appointment to calculate a participants' BMI and waist-to-hip-ratio. On the second study appointment, taste intensity for sucrose and sodium chloride concentrations (100, 200, and 300 mM each) were rated on labeled magnitude scales following the fat detection threshold measurement. Data was collected using Compusense Cloud Software as part of the Compusense Academic Consortium.

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Fat detection thresholds

Non-fat milk (Devondale), gum arabic (Tic Gums), EDTA (Titriplex III, Merck KGaA), oleic acid (Sigma-Aldrich Chemie GmbH), paraffin oil (Sanofi Consumer Healthcare) and canola oil (Coles) were all food grade quality and purchased from commercial vendors. All samples were freshly prepared on the morning of each testing day.

In Table 1 the fat concentrations for the 4 stimuli are depicted. Oleic acid concentrations were based on an established protocol (Haryono et al. 2014), whereas the concentrations used for the other 3 stimuli, were determined in pilot studies using 0.15 log steps with a starting point of 1.00% fat in the samples. The lowest (0.30% fat in the sample) and the highest concentration (50% fat in the sample) were extrapolated. For the 4th stimulus, canola oil spiked with oleic acid, increasing concentrations of canola oil based on the pilot studies with a fixed amount of oleic acid (3.80 mM), based on the average detection thresholds of oleic acid in previous studies (Stewart et al. 2010; Stewart and Keast 2012b; Newman 2013) were used.

For each of the 4 stimuli, a base solution with emulsified fat containing non-fat milk and 5% gum Arabic was prepared and homogenized at 12 000 rpm for 30 s per 100 mL. To prepare the 13 concentrations, the respective amount of fat (Table 1) was added to 13 beakers, filled up with base solution and homogenized again at 12 000 rpm for 30 s per 100 mL. When oleic acid was used, 0.01% EDTA (as an anti-oxidant) was added to the base solution and 5% of paraffin oil (to increase viscosity) was added to each beaker. Control samples were prepared in the same way but without adding a respective stimuli.

Sensory testing was conducted under red light and participants were instructed to wear nose clips to avoid visual and olfactory cues. To roughly test a participants' sensitivity and to reduce sensory fatigue, a sensory training was introduced at the start of each appointment. In this training, participants were presented with a labeled control and test sample and asked whether they could perceive a difference between these 2 samples. Training would always start at concentration step 7. When a participant could not detect a difference between the labeled test and control samples in the training, additional training steps with higher concentrations of the respective stimulus (concentration step 10 and 13, respectively) were introduced. Whenever a participant perceived a difference between the 2 samples, the actual determination of an individuals' threshold started. Based on the sensory training, the detection threshold test had different starting points—following a perceived difference at concentration step 7, the actual test started at concentration step 1, a successful training at concentration step 10 would set concentration step 7 as a starting point and a perceived difference at concentration step 13 would have concentration step 10 as the starting concentration.

Fat detection thresholds were determined using an ascending 3-alternative forced choice triangle test based on previous studies (Mattes 2009b; Stewart et al. 2010; Stewart and Keast 2012a; Newman 2013; Haryono et al. 2014; Tucker et al. 2014; Sayed et al. 2015). Participants received a set of 3 samples (2 control and 1 test sample containing the stimulus) in randomized array and were instructed to identify "the odd one out" Whenever a participants' answer was incorrect, the concentration of the test sample was increased. When a participant picked the test sample correctly, another set of samples containing the same fat concentration was presented. An individuals' detection threshold was defined as the first concentration a participant could correctly identify among the control samples over 3 consecutive times.

Viscosity measurements

To examine textural differences between and within the stimuli, rheological measurements of the final samples (emulsions) were performed based on the protocol of Krzeminski and colleagues (Krzeminski et al. 2011). In 3 consecutive measurements, viscosity of the concentration steps 1, 5, 9, and 13 (Table 1) were determined reflecting every fourth concentration within the 13 concentration steps that were used. The viscosity measurements were conducted at room temperature (20 °C), similar to the temperature in the sensory lab. A stress-controlled rheometer (Phyisca MSC 301, Anton Paar) with a double gap system (measuring system DG27/T200/SS) was used for the rheological measurements. Within a thixotropic loop experiment, shear rate was linearly increased from $\dot{\gamma} = 0$ to $\dot{\gamma} = 500\ s^{-1}$ within 3 min. The shear rate was hold for 3 min at $\dot{\gamma} = 500\ s^{-1}$ and then decreased from $\dot{\gamma} = 500\ s^{-1}$ to $\dot{\gamma} = 0\ s^{-1}$ within 3 min. To reflect the sensory perception in the oral cavity the apparent viscosity at the shear rate of at $50\ s^{-1}$ (τ <50 s^{-1}>) from the upward curve was used (Shama and Sherman 1973; Skriver 1999).

Table 1. Fat concentrations for the 4 stimuli

	Oleic acid		Paraffin oil		Canola oil		Canola oil + oleic acid	
	mM[a]	%	mM	%[b]	mM	%[b]	mM	%[b]
1	0.02	0.001	5.41	0.30[c]	9.90	0.30[c]	9.90 + 3.80	0.30[c] + 0.119
2	0.06	0.002	17.88	1.00	32.69	1.00	32.69 + 3.80	1.00 + 0.119
3	1.00	0.032	25.21	1.41	46.09	1.41	46.09 + 3.80	1.41 + 0.119
4	1.40	0.044	35.76	2.00	65.37	2.00	65.37 + 3.80	2.00 + 0.119
5	2.00	0.063	50.42	2.82	92.18	2.82	92.18 + 3.80	2.82 + 0.119
6	2.80	0.088	71.15	3.98	130.10	3.98	130.10 + 3.80	3.98 + 0.119
7	3.80	0.119	100.47	5.62	183.70	5.62	183.70 + 3.80	5.62 + 0.119
8	5.00	0.158	141.95	7.94	259.54	7.94	259.54 + 3.80	7.94 + 0.119
9	6.40	0.202	200.59	11.22	366.75	11.22	366.75 + 3.80	11.22 + 0.119
10	8.00	0.250	283.37	15.85	518.10	15.85	518.10 + 3.80	15.85 + 0.119
11	9.80	0.309	400.29	22.39	731.87	22.39	731.87 + 3.80	22.39 + 0.119
12	12.00	0.380	565.30	31.62	1033.58	31.62	1033.58 + 3.80	31.62 + 0.119
13	20.00	0.631	893.90	50.00[c]	1634.37	50.00[c]	1634.37 + 3.80	50.00[c] + 0.119

[a]Based on Haryono et al. (Haryono et al. 2014).

[b]Based on pilot studies, using 0.15 log steps with a starting point of 1.00.

[c]Extrapolation. Concentrations in millimolar were calculated on the basis of the density at 25 °C and the molecular weight of the respective stimulus. Concentrations highlighted in grey were used for sensory training to roughly test an individuals' fat sensitivity.

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Statistical analysis

Analysis was performed using SPSS, version 22 (IBM SPSS). Because fat detection thresholds were not normally distributed, non-parametric tests were used. Friedman-Tests were used to examine differences over repeated measurements. To evaluate differences between the 3 measurements within one stimulus, Wilcoxon signed rank tests were performed. Intraclass correlations were calculated to assess the consistency of detection thresholds over the 3 measurements per stimulus. To test for differences between the different stimuli and for fat sensitivity-specific differences, the Mann-Whitney-*U* test was used. Spearman correlations were used to evaluate associations between variables. Differences in viscosity were examined using a post hoc Bonferroni corrected univariate ANOVA with viscosity as dependent variable and concentration steps and stimuli as independent variables. Significance was accepted at P values < 0.05. Values are expressed as means ± standard error of the mean (SEM).

Results

Baseline characteristics

In total, 30 participants (24 females, 28.3 ± 1.3 years old, had a waist-to-hip ratio of 0.77 ± 0.02 and a BMI of 24.0 ± 0.8 kg/m^2) completed the study.

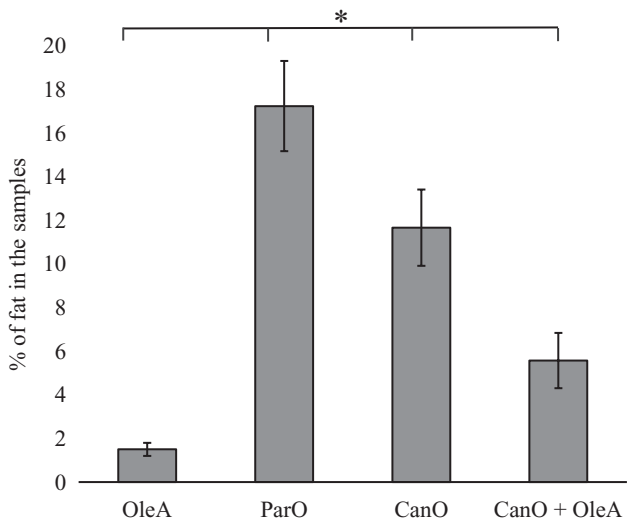


Figure 1. Differences in detection thresholds of the 4 stimuli over the mean of all 3 measurements. *All stimuli are significantly different from each other (P < 0.05). For visualization, oleic acid was scaled up by the factor 10. Data are depicted as mean ± SEM. *OleA: Oleic Acid, ParO: Paraffin Oil, CanO: Canola Oil, CanO + OleA: Canola Oil + Oleic Acid.*

Detection thresholds

Figure 1 depicts the average percentage of fat in the samples that was detected for each stimuli over the course of 3 measurements (oleic acid: 0.15 ± 0.03% [range 0.02–0.55%], paraffin oil: 17.24 ± 2.07% [range 1.47–50.00%], canola oil: 11.66 ± 1.75% [range 0.87–29.08%], canola oil + oleic acid: 5.57 ± 1.27% [range 0.30–23.05%]). It was observed that the FFA oleic acid was perceivable at significantly lower concentrations compared to the fat-like stimulus paraffin oil, and the fatty stimuli canola oil and canola oil + oleic acid (all P < 0.001). When spiked with oleic acid, canola oil was detected at significantly lower concentrations than canola oil on its own (P < 0.001) and paraffin oil (P < 0.001). Detection thresholds for canola oil were also significantly lower than those for paraffin oil (P = 0.017).

In Table 2, detection thresholds for the 4 stimuli over all 3 measurements are depicted. For oleic acid, paraffin oil and canola oil no significant differences between the measurements were observed. Detection thresholds for canola oil + oleic acid were significantly lower on the third measurement compared with the first measurement (P = 0.023). A similar trend for improvement over repeated measurements was observable for oleic acid although this didn't reach significance (P = 0.069). Nonetheless, oleic acid detection thresholds were significantly lower on the third measurement compared with the first measurement (P = 0.047). The intra-class correlations (ICC) for detection thresholds measured in percentages of fat in the samples were as follows: canola oil: ICC = 0.264, P = 0.009, paraffin oil: ICC = 0.221, P = 0.023, oleic acid: ICC = 0.461, P < 0.001, canola oil + oleic acid: ICC = 0.316, P = 0.002. Considering associations between the 4 stimuli with regards to the means of all 3 measurements, a significant correlation between detection thresholds for oleic acid and canola oil + oleic acid was found (R = 0.497, P = 0.005). Additionally, a trend for a correlation between detection thresholds of canola oil and canola oil + oleic acid was observed (R = 0.320, P = 0.085).

To further examine associations between detection thresholds for the 4 stimuli and possible differences in fat sensitivity, we classified our participants into 2 groups. Based on previous work (Haryono et al. 2014), participants with a mean detection threshold for oleic acid below 3.8mM were classified as hypersensitive (N = 18), those with a mean detection threshold ≥3.8 mM were classified as hyposensitive (N = 12). Accordingly, oleic acid detection thresholds were significantly higher in the hyposensitive group (first measurement: P = 0.002; second measurement: P = 0.001; third measurement: P < 0.001; mean over all 3 measurements: P < 0.001; Figure 2). Moreover, participants being hypersensitive for oleic acid had significantly lower detection thresholds for canola oil spiked with 3.8mM oleic acid (first measurement: P = 0.004; second measurement: P = 0.015; third measurement: P = 0.043; mean of all 3

Table 2. Detection thresholds over all 3 measurements

	% of fat in the samples			
	First measurement	Second measurement	Third measurement	*P*-value
Oleic acid	0.19 ± 0.04[a]	0.16 ± 0.04	0.11 ± 0.03[a]	0.069
Paraffin oil	17.80 ± 3.16	19.46 ± 3.01	14.45 ± 2.79	0.179
Canola oil	13.35 ± 2.65	10.90 ± 2.64	10.74 ± 2.02	0.410
Canola oil + oleic acid	7.51 ± 1.91[a]	5.12 ± 1.39	4.07 ± 1.84[a]	0.047

[a]Significant difference between the first and third measurement of detection thresholds of oleic acid (P = 0.047) canola oil + oleic acid (P = 0.023). Data are depicted as mean ± SEM.

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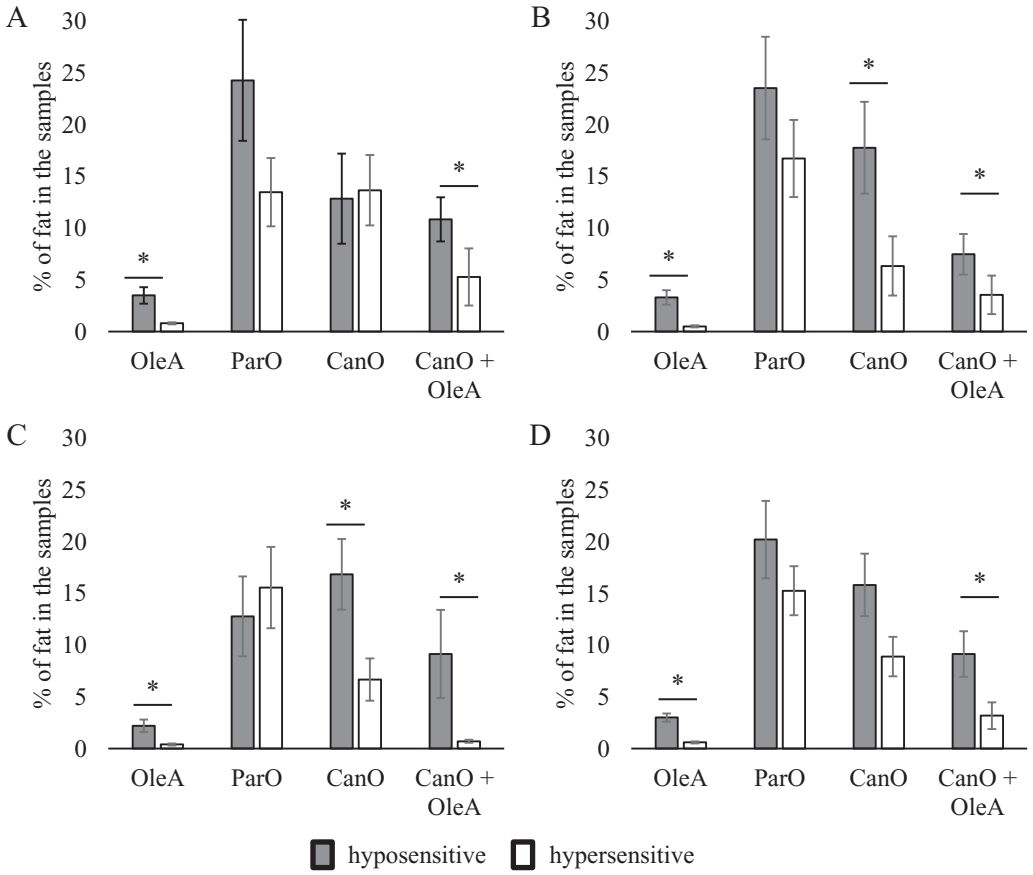


Figure 2. Differences in fat detection thresholds between hyper- and hyposensitive participants. A: first measurement, B: second measurement, C: third measurement, D: mean of all 3 measurements. *Significant differences between hyper- and hyposensitive participants ($P < 0.05$). For visualization, oleic acid was scaled up by the factor 10. Data are depicted as mean ± SEM. *OleA: Oleic Acid, ParO: Paraffin Oil, CanO: Canola Oil, CanO + OleA: Canola Oil + Oleic Acid.*

measurements: $P = 0.004$). Theoretically, the 18 hypersensitive participants that detected oleic acid at concentrations < 3.8 mM could have been able to detect the lowest concentration of canola oil + oleic acid 54 times out of 90 measurements (60%), since this concentration was spiked with the fixed amount of 3.8 mM oleic acid. Concurrently, among the 90 measurements of canola oil + oleic acid, 38 times (42.2%) the lowest concentration was determined as detection threshold. In the remaining 52 measurements higher concentrations of canola oil + oleic acid were required to identify the test sample among the fat-free control samples, stretching over the whole concentration range (0.3–50% fat in the samples). Whereas no significant correlations between the means of all 3 measurements in hypersensitive participants could be observed, hyposensitive participants showed significant correlations between detection thresholds of canola oil and oleic acid ($R = 0.587$, $P = 0.045$) and between canola oil and canola oil + oleic acid ($R = 0.608$, $P = 0.036$). Apart from oleic acid and canola oil + oleic acid, hyper- and hyposensitive participants also differed in their detection thresholds for canola oil (second measurement: $P = 0.035$; third measurement: $P = 0.019$; mean over all 3 measurements: $P = 0.087$), with hypersensitive participants detecting canola oil at lower concentrations (Figure 2). In contrast, detection thresholds for paraffin oil did not differ significantly between hyper- and hyposensitive participants.

Figure 3 depicts the viscosity measurements of the emulsions at concentration step 1, 5, 9, and 13 for each of the 4 fatty stimuli. The maximal viscosity differences between the means of the 4 stimuli that were measured 3 consecutive times were 4.41 mPas (concentration step 1), 3.85 mPas (concentration step 5), 2.55 mPas (concentration step 9), and 443.17 mPas (concentration step 13). An ANOVA with the independent variables stimuli and concentration steps and the dependent variable viscosity revealed that canola oil showed a trend for higher viscosity compared with canola oil spiked with oleic acid ($P = 0.070$), whereas the viscosity of all other stimuli differed significantly from each other (all $P < 0.001$). Moreover, a significant viscosity difference between the concentration steps ($P < 0.001$) was found. However, the differences between the stimuli and the concentration steps was based on an interaction effect between the measured stimuli and the measured concentration steps ($P < 0.001$). This effect was driven by concentration step 13 (Figure 3) in which the fat content was extrapolated to ensure a taste sensation for each participant. Accordingly, the viscosity at concentration step 13 was found to be significantly higher compared with the viscosity at concentration steps 1, 5, and 9 (all $P < 0.001$). In contrast, no significant difference between concentration steps 1, 5, and 9 was observed.

Concerning oral fat perception and sensitivity for primary taste modalities, no significant associations between fat detection thresholds and the taste intensity ratings for sucrose and sodium chloride were found, nor did hyper- and hyposensitive participants differ significantly in their ratings on labeled magnitude scales.

Discussion

This study compared detection thresholds for oleic acid, paraffin oil, canola oil, and canola oil spiked with oleic acid presented in

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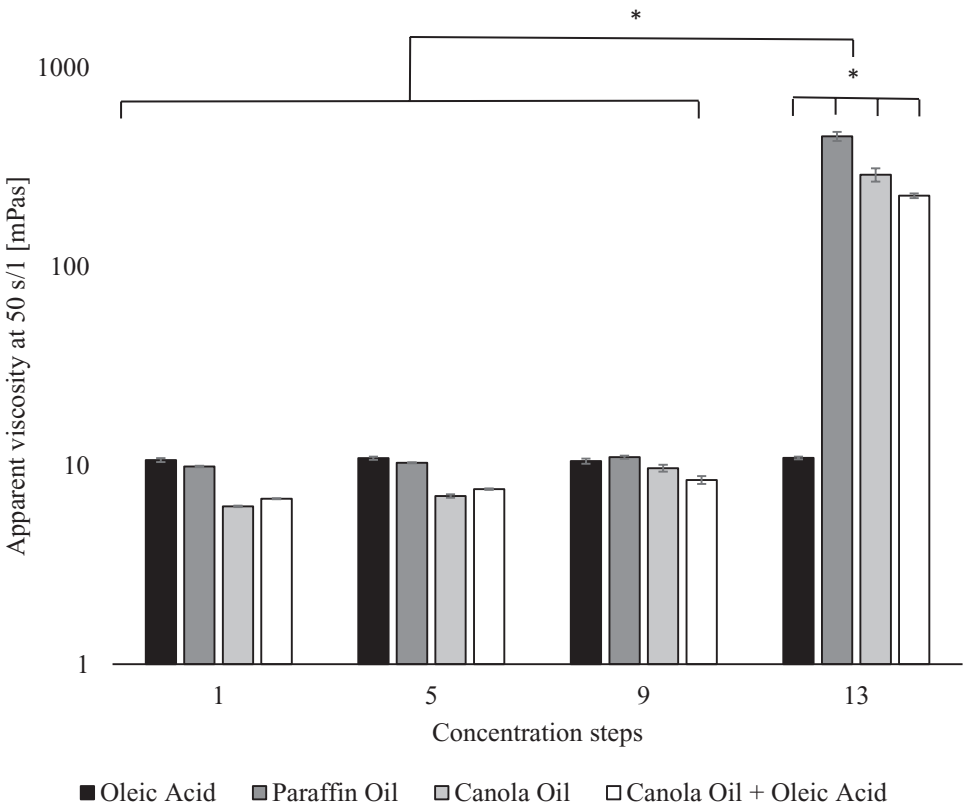


Figure 3. Viscosity differences between the measured concentration steps and the 4 stimuli. The mean apparent viscosity at 50 s^{-1} is depicted on a logarithmic scale. An interaction effect between stimuli and concentration steps was observed ($P < 0.001$) which was mainly driven by the highest fat content that was added in concentration step 13. No significant difference between concentration steps 1, 5, and 9 was found. The viscosity between the 4 stimuli differed significantly based on the differences observed at concentration step 13.

emulsions in a repeated measurements design to examine possible associations between detection thresholds for the different fatty stimuli. The results of this study revealed that the higher the concentrations of FFAs in the stimulus, the lower the individual fat detection threshold. As expected, the FFA oleic acid was perceivable at significantly lower concentrations than the fatty stimuli canola oil + oleic acid, canola oil and the fat-like stimulus paraffin oil. Moreover, the results of this study show that detection thresholds for canola oil + oleic acid are also significantly lower compared to detection thresholds of canola oil on its own. In comparison to paraffin oil detection thresholds, detection thresholds for canola oil were significantly lower. Additionally, it was observed that participants being more sensitive for oleic acid, were able to detect significantly lower concentrations of canola oil + oleic acid and canola oil, whereas there was no difference in paraffin oil detection thresholds.

Dietary fat can be perceived because of its textural properties (Tomaschunas et al. 2012; Sonne et al. 2014) but also because FFAs evoke unique bitter, pungent, and fatty taste signals (Mattes 2009a; Stewart et al. 2010; Galindo et al. 2012;Running et al. 2015). The effective stimuli to interact with specific receptors located in taste buds on the tongue surface and to evoke those taste signals have been identified as FFAs (Gilbertson et al. 2005; Gilbertson et al. 2010; Mattes 2011). However, the concentration of FFAs in the food supply is generally low and fat is mainly consumed in form of TAGs (Lawson 1995) which can be hydrolyzed by lingual, gastric, and pancreatic lipases to FFAs and glycerol. In rodents lingual lipases seem to be the dominant form, whereas in humans gastric lipases have been identified to be the predominant form (Denigris et al. 1988). Potentially, this oral enzyme is not necessary for the digestive breakdown of fats to enable absorption into enterocytes but rather for interactions with taste receptors to initiate digestive processes such as the peripheral release of gastric and pancreatic lipases. Although it was shown that lingual lipase might not always be active (Kulkarni and Mattes 2014), studies have reported that human lingual lipase activity ranged between 1–12 µmol fatty acids min/L (Stewart et al. 2010) and that different activity levels affected human fat sensitivity (Voigt et al. 2014). Moreover, the tasteless lipase inhibitor orlistat only increased detection thresholds for the TAG triolein but not for the FFA oleic acid (Pepino et al. 2012). In general, oleic acid was perceivable at significantly lower concentrations than triolein (Pepino et al. 2012; Voigt et al. 2014). The results of our studies are consistent with this finding. We observed that oleic acid detection thresholds were significantly lower than detection thresholds for paraffin oil, canola oil and canola oil + oleic acid. This outcome was expected since the oleic acid concentrations that were based on an established procedure (Haryono et al. 2014) were already much lower than the concentrations needed for detection of canola oil and paraffin oil as determined in pilot studies. Nevertheless, oleic acid was detectable at very low concentrations that are comparable to the low concentration also present in dietary oils (1–2%) (Gunstone and Norris 1983; Koriyama et al. 2002). One reason which might explain why canola oil detection thresholds are significantly higher than oleic acid detection thresholds could be the composition of FFAs. In canola oil a mixture of FFAs can be present, whereas in oleic acid only this long-chain fatty acid is available. Previous studies have shown that long chain polyunsaturated fatty acids compared to saturated or short

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chain fatty acids have greater affinity for some fatty acid receptors (Hirasawa et al. 2005; Galindo et al. 2012). Furthermore, in a recent study the effect of adding FFAs of varying degree of saturation to liquid chocolate was examined. It was reported that the polyunsaturated FFA linoleic acid reached rejection of the FFA-infused chocolate at lower concentrations than the monounsaturated FFA oleic acid (Running et al. 2017). Hence, apart from the chain length, the degree of saturation might also affect affinity for fatty acid receptors or lingual lipase and this might explain why oleic acid results in stronger taste signals than canola oil.

Apart from the FFA-stimulus oleic acid, we also observed that the stimulus rich in FFAs and TAGs, canola oil spiked with 3.80 mM oleic acid, was detectable at significantly lower concentrations than the TAG-rich stimulus canola oil. This observation can partly be explained by the presence of 18 participants that were hypersensitive for oleic acid and had a mean oleic acid detection threshold below 3.80 mM. We predicted that these hypersensitive participants would identify the lowest concentration of canola oil + oleic acid, whereas hyposensitive participants (mean oleic acid detection threshold ≥3.80 mM) were unlikely to be sensitive for the added oleic acid. The detection thresholds for canola oil + oleic acid in hyposensitive participants were expected to be comparable to canola oil detection thresholds. Indeed, we observed a peak at the lowest concentration of canola oil + oleic acid but also detection thresholds spreading into the highest concentrations used. For hyposensitive participants, significant correlations between detection thresholds for canola oil, oleic acid and canola oil + oleic acid were found. Additionally, hyposensitive participants were significantly less sensitive for canola oil compared with hypersensitive participants on 2 of the 3 measurements. These observations lead to the assumption that humans that can detect low concentrations of FFAs are also more sensitive for fats rich in TAGs. Whereas hypersensitive participants might experience more taste sensations evoked by FFAs, hyposensitive participants might be more dependent on textural cues that increase with higher fat content. The reason why some individuals are hyper- or hyposensitive for fats cannot be explained in this study. Based on the results of previous studies, it can be assumed that fat sensitivity can differ because of varying factors. Among others, increased lingual lipase activity might lead to increased fat sensitivity (Voigt et al. 2014), genetic differences in taste receptors can affect the taste for fat (Keller et al. 2012; Sayed et al. 2015), but also the fat content in the diet can influence fat detection thresholds (Stewart and Keast 2012b; Newman et al. 2016) (for a review see Heinze et al. 2015).

Paraffin oil and canola oil were chosen as stimuli in this study to evaluate fat perception based on textural cues. The fat-like stimulus paraffin oil, a mixture of hydrocarbons, cannot be hydrolyzed by human lipases. Hence, it can only be perceived by textural attributes such as oiliness or viscosity of the samples. In contrast, the TAGs in canola oil can be hydrolyzed by human lipases into glycerol and fatty acids. Thus, the amount of FFAs can be elevated within the oral cavity which might increase taste sensations evoked by an interaction between taste receptors and FFAs. If TAG-rich fats would be predominately perceived over textural features, we expected to find similar detection thresholds for paraffin oil and canola oil. In general, TAGs are more viscous than FFAs (Valeri and Meirelles 1997). With increasing viscosity fluids become thicker and the perception of textural cues can increase. Therefore, TAG-rich fats might be more likely to be discriminated due to textural attributes than fat rich in FFAs. However, when comparing samples high or low in viscosity, Le Calve and colleagues observed that fat perception can differ from viscosity differences. Whereas participants didn't perceive a difference between samples of 15% and 10% fat, respectively, with a viscosity difference of 8 mPas, a difference between the 15% fat and a 7.5% fat sample with a viscosity difference of 9 mPas was noticed (Le Calve et al. 2015). In the current study a viscosity difference of 2.55–4.41 mPas was observed between the mean of the 4 stimuli when the fat content varied between 0.03% and 11.22 + 0.119% fat. However, at the highest fat concentration containing 5.631% (oleic acid with 5% paraffin oil, see 2.3 Fat Detection Thresholds) and 50.119% fat (50% canola oil spiked with 0.119% oleic acid), the viscosity difference between the stimuli was 443.17 mPas. Accordingly, an interaction effect between concentration steps and stimuli was found to affect the viscosity of the samples. In line with our results, it was reported that with increasing fat content, viscosity differences increase as well and are more likely to be detected (Tomaschunas et al. 2012; Le Calve et al. 2015). Whereas the fat content in samples with low viscosity could be increased or decreased by 50% without affecting fat discrimination, the fat content in samples high in viscosity was detected at much lower concentration differences (Le Calve et al. 2015). Subsequently, in the current study it was expected that those participants that needed higher fat concentrations to detect the stimuli were more likely to refer to textural cues than to taste sensations, especially for canola oil and paraffin oil that were comparable in their viscosity at room temperature for fat concentrations between 0.30–11.22% (Figure 3). Nonetheless, canola oil was detectable at significantly lower concentrations than paraffin oil. Giving the fact that lipases can hydrolyze TAGs in canola oil and liberate FFAs, it is likely that additional taste sensations facilitate the perception of canola oil in comparison to paraffin oil and are predominant over textural features. This assumption can be supported by the finding of participants being hypersensitive for oleic acid were also more sensitive for canola oil compared with hyposensitive participants whereas no difference between both groups was found for paraffin oil detection thresholds.

Considering the important question of which fat attributes might be the major contributors that are perceived in the human oral cavity our results are in line with previous studies (Pepino et al. 2012; Voigt et al. 2014). By examining detection thresholds for different fat types, the results of the current study support the theory that FFAs are the major contributors in fat perception. Although dietary fat can also be perceived via textural cues as paraffin oil detection thresholds have shown, the presence of FFAs seems to facilitate fat discrimination and leads to lower detection thresholds with increasing FFA-concentration, as was shown in the significant differences between detection thresholds of canola oil, canola oil spiked with oleic acid and oleic acid.

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