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Proton Transfer Reaction Mass Spectrometry and Time Intensity Perceptual Measurement of Flavor Release from Lipid Emulsions Using Trained Human Subjects

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ABSTRACT: The effect of the fat component of liquid emulsions on dynamic "in-nose" flavor release was examined using a panel of trained human subjects ($n = 6$), proton transfer reaction mass spectrometry (PTR-MS), and time intensity (TI) sensory evaluation. A rigorous breathing and consumption protocol was developed, which synchronized subjects' breathing cycles and also the timing of sample introduction. Temporal changes in volatile release were measured in exhaled nostril breath by real-time PTR-MS. Corresponding changes in the perceived odor intensity could also be simultaneously measured using a push button TI device. The method facilitated accurate examination of both "preswallow" and "postswallow" phases of volatile release and perception. Volatile flavor compounds spanning a range of octanol/water partition coefficient ($K_{o/w}$) values (1−1380) were spiked into water (0% fat) or lipid emulsions with various fat contents (2, 5, 10, and 20% fat). Replicate samples for each fat level were consumed according to the consumption protocol by six subjects. Statistical comparisons were made at the individual level and across the group for the effects of changes in the food matrix, such as fat content, on both pre- and postswallow volatile release. Significant group differences in volatile release parameters including area under the concentration curve (AUC) and maximum concentration (I_{max}) were measured according to the lipid content of emulsions and volatile $K_{o/w}$. In a second experiment, using single compounds (2-heptanone, ethyl butanoate, and ethyl hexanoate), significant decreases in both in-nose volatile release and corresponding perceived odor intensities were measured with increasing fat addition. Overall, the effect of fat on in vivo release conformed to theory; fat had little effect on compounds with low $K_{o/w}$ values, but increased for volatiles with higher lipophilicity. In addition, significant pre- and postswallow differences were observed in AUC and I_{max}, as a result of changing fat levels. In the absence of fat, more than half of the total amount of volatile was released in the preswallow phase. As the content of fat was increased in the emulsion systems, the ratio of volatile released postswallow increased compared to preswallow. These data may provide new insights into why low-fat and high-fat foods are perceived differently.

KEYWORDS: flavor release, PTR-MS, time intensity, fat/lipid, emulsion, low-fat, obesity

INTRODUCTION

An important part of the sensory impression of eating is determined by the amount and rate of aroma released from the food matrix. In this regard, it is well-known that fat can have an effect on the temporal release of volatile compounds and their subsequent perception.[1−7] The unique textural and mouthfeel characteristics of fat are also associated with high hedonic value;[8−10] overconsumption of pleasurable high-fat foods is one factor contributing to the rising tide of global obesity. The food industry can play a role in reducing the fat content of manufactured foods; however, creating low-fat food products with full-fat flavor remains a considerable challenge.[11−14] In a multiphase system or an emulsion, depending on lipophilicity, aroma volatile compounds are partitioned to a greater or lesser extent in the fat phase, which can act as an aroma "sink". This reduces the release of lipophilic volatiles into the headspace of the food and their subsequent perception.[2] When fat is removed from the food matrix, lipophilic aroma compounds are released in a rapid burst that fades quickly, which has been associated with "unbalanced" flavor perception.[1,2,15] In the quest to find new technologies to reduce or replace fat in foods without compromising flavor, a better understanding of the fundamental in vivo dynamics of volatile release and concomitant perception using a standardized approach is required. This paper discusses recent experiments using proton transfer reaction mass spectrometry (PTR-MS) and a panel of human subjects to measure the effect of lipid emulsions on volatile flavor release and perception.

Flavor Release. The amount, timing, and rate of volatile compounds released from a food matrix are critical to create the characteristic sensory impression of a food. Food aroma is complex, normally composed of a mixture of volatile compounds that vary in their volatility (vapor pressure) and lipophilicity. Both these and other factors, such as mass transfer in the liquid and gas phases, as well as changes in surface area over time during mastication and swallowing affect the in-mouth temporal release of volatiles.[16,17] The fat component in food acts as a solvent or sink for lipophilic flavors, affecting both the amount and timing of release from the food matrix.[18,19] For volatile compounds with octanol/water partition coefficients ($K_{o/w}$) > 1, release from the food matrix generally decreases with increasing fat content.[1−5] As the $K_{o/w}$ values of volatile compounds increase, the degree of partitioning into the fat phase increases. Very hydrophilic compounds ($K_{o/w} < 1$) are generally unaffected by the presence of fat. In a fat-containing system, the amount of volatile release is

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Table 1. Physicochemical Properties of the Seven Volatile Aroma Compounds Used in Experiments[a]

	C	molar concn	$K_{o/w}$	MW (g/mol)	bp (°C)	VP (Pa at 25 °C)	m/z M + H$^+$
2-butanone	4	2.80×10^{-4}	1	72	79	11875[b]	73
2-pentanone	5	2.35×10^{-4}	6	86	102	4718[c]	87
2-heptanone	7	1.77×10^{-4}	73	114	151	514[c]	115
2-octanone	8	1.60×10^{-4}	234	128	175	187[b]	129
2-nonanone	9	1.44×10^{-4}	1380	142	195	27[d]	143
ethyl butanoate	6	1.89×10^{-4}	80	116	121	1510[b]	117
ethyl hexanoate	8	1.52×10^{-4}	641	144	168	215[b]	145

[a] Carbon chain number (C), molar concentration based on 25 mg/L added to water or emulsion (mol/L), octanol/water partition coefficient ($K_{o/w}$), molecular weight (MW), boiling point (bp), vapor pressure (VP), measured ion by PTR-MS (m/z M + H$^+$). [b] Corvarrubias-Cervantes et al.[42] [c] Rathbun and Tai.[33] [d] Voilley et al.[43]

defined by the air/product partition coefficient ($K_{a/p}$), which is directly related to the air/water partition coefficient ($K_{a/w}$) and inversely related to $K_{o/w}$, where ϕ_o is the phase volume of oil (eq 1). Equation 1 predicts that as ϕ_o decreases, for compounds with $K_{o/w} > 1$, the rate of volatile release in the air phase will increase.

$$K_{a/p} = \frac{K_{a/w}}{\phi_o(K_{o/w} - 1) + 1} \quad (1)$$

Furthermore, $K_{a/w}$ is also related to the vapor pressure. Complex models may be applied to the mass transfer of volatiles.[20] When the volatility of a compound is low, the mass transfer is gas-phase limited, but when the volatility of a compound is high, the release is limited by the contact area between the liquid and air phases. In vivo mass transfer phenomena are complex and require sophisticated modeling because of interacting factors such as the mucus layer lining the mouth and airways.[21]

In Vivo Measurement of Volatile Flavor Release. Real time techniques such as atmospheric pressure chemical ionization mass spectrometry (APCI-MS) and PTR-MS have been applied to the measurement of in vivo volatile release.[1−3,7,8,22−24] Most researchers measure retronasal release indirectly through "in-nose" measurement of exhaled volatiles through the nostrils. During mastication and swallowing, odorants are transported from the oral to the nasal cavity and receptors in the olfactory epithelium.[22] In vivo methods allow the most accurate indication of food interaction with the human olfactory system. Ideally the sensory impression can be simultaneously measured using a technique such as time intensity, which characterizes continuous perceived odor intensity over the consumption period. The objectives of this paper were (i) to develop a rigorous standardized breathing and consumption protocol using a panel of human subjects interfaced with in-nose PTR-MS measurement, (ii) to apply the protocol to measure pre- and postswallow volatile release and simultaneous aroma perception in lipid emulsions, and (iii) to better understand the in vivo effects of lipids on volatile release and perception in simple emulsion systems. The consumption protocol was designed to mainly understand differences in flavor release due to the food structure/composition (i.e., fat content) and to minimize the influence of idiosyncratic subject differences.

MATERIALS AND METHODS

Flavor Chemicals. Five homologous odor-active methyl ketones and two ethyl esters commonly found in foods and beverages were sourced from Firmenich (Balgowlah, Australia). Methyl ketones are often formed in lipid-rich foods such as mold-ripened and hard cheeses (Parmesan, Cheddar).[25] Esters are potent odor compounds present in a wide range of common foods and beverages. The volatile compounds selected represented a range of physicochemical parameters, such as $K_{o/w}$, boiling point (bp), and vapor pressure (VP) (Table 1). The aroma volatile compounds were diluted in food grade ethanol such that a 5−20 μL aliquot could be added to bulk phase samples to reach an appropriate final concentration (1−50 mg/L).

Lipid Solutions. Oil-in-water solutions were made by diluting a commercially available soybean oil based emulsion, IVELIP 20% (Baxter Health, Old Toongabbie, Australia). This commercial lipid emulsion has a tightly controlled formulation and is packaged sterile, making it suitable for human consumption. The variation of the product was small, and the stability of the emulsion ensured consistency for cross-experimental purposes. The emulsion had a Sauter mean diameter, $D_{3,2}$, of 0.25 μm, and a volume moment mean diameter, $D_{4,3}$, of 0.3 μm. The lipid emulsion was diluted with deionized water to achieve a series of lipid contents, 0, 2, 5, 10, and 20%. The emulsion did not undergo phase separation at any dilution. A 5 μL aliquot of the volatile mixture was added to a 10 mL volume of emulsion in a plastic 20 mL syringe (Terumo Corp., Macquarie Park, Australia) and capped and equilibrated in a refrigerator at (4 °C) overnight prior to experiments.

PTR-MS Conditions. Volatile release was measured using a high-sensitivity quadrupole model PTR-MS (IONICON Analytik GmbH, Innsbruck, Austria). The design and operation of the PTR-MS have been comprehensively described elsewhere;[26] however, a brief description is provided. PTR-MS is based on gas-phase chemical ionization, specifically proton transfer reactions, normally with H_3O^+ as the primary reactant ion. Primary reactant ions [H_3O^+] are generated from water vapor and passed into a drift tube; volatile organic compounds (VOCs), such as aroma volatiles, are introduced at a fixed rate into the drift tube and may undergo a proton transfer reaction, producing an RH^+ ion. Normal air components do not react; however, proton transfer reactions occur readily for VOCs with a proton affinity greater than that of H_2O. Most VOCs undergo ionization with minimal fragmentation; RH^+ ions generated in this way are analyzed by a conventional quadrupole mass detector. Because fragmentation is limited, target protonated ions can be measured in real time at microgram per liter concentrations. These characteristics make PTR-MS ideal for in vivo measurement of breath volatiles.

The sample headspace gas was drawn through the inlet tubing at a rate of 400 mL/min., with 15 mL/min drawn into the reaction chamber of the PTR-MS instrument. The transfer tubing was held at 60 °C, the reaction chamber was held at 70 °C (2.19 mbar), and the drift tube voltage was set at 600 V. Initial experiments indicated that the fresh emulsion did not contain volatile compound ions corresponding to the target mass/charge ratios (m/z). Preliminary experiments indicated that the methyl ketones did not undergo significant fragmentation; however, some fragmentation was observed for the esters; this was consistent with

the findings of others.[27] The PTR-MS was used in the multiple ion detection (MID) mode, and the following ions were measured; protonated water isotope ($H_3{}^{18}O^+$; m/z 21), water cluster ($H_3O^+ \cdot H_2O$; m/z 37), acetone (m/z 59) and the volatile ions listed in Table 1. Target volatiles were all measured with a dwell time of 50 ms, and the system was programmed to measure the full range of target volatiles every 500 ms, that is, two scans per second.

In Vivo Volatile Measurement. Approval to use human subjects in the PTR-MS experiments was obtained from the CSIRO low-risk ethics committee. Six members were recruited from the trained sensory panel for in vivo experiments. Five females and one male were selected on the basis of previous participation in PTR-MS in vivo pilot studies and successful past performance in descriptive sensory panels for various products. Exhaled volatiles were measured by fitting a sterile, disposable, soft-plastic medical nasal cannula (Mayo Healthcare, Roseberry, Australia) into the nostrils of each subject and connecting it to the transverse flow of the PTR-MS inlet via a ~1 m length of PEEK tubing (i.d. = 0.4 mm). A new cannula was used for each subject. Pilot experiments confirmed that volatile loss or carry-over was not significant using the disposable cannula (data not shown).

Breathing Regulation. Potential variation in data obtained in human "in nose" flavor release experiments relates to individual differences in the timing and rate of breathing. To minimize this variation, subjects were trained to follow a strictly regulated breathing protocol, based on a visual animation developed for this purpose. The animation was developed using Flash CS2 (Adobe, Chatswood, Australia), such that precisely timed inspiration and expiration cycles were displayed by way of an animated graphic of an expanding and contracting disk lasting 1.5 s for a total of 3 s for each breathing cycle. The animation included a 10 s countdown, with the numbers 10 through 1 displayed on screen, before displaying a series of 25 precisely timed numbered breathing cycles. After five "presample" inspiration and expiration cycles (15 s), the animation indicated when the sample was introduced into the subjects' mouth. During the sixth breathing cycle the liquid emulsion sample was taken into the mouth (by way of a preloaded syringe) and held in the mouth with swishing and jaw movement for a further 10 breathing cycles (30 s, "preswallow" phase) without swallowing. Finally, the sample was swallowed, and the subjects were instructed to breathe for a further 10 cycles (30 s, "postswallow" phase). Hence, each complete experiment consisted of 25 breathing cycles, totaling 75 s duration.

Time Intensity Measurement. Time intensity (TI) measurement of real time changes in the perceived intensity of odor stimulus (simultaneous with the in-nose volatile release measurement) was achieved by way of a button-activated joystick (Olfactory Intensity Device (OID), Gerstel, Mühlheim an der Ruhr, Germany). The OID was connected to the analogue input slot (AI 421) of the PTR-MS quadrupole. The 1 V output was converted to a digital signal, which was acquired in a dedicated channel in the PTR-MS software together with volatile data. The panelists were instructed to familiarize themselves with the device and trained in the amount of pressure required to fully depress the button (100%, maximum intensity), lower levels (75, 50, and 25%), and no perceived stimulus (0%). After acquisition, the TI data were exported into Excel (Microsoft) for further manipulation and statistical analysis.

Time Intensity Training Experiment. Subjects were asked to familiarize themselves with the odor of an aqueous ethyl butanoate solution (10 mg/L, presented in a sealed plastic cup) and describe the odor. As expected, all of the subjects could readily perceive and describe the compound; descriptors such as "fruity", "strawberry", and "bubblegum" were used. Subjects were then presented with a water blank and then a series of four ethyl butanoate aqueous solutions made up in sealed plastic cups (1, 10, 20, and 50 mg/L). Pilot testing of the solutions with four staff members indicated that these concentrations represented a good range from mild to strong odor. Samples were labeled with a random code. Subjects were instructed to rank the solutions in increasing intensity after taking a sip and swirling it around in the mouth. After completion of the ranking task, subjects were then given a demonstration of the TI device and asked to familiarize themselves with the use of it. After taking a mouthful (10 mL) of the 1 mg/L solution, subjects were instructed to rate the intensity using the TI device after being informed that this was the lowest intensity solution (low-concentration anchor). Subjects were then given a 10 mL volume of the 50 mg/L solution (high-concentration anchor) and asked to rate the intensity using the TI device. After palate cleansing with plain crackers and filtered water, subjects were given a mandatory 15 min break. After the break, the subjects were set up for PTR-MS in-nose measurements. Subjects were presented with four replicate samples of each of the four concentrations (1, 10, 20, and 50 mg/L) given in randomized order, with only a ~30 s break between replicates. A 2 min break was given between each concentration level, with palate cleansing. The "training experiment" was continued until all 16 samples were completed within the 2 h session.

Time Intensity in Lipid Emulsions Experiment. The aroma impact of single volatiles at a specific time was assessed during emulsion consumption. Two compounds of similar intermediate lipophilicity, 2-heptanone ($K_{o/w}$ = 73) and ethyl butanoate ($K_{o/w}$ = 80), as well as a more lipophilic ester, ethyl hexanoate ($K_{o/w}$ = 641), were used. The volatile compounds were added to the emulsions at a concentration of 50 mg/L; preliminary experiments were performed to ascertain a reasonable concentration threshold for retronasal perception in the maximum fat sample, 20%. At a lower concentration (e.g., 25 mg/L) it was difficult to perceive the volatile compounds in 20% fat emulsions against the mild background aroma of the emulsion. The odor of 2-heptanone was described by subjects as "varnish" and "blue-cheese-like", and the two esters were given "fruity", "wine", and "sweet" descriptors. It was hypothesized that the more highly lipophilic ester compound, ethyl hexanoate, would be released less in a lipid-containing system, leading to a lower sensory impression, or TI rating. Six subjects were presented with three replicates of each of the five fat levels (0, 2, 5, 10, and 20%).

Volatile Release in Lipid Emulsions Experiment. Water or emulsion samples were spiked with the seven flavor volatiles, each at a final concentration of 25 mg/L in the samples. A clear volatile signal for each compound could be measured at this concentration by the PTR-MS at all fat contents in the emulsions, and the combined overall odor was not too strong or unpleasant for subjects. Replicate volumes of 10 mL of spiked sample were drawn into labeled sterile 20 mL plastic syringes and capped and refrigerated overnight for use in experiments on the following day. The spiked samples were presented in randomized order; however, all replicates (n = 4) for a particular fat level were given sequentially. Volatile data were measured for the replicate samples at each fat level with only a 30 s break between each experiment, with no opportunity for palate cleansing. After completing all samples for a given fat level, subjects were then required to consume a plain cracker and drink at least 20 mL of filtered water to remove any residual fat carry-over that may have affected release. After a further 5 min pause, the next series of replicates were performed. All experiments were performed within a single 2 h session.

Data Processing and Statistical Analysis. Volatile and TI release curves are normally defined by three key parameters: area under the concentration curve (AUC) during a defined time interval, a measure of overall release and release rate; the maximum concentration reached or I_{max}; and also the time to reach I_{max}, which is T_{max}. Volatile concentration (μg/L) was calculated by the PTR-MS software according to the method of Lindinger et al.[26] The PTR-MS data files were imported into Excel (Microsoft). TI data were recorded on a 0−1 V scale. Raw data were multiplied by 100 to express as a percentage value. Time−concentration and TI data were then imported into Excel. The Excel "MAX" and "LOOKUP" functions were used to find maximum intensity (I_{max}) and time to maximum (T_{max}) values for the "preswallow" (15−45 s) and "postswallow" (45−75 s) periods. The timing of pre- and

Table 2. Subject Mean (n = 16) Breath Acetone (m/z 59) Total Area Under the Curve (AUC) Data Measured during the "Time Intensity Training Experiment" (Training) and Mean (n = 15) Total AUC Acetone Data for the "Time Intensity in Lipid Emulsions Experiment" (Lipid) for the Six Subjects[a]

	total AUC m/z 59		pre-AUC m/z 117		post-AUC m/z 117		total AUC m/z 117	
subject	training	lipid	training	lipid	training	lipid	training	lipid
1	14093 ± 3.2%	14067 ± 3.8%	565	612	250	552	816	1164
2	16968 ± 4.5%	15257 ± 4.6%	2708	5349	532	2317	3240	7666
3	23534 ± 3.8%	23835 ± 3.5%	401	1716	95	920	496	2636
4	17642 ± 9%	15257 ± 4.6%	365	331	492	1841	857	2172
5	14153 ± 5.3%	12755 ± 4.7%	595	1063	291	1303	885	2366
6	24820 ± 4.2%	14543 ± 5.9%	249	722	78	697	327	1419
correlation				0.96		0.89		0.96
p value				0.002		0.019		0.003

[a] Corresponding subject mean preswallow (pre), postswallow (post) and total AUC data for ethyl butanoate (m/z 117) released during the training experiment (n = 16) and the lipid experiment (n = 15). Correlation between release parameters for both sets of data and associated P value. Experiments were performed 1 week apart. Acetone AUC data were used to assess reproducibility of breathing throughout experiments. AUC for ethyl butanoate was used as an index of "relative release".

postswallow T_{max} varied considerably between subjects and replicates for a given fat level. No attempt was made to smooth or preprocess volatile or TI data before analysis. A rectangular integration function (IGOR Pro Software, WaveMetrics Inc., Lake Oswego, OR) was used to calculate volatile and TI AUC values during the preswallow period (15−45 s), the postswallow period (45−75 s), and also the total AUC, 0−75 s. Individual replicate release curve data parameters (pre- and postswallow AUC, I_{max}, and T_{max}) were analyzed by multivariate analysis of variance (MANOVA) to determine the significance of single effects such as fat content, subject, and volatile compound and interactions when appropriate (Genstat, version 13, VSN International, Hempstead, U.K.). Least significant difference (LSD) values were calculated by the software at the 5% significance level for the appropriate effect or interaction. The product moment correlation (r) and estimation of significance (two-sided t test against zero) of the relationship between data sets were performed using the correlation function in Genstat. Average volatile release curves and TI profiles were obtained by plotting the average data for each time point; hence, I_{max} values read directly from curves may not correspond with statistically determined values presented in data tables.

RESULTS AND DISCUSSION

Breathing Regulation and Panel Performance. An objective of the present study was to establish a generic regulated breathing protocol for use in "in-mouth" release experiments to examine mainly the group average effect of differences in food structure and composition on volatile release. Furthermore, the objective was to minimize idiosyncratic differences in breathing and chewing and to facilitate statistical analysis. To this end, acetone, a normal volatile component of human breath, was monitored during all in-nose experiments, in addition to the target flavor volatiles, as a marker for breathing rate. Acetone was assumed to be the only positively charged volatile ion with a mass-to-charge ratio (m/z) of 59 present on the breath. The absence of any interference volatiles with m/z 59 in the IVELIP solution was confirmed by PTR-MS. Breath acetone was used as a marker to monitor the effectiveness and reproducibility of the breathing regulation protocol. The average of (n = 16) breath acetone (m/z 59) measured throughout the time intensity training experiments for each individual subject was plotted. The group mean (n = 96) acetone profile was also calculated (data not shown). Comparison of individual and group mean acetone profiles indicated that all subjects were able to breathe according to the regulated timing protocol; each individual trace indicated clearly defined breath cycles with good alignment of the peaks and troughs. Some small perturbations from the ideal regular frequency were observed after the liquid sample bolus was introduced; however, the regular breathing cycle was quickly re-established (data not shown). Assuming minimal drift in the concentration of baseline breath acetone throughout the experimental session, the subjects showed a high degree of reproducibility in terms of depth of inspiration and expiration from breath to breath.

Table 2 contains data corresponding to average AUC for total measured breath acetone obtained during two experiments; the "time intensity training" experiment and the "time intensity in lipid emulsion" experiment for ethyl butanoate (described in a later section) for the full duration of the experiments (75 s). The two experiments were separated by 1 week. Mean AUC values for acetone for each subject together with percentage deviation are shown in Table 2. The total AUC was highly reproducible within an experiment for each subject, generally with a percent coefficient of variation (% CV) < 5, indicating that the subjects were able to adhere to the timing protocol and breathe a similar volume of air for each experiment; that is, the methodology was highly reproducible from experiment to experiment. Because the amount of aroma volatile released on the breath is dependent to a large extent on the rate and volume of breathing, it was important to ensure that breathing was consistent. Individual acetone total AUC data were similar in magnitude in both experiments, indicating that this endogenous breath volatile remained fairly constant for most subjects (subject 6 was an exception). It is known, however, that the breath acetone concentration can fluctuate from day to day within an individual depending on dietary[28,29] and physiological factors.[30] The primary purpose of measuring acetone was to establish that the breathing was consistent, that is, having a low % CV within a series of experiments on a given day.

To ascertain the "relative release" of each subject, the subject mean (n = 16) preswallow, postswallow, and total AUC for ethyl butanoate (m/z 117) (averaged across all four concentrations) in

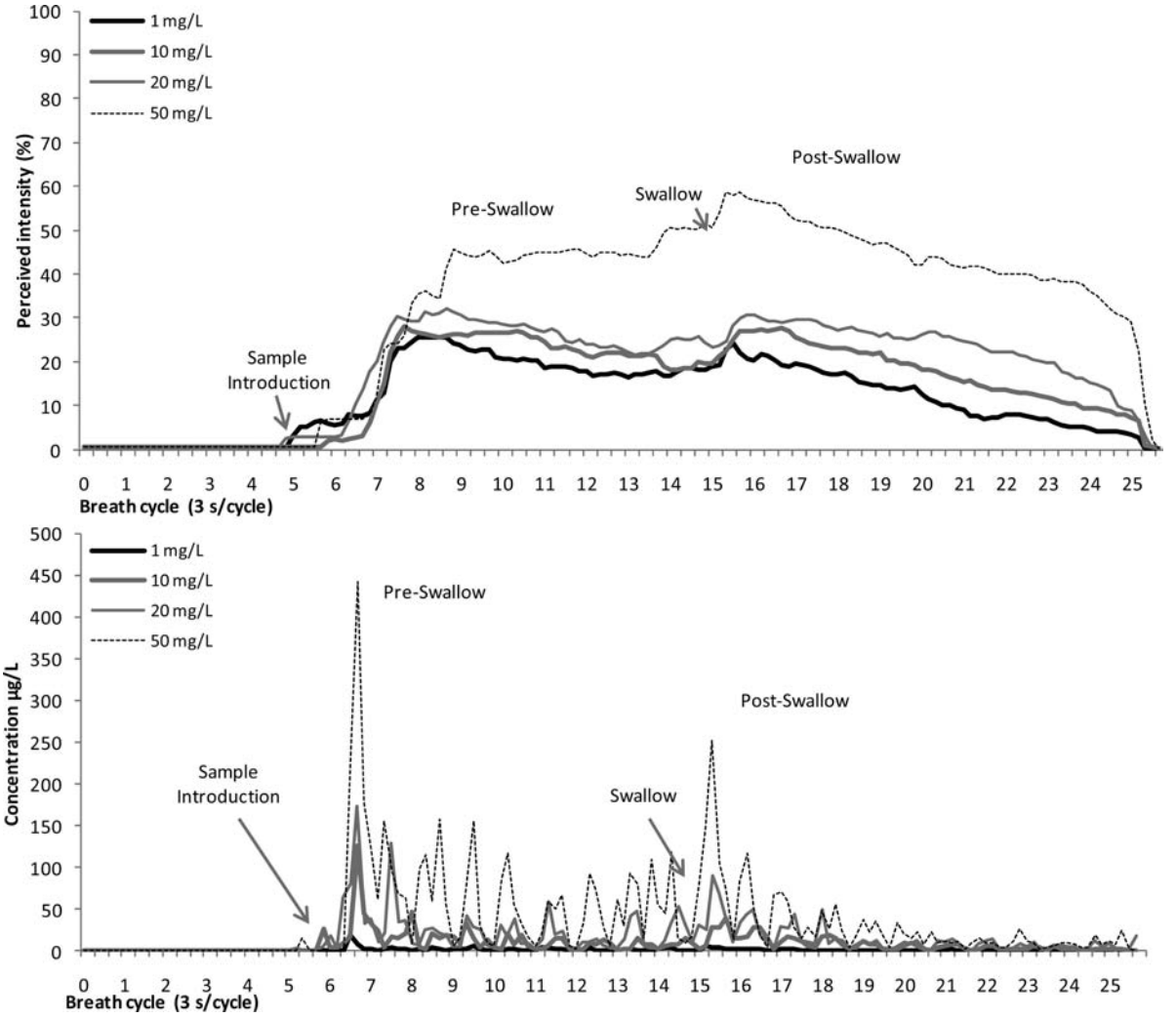

Figure 1. Time point group average (n = 24) time intensity profiles of perceived intensity (100% scale; top) and corresponding volatile profiles (bottom) of ethyl butanoate (m/z 117) in aqueous solutions at 1, 10, 20, and 50 mg/L. Clear I_{max} values were apparent during both the preswallow and postswallow periods for both the sensory and volatile data.

the "Time Intensity Training" experiment and the subject mean (n = 15) preswallow, postswallow, and total AUC (m/z 117) for the ethyl butanoate series from the "Time Intensity in Lipid Emulsions" experiment (averaged across all fat levels) were compared. The average pre- and postswallow and total AUC for each subject were of similar magnitude in each experiment and positively correlated (Table 2), indicating a degree of consistency in the "relative release" across time for individuals, although subject 2 clearly had quite high leverage as a consistently "high releaser". The relative release data for ethyl butanoate in both sessions was not perfectly consistent; however, subject 6 was always a "low releaser" and subjects 4 and 5 were "intermediate releasers". The remaining subjects, 1 and 3, varied somewhat but could be rated a "low" and "intermediate" releasers on the basis of the lipid emulsion release data. Depending on the experiment, subject 2 released between approximately 6- and 10-fold higher ethyl butanoate compared to subject 3. Similar large interindividual differences in the amount of volatile release have been reported by other groups.[22−24,31] Although not expected, there was not a positive correlation between the magnitude of ethyl butanoate (m/z 117) released and endogenous breath acetone.

Time Intensity Training. All six subjects were able to correctly rank the aqueous solutions of ethyl butanoate in order of increasing concentration after taking a sip of the liquid. This indicated that clear differences in the magnitude of the retronasal olfactory stimulus could be perceived within the concentration range proposed for use in PTR-MS time intensity measurements.

Group-average TI and volatile (m/z 117) profiles (n = 24) for ethyl butanoate registered two distinct preswallow and postswallow I_{max} events during the consumption protocol (Figure 1). A concentration-dependent response in both in-nose volatile release and perceived sensory stimulus was measured. Similar pre- and postswallow I_{max} events have been described in semi-solid and solid foods,[23,32] but generally not in thin liquids. Significant (p < 0.001) "concentration" effects were found for all release parameters (Table 3). The effect of concentration on T_{max} was not significant (data not shown); as the training system was aqueous, no differences in T_{max} were expected. Significant concentration-dependent increases in both volatile release and perception were measured. On average, there appeared to be a greater proportion of ethyl butanoate released in the preswallow phase compared to the postswallow phase. Group comparisons

indicated that volatile AUC and I_{max} values for the preswallow period were significantly higher than the postswallow period for the three highest concentration levels in the aqueous solutions ($p < 0.001$). At the individual level, the preswallow volatile AUC and I_{max} values were higher than postswallow values for five of the six subjects (individual data not shown). For the corresponding TI data, however, no clear pre- and postswallow differences for AUC or I_{max} were measured. A significant positive correlation between group mean volatile AUC data (m/z 117) and group mean sensory AUC data (TI) at the five fat levels was indicated: AUC preswallow (0.99, $p = 0.002$), AUC postswallow (0.97, $p = 0.009$), I_{max} preswallow (0.99, $p = 0.001$), and I_{max} postswallow (0.99, $p = 0.004$). A linear response of perceived intensity with an increasing in-nose concentration of ethyl butanoate was demonstrated on the group level. It was concluded from the training experiments that subjects could be calibrated to provide meaningful group data in further experiments.

Table 3. Group Mean (n = 24) Time Intensity and Volatile (m/z 117) Data Averaged across Six Subjects for 1, 10, 20, and 50 mg/L Aqueous Solutions of Ethyl Butanoate Used in the Training Experiment[a]

	1 mg/L	10 mg/L	20 mg/L	50 mg/L
Time Intensity Data				
AUC $_{preswallow}$	485	557	648 b	979 b
AUC $_{postswallow}$	408	561	751 b	1442 b
$I_{max\ preswallow}$	31 d	34	41 d	61 b
$I_{max\ postswallow}$	26	31	37 c	61 b
Volatile Data (m/z 117)				
AUC $_{preswallow}$	52	327 b,d	716 b,d	1816 b,d
AUC $_{postswallow}$	28	253	458	946
$I_{max\ preswallow}$	27	216 b,d	421 b,d	872 b,d
$I_{max\ postswallow}$	12	95	197	448 b

[a] The effect of concentration was significant ($p < 0.001$) on the time intensity and volatile parameters listed. AUC, area under the curve; I_{max}, maximum intensity. Least significant difference calculated for the effect of intensity and the comparison of pre- or postswallow data. b, significantly higher than previous concentration level; c, significantly higher than 1 mg/L; d, significantly higher preswallow compared to postswallow.

In-Nose Release and Time Intensity in Lipid Emulsions. In-nose TI experiments were performed for 2-heptanone, ethyl butanoate, and ethyl hexanoate in emulsions with various fat contents. The group mean TI and corresponding volatile data (m/z 145) for the ethyl hexanoate experiments are shown in Figure 2. Similar to the training experiment, two clear I_{max} events were measured in the preswallow and postswallow phases. Once again, a significant proportion of the volatile was released in the preswallow phase regardless of fat level. For both the TI and volatile data, AUC and I_{max} generally decreased as the fat content increased in the emulsions (Figure 2). A complete list of the

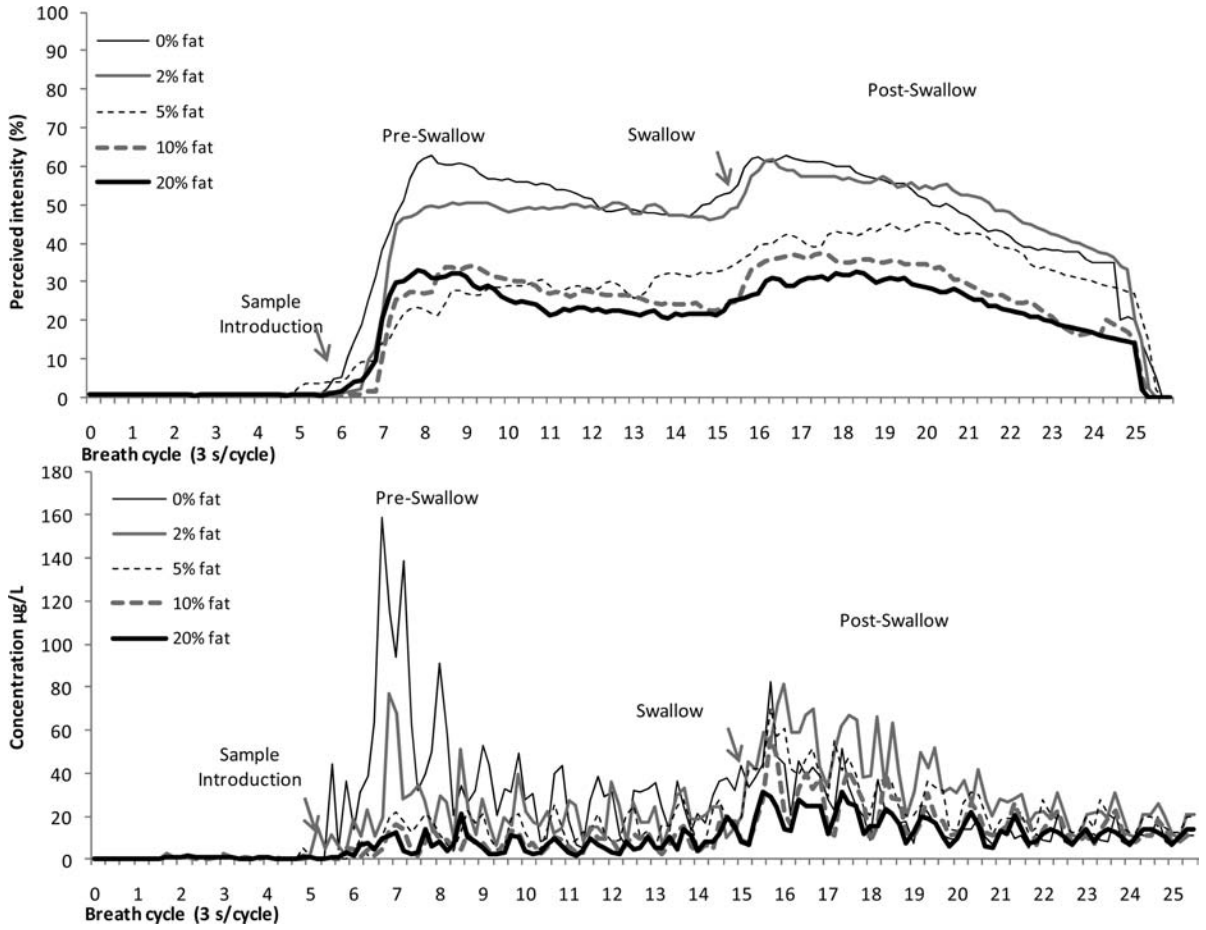

Figure 2. Time point group average (n = 18) time intensity profiles of perceived intensity (100% scale; top) and corresponding volatile profiles (bottom) of ethyl hexanoate (m/z 145) spiked at a concentration of 50 mg/L in 0, 2, 5, 10, and 20% fat emulsion systems. Clear I_{max} values were apparent during both the preswallow and postswallow periods for both the sensory and volatile data.

Table 4. Group Mean Data (n = 18) Averaged across Six Subjects Showing the Effect of Increasing Fat Level on Time Intensity (TI) and Volatile Release Data for 2-Heptanone, Ethyl Butanoate, and Ethyl Hexanoate[a]

	fat level					
	0%	2%	5%	10%	20%	p value
2-Heptanone						
TI Data						
AUC $_{preswallow}$	1358	893 a	911 b	890 b	663 a	<0.001
AUC $_{postswallow}$	1494	1024 a	962 b	918 b	1032 b,z	<0.001
$I_{max\ preswallow}$	71	48 a	49 b	50 b	39 a	<0.001
$I_{max\ postswallow}$	72	50 a	49 b	47 b	48 b	<0.001
Volatile Data						
AUC $_{preswallow}$	2923	1946 a	1243 b	1061 b	812 a	<0.001
AUC $_{postswallow}$	3409	2608 a,z	2160 b,z	2042 b,z	1856 b,z	<0.001
$I_{max\ preswallow}$	741	452	273	218	137	<0.001
$I_{max\ postswallow}$	670	496	359	314	260z	<0.001
Ethyl Butanoate						
TI Data						
AUC $_{preswallow}$	1466	880 a	659 a	645 b	664 b	<0.001
AUC $_{postswallow}$	1606	958 a	753 b	604 b	660 b	<0.001
$I_{max\ preswallow}$	72	48	36	34	92	ns
$I_{max\ postswallow}$	71	99	40	32	34	ns
Volatile Data						
AUC $_{preswallow}$	2412 z,x	2009 a,z,x	1793 a,z,x	1233 a,x	772 a,x	<0.001
AUC $_{postswallow}$	1109 x	1350 x	1320 x	1365 x	1207 z,x	0.037
$I_{max\ preswallow}$	1146 z,x	716 a,z,x	621 x	442 a,x	220 x	<0.001
$I_{max\ postswallow}$	395 x	448 x	405 x	414 x	280 a,x	0.036
Ethyl Hexanoate						
TI Data						
AUC $_{preswallow}$	1396	1237	724 a	693 b	651 b	<0.001
AUC $_{postswallow}$	1420	1482 z,x	1144 a,z,x	883 a,x	748 b	<0.001
$I_{max\ preswallow}$	74	67 a	47 a	40 a	44 b	<0.001
$I_{max\ postswallow}$	69	67	52 a	45 b	40 a	<0.001
Volatile Data						
AUC $_{preswallow}$	1073 z	643 a	422 b	230 b	205 b	<0.001
AUC $_{postswallow}$	570	1058 a,z	812 a,z	598 a,z	446 a,z	<0.001
$I_{max\ preswallow}$	381 z	179 a	109 b	56 a	51 b	<0.001
$I_{max\ postswallow}$	147	203	151 a	93 a	57 b	<0.001

[a] AUC, area under the curve; I_{max}, maximum intensity. Least significant difference calculated for the effects of fat and the comparison of pre- or postswallow data. p value (right-hand column) for the overall effect of fat on a given parameter. Across row comparisons: a, significantly lower than previous fat level; b, significantly lower than 0% fat sample. Down column comparisons: z, significantly higher in pre- vs postswallow comparison; x, significantly higher in ethyl butanoate vs ethyl hexanoate comparison.

group mean values for both the TI and volatile data for the three flavor compounds are listed in Table 4. The main effects of "fat", "'preswallow", "postswallow", "volatile", and "assessor" were all significant ($p < 0.001$) for volatile AUC and I_{max}. For the corresponding TI data, all main effects for AUC were significant ($p < 0.001$). For I_{max} only "fat" and "assessor" were overall significant ($p < 0.001$).

In most cases, corresponding step decreases in volatile AUC were measured as the fat content increased both pre- and postswallow (Table 4). The pre- or postswallow volatile AUCs were compared. For 2-heptanone the postswallow volatile AUC was higher than the preswallow AUC for all samples; however, the difference became significant and increased only after the addition of fat (Figure 3). An overall significant pre- and postswallow effect for the TI AUC data was also measured ($p < 0.001$), suggesting that the volatile differences were accompanied by a perceptual difference. Higher TI AUC was measured at all fat levels postswallow compared to preswallow; however, the difference was significant only at 20% fat addition. Significant correlations between group mean volatile (m/z 115) and group mean perceived stimulus (TI) data for the five fat levels were measured for all parameters: AUC preswallow volatile (0.92, p = 0.03), AUC postswallow (0.90, p = 0.04), I_{max} preswallow (0.92, p = 0.03), and I_{max} postswallow (0.89, p = 0.04).

For ethyl butanoate, the timing of swallow, pre or post, on volatile AUC was overall significant ($p < 0.001$); however, the differences between pre- and postswallow were not as large as for 2-heptanone, and a different trend was apparent. The preswallow volatile AUC was significantly higher at 0, 2, and 5% fat levels, but switched to a higher postswallow AUC beyond 10% fat addition (Figure 3). No significant differences were measured between the pre- and postswallow TI AUC data for ethyl butanoate. Positive correlations between group mean m/z 117 and group mean TI data at the five fat levels were found for AUC preswallow (0.77, p = 0.12) and I_{max} postswallow (0.56, p = 0.33); however, the trends were not significant.

For ethyl hexanoate, the effect of pre- or postswallow on volatile AUC was highly significant ($p < 0.001$); higher postswallow volatile AUC values were measured at all levels of fat, except for 0%. Higher perceived postswallow intensity was also reflected in the corresponding TI data (Figure 3) for the samples containing fat. Positive correlations between the group mean volatile release (m/z 145) and group mean perceived stimulus (TI) over the five fat levels were measured for all parameters: AUC preswallow (0.94, p = 0.02), AUC postswallow (0.68, p = 0.2), I_{max} preswallow (0.92, p = 0.03), and I_{max} postswallow (0.86, p = 0.06).

In summary, the effect of fat on volatile release was overall highly significant for I_{max} values for the three volatiles studied (Figure 2). Decreases in both pre- and postswallow volatile I_{max} were measured with increasing fat content (Table 4). Interestingly, as fat content increased, decreases in volatile I_{max} were generally more apparent during the preswallow phase compared to postswallow.

Decreases in the perceived TI I_{max} according to increasing fat were observed especially in the preswallow phase, although the effect of fat on TI I_{max} was not significant for ethyl butanoate (Table 4). Generally no differences were found when TI I_{max} preswallow and postswallow were compared; this was in broad agreement with the volatile I_{max} data. The purpose of including the two ethyl esters (ethyl butanoate vs ethyl hexanoate) was to test the hypothesis that the release of the more lipophilic ethyl hexanoate would be less in fat-containing emulsions with a corresponding lower perceived intensity by TI. Significantly higher ($p < 0.001$) AUC and I_{max} volatile data were measured for ethyl butanoate compared to ethyl hexanoate, as expected (see Figure 3). This was, however, not reflected in the corresponding TI data. The TI AUC for ethyl hexanoate (postswallow) was in fact higher than for ethyl butanoate. No differences in TI I_{max} were found between the two esters.

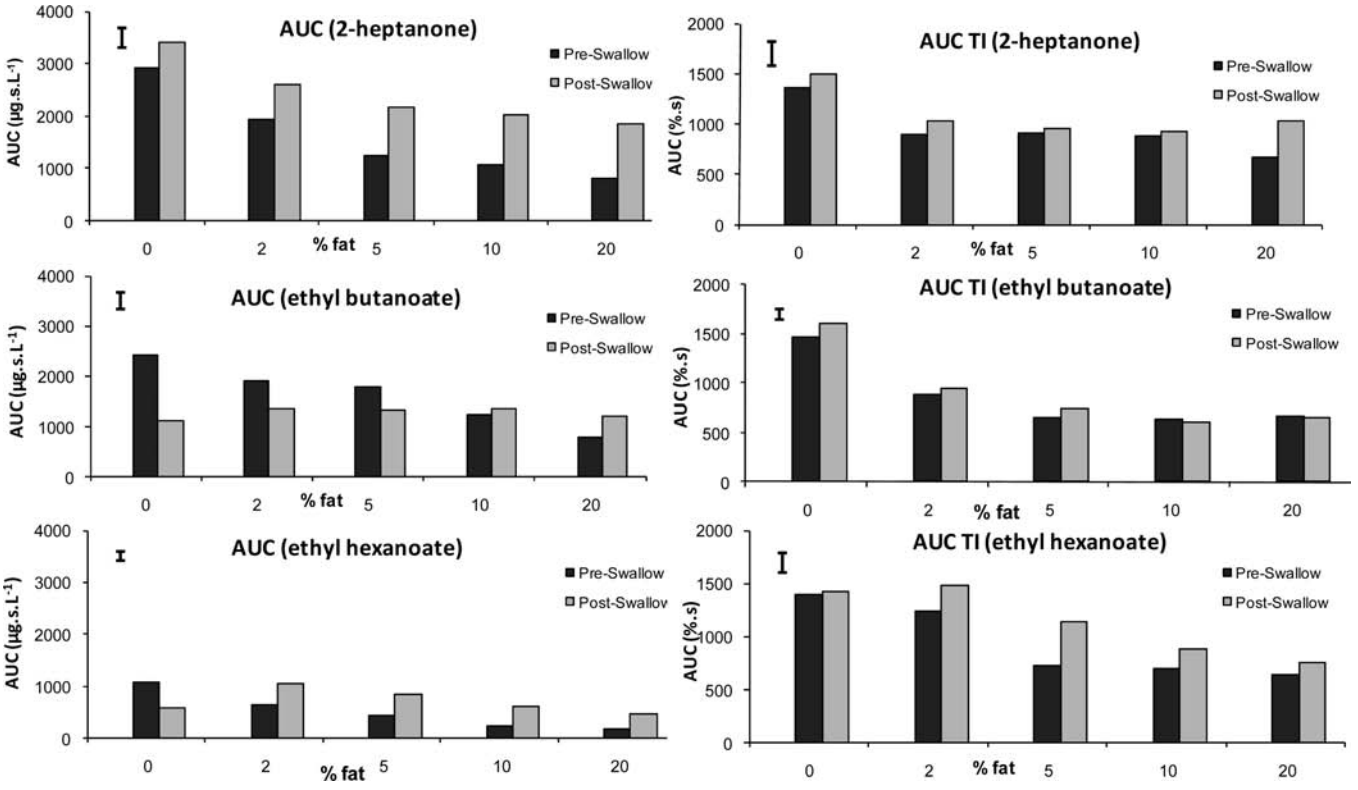

Figure 3. Graphical representation of group mean ($n = 18$) pre- and postswallow volatile and time intensity AUC data as a function of fat. Volatiles were spiked at a concentration of 50 mg/L. The LSD (represented by the bar) was calculated for the comparison of pre- and postswallow data at the 5% significance level.

No significant differences in pre- or postswallow volatile or TI T_{max} were measured for 2-heptanone, ethyl butanoate, or ethyl hexanoate (data not shown). In a previous study,[1] differences in T_{max} data were not found for volatiles as a result of increasing fat; however, differences in the corresponding perceived intensity were recorded.

In-Nose Volatile Release from Lipid Emulsions. The final experiment systematically examined the effect of increasing additions of fat on in-nose release across all seven flavor volatiles without the corresponding time intensity. Data from only five subjects were used in the statistical analysis as one participant (subject 2, "high releaser") was unable to complete the study. Group-mean volatile AUC and I_{max} data for the pre- and postswallow phases are summarized in Table 5. As for the previous studies, significant release during both the pre- and postswallow periods was measured. Comparisons for the effect of fat on each parameter can be made by reading down the data columns. For 2-butanone and 2-pentanone, the compounds with lowest lipophilicity, a trend in decreasing preswallow AUC and I_{max} values was measured with increasing fat. The decreasing preswallow trend was offset by increasing postswallow release; the total amount of 2-butanone and 2-pentanone (pre- and postswallow combined) did not change significantly, consistent with eq 1; compounds with low lipophilicity are not predicted to be affected by the presence of fat (eq 1). The differing distribution into pre- and postswallow phases as the fat content increased does indicate a significant interaction with fat, however. With increasing $K_{o/w}$ the in vivo AUC and I_{max} values were more strongly affected by the presence of fat, stepwise in accordance with increasing ϕ_o as predicted by theory. Similar observations have been demonstrated in vitro[3,5] and in vivo.[1,3]

Comparison of pre- and postswallow data indicated significantly higher group postswallow AUC at higher fat levels. For 2-butanone and 2-pentanone, the volatiles with the lowest $K_{o/w}$ values, as the fat level increased in the emulsions, the preswallow AUC decreased, whereas the postswallow AUC increased. Although the total AUC (pre- and postswallow AUC combined) remained similar, the relative partitioning pre- or postswallow systematically changed. As the $K_{o/w}$ value of volatile compounds increased, from 2-heptanone to 2-nonanone, the presence of fat had a more pronounced effect on release; the preswallow AUC and, to a lesser extent, the postswallow AUC decreased systematically as the fat content increased. Similar trends were also measured for the two ethyl esters. Of particular interest was the fact that generally the effect of fat on volatile release was most obvious in the preswallow phase compared to postswallow (Table 5); the differences in preswallow AUC and I_{max} between fat levels were on average more pronounced. The preswallow mean volatile data for each of the seven volatiles across the fat series are shown graphically in Figure 4 for ease of interpretation.

Figure 4 clearly shows that the AUC in water (no fat interaction) decreased with the increasing chain length for the methyl ketone series; 2-butanone was largest and 2-nonanone was lowest. This was also reflected in the I_{max} data. Rates of volatilization of ketones across the air/water interface using a two-film mass transfer model were described by Rathbun and Tai.[33,34] They demonstrated a direct relationship with vapor pressure and an inverse relationship with molecular weight consistent with the decreasing PTR-MS response with increasing chain length. Furthermore, the molar concentration of the reactive aldehyde moiety decreased with increasing chain length (Table 1), further decreasing the PTR-MS response from 2-butanone to 2-nonanone.

Table 5. Influence of Increasing Fat Level on In-Nose Release for Seven Volatiles[a]

		AUC		I_{max}	
volatile	% fat	preswallow	postswallow	preswallow	postswallow
2-butanone	0	974	1189	391	326
	2	1015	1293 z	452	358
	5	794	1382 z	443	377
$K_{o/w}$ = 1	10	806	1458 z	237	364
	20	589	1573 z	358	372
p value (fat)		ns	ns	ns	ns
2-pentanone	0	553	601	249	174
	2	465	650	299	194
	5	468	739 z	257	229
$K_{o/w}$ = 6	10	474	726 z	177	191
	20	317	770 z	136	185
p value (fat)		ns	ns	ns	ns
2-heptanone	0	435	380	211	116
	2	267 a	382	168	107
	5	219 b	395 z	114	107
$K_{o/w}$ = 73	10	164 a	346 z	49 b	76
	20	125 b	335 z	43 b	65
p value (fat)		0.008	ns	<0.001	ns
2-octanone	0	299	241	139	66
	2	155 a	251 z	72	55
	5	116 b	245 z	40	46
$K_{o/w}$ = 234	10	69 b	204 z	19	34
	20	82 b	190 z	17	26
p value (fat)		<0.001	ns	<0.001	<0.001
2-nonanone	0	156	120	65	25
	2	81 a	146 z	20 a	21
	5	56 b	127 z	11 b	16 b
$K_{o/w}$ = 1380	10	40 b	108 z	6 b	13 b
	20	37 b	92 z	6 b	9 b
p value (fat)		<0.001	0.003	<0.001	<0.001
ethyl butanoate	0	220 z	129	169 z	67
	2	128 a	114	134 z	52
	5	93 b	125	53 b	61
$K_{o/w}$ = 80	10	58 b	122 z	50 b	49 b
	20	80 b	123	47 b	36
p value (fat)		<0.001	ns	<0.001	ns
ethyl hexanoate	0	166 z	63	131 z	34
	2	56a	83	38 a	26
	5	43	83	31 b	23 a
$K_{o/w}$ = 641	10	24	76 z	11 b	19 b
	20	29	68 z	9 b	12 b
p value (fat)		<0.001	ns	<0.001	0.006

[a] Group mean data (n = 20) averaged across five subjects. Least significant difference calculated for the effects of fat and the comparison of pre- or postswallow data. Down column comparisons for the effect of fat: a, significantly lower than the previous fat level; b, significantly lower than 0% fat. Across row comparisons of pre- and postswallow phases: z, significantly higher pre- or post swallow.

Finally, differences in T_{max} as a result of fat were examined for each volatile individually across subjects. No preswallow differences in volatile T_{max} were measured for any of the seven volatiles. Postswallow fat effects on volatile T_{max} were measured for only the most lipophilic volatiles: 2-nonanone (p = 0.04) and ethyl hexanoate (p = 0.04). In both cases T_{max} was highest at 20% fat and lowest at 0% fat, with an increasing trend with increasing fat (data are not shown). There was a high degree of variation in the position of pre- and postswallow T_{max} within as well as between subjects for a given fat level; hence, limited differences were found for volatile T_{max}. These observations are consistent with data reported by others.[6,8,23] Malone et al.[1] observed a significant delay in perceived 2-heptanone intensity, without obvious changes in volatile T_{max}.

Proportionality of In-Nose Release. The release data from each of the five subjects were averaged for each of the seven volatiles across the fat levels with the purpose of obtaining a single index of the consistency of "relative release ratio" of each individual. The mean data for each volatile for each subject were scaled as a percentage compared to the release of 2-butanone in a 0% fat solution, the volatile of maximum release for each subject in all cases. Table 6 shows the scaled release ratios of the six volatiles compared to 2-butanone in water. Very consistent ratios were obtained across the five subjects for both AUC and I_{max}, although, as previously described, the absolute magnitude of release varied considerably across subjects. These findings are in direct agreement with Delahunty and Guilfoyle.[35]

Consistency of Volatile Release Across Subjects. The mean (n = 20) total, pre-, and postswallow volatile release for each subject for ethyl butanoate, ethyl hexanoate, and 2-heptanone averaged across all the fat levels (0, 2, 5, 10 and 20%) in the time intensity experiments is shown graphically in Figure 5. Importantly, the data corresponding to each volatile were obtained in separate experiments performed on different days, unlike the volatile data discussed in the previous section. The relative proportion of pre- and postswallow release was remarkably consistent for each subject for the three volatiles. Moreover, significant positive correlations between the total AUC for each volatile in the time intensity experiments and the total AUC release data (ethyl butanoate in water) obtained in the training experiment (Table 2) were found across the six subjects: ethyl butanoate (0.96, p = 0.002), 2-heptanone (0.96, p = 0.002), and ethyl hexanoate (0.94, p = 0.005). The total amount of release and the proportion of pre- and postswallow release remained relatively consistent across subjects for each experiment, regardless of the food composition (aqueous solution or emulsions of various fat compositions).

Evaluation of the Consumption and Measurement Protocol. In reality, a liquid food remains in the oral cavity for only a short amount of time (~1−3 s) before swallowing. Hence, the current protocol is experimental; subjects were required to hold the liquid in their mouth for 10 breath cycles (30 s) before swallowing. In other studies, similar or more prolonged preswallow periods[23,24] or slower breathing cycles have been reported.[18] This experimental eating protocol is intended to be used with solid foods in the future; a 30 s preswallow period was determined to be sufficient time for efficient mastication of most (solid) samples.

It has been demonstrated that much of the reported interindividual variation in in vivo release is due to how often the velum opens during the preswallow phase; most studies are based mainly on mastication of semisolid and solid foods.[23,36] In the

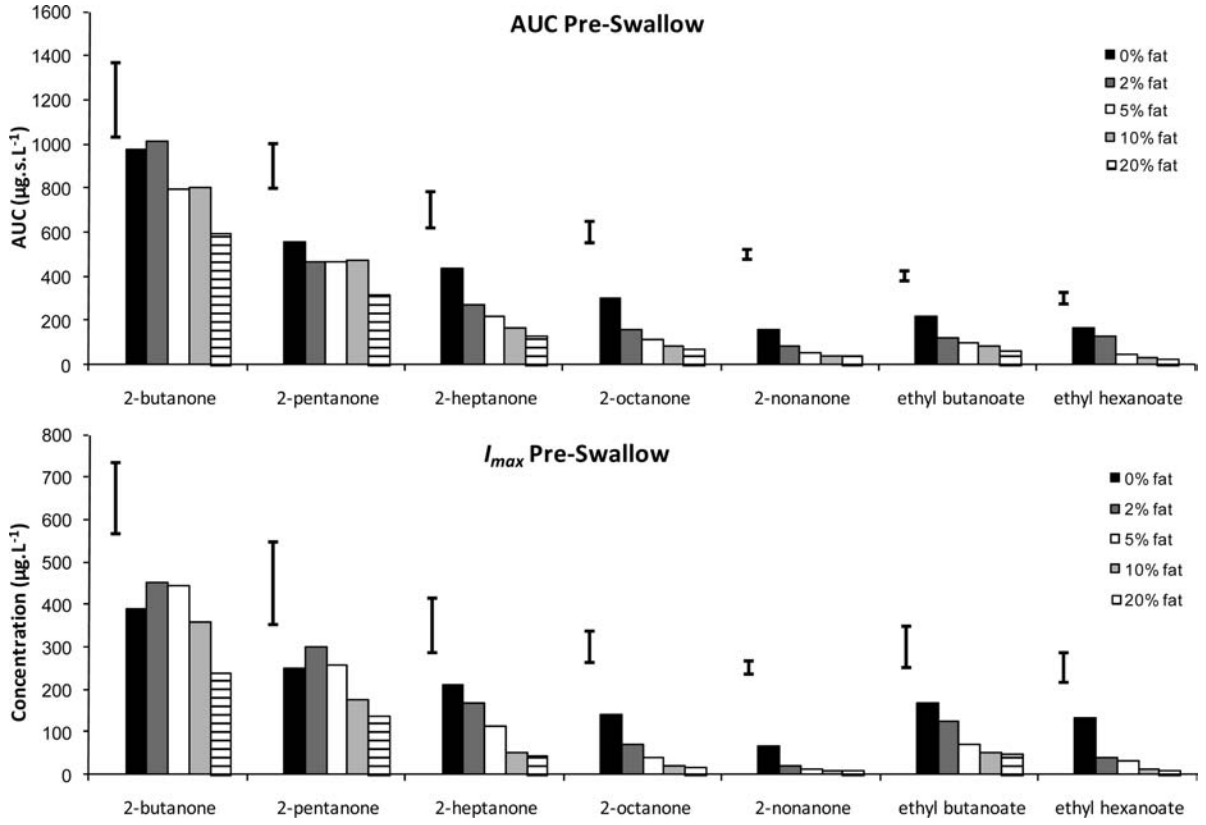

Figure 4. Graphical representation of group mean ($n = 20$) preswallow volatile AUC and I_{max} data for each of the seven volatiles (25 mg/L) as a function of fat. The LSD (represented by the bar) was calculated for the effect of fat at the 5% significance level.

Table 6. Individual Subject Mean ($n = 20$) Total AUC and Pre- and Postswallow I_{max} Data for Each Volatile Averaged across All Fat Levels (0, 2, 5, 10, and 20%) from the Volatile Release in Lipid Emulsions Experiment[a]

	subject				
	1	3	4	5	6
			AUC		
2-butanone	100	100	100	100	100
2-pentanone	53	52	54	47	54
2-heptanone	25	28	31	24	28
2-octanone	16	18	19	14	16
2-nonanone	9	11	9	8	8
ethyl butanoate	9	9	15	9	10
ethyl hexanoate	5	6	8	5	5
			I_{max}		
2-butanone	100	100	100	100	100
2-pentanone	50	54	59	55	62
2-heptanone	23	26	33	25	31
2-octanone	11	14	16	12	14
2-nonanone	4	5	6	4	5
ethyl butanoate	13	15	25	15	21
ethyl hexanoate	5	6	12	7	9

[a] For AUC and I_{max}, the proportionality remained very constant across individuals. The data were adjusted to the volatile of maximum AUC and I_{max} (always 2-butanone in water) and are expressed as a percentage proportion of 2-butanone release.

case of liquid foods, the transport of aroma from the oral to the nasal cavity is thought to be limited before swallowing, compared to solid foods, not just because of the shorter residence time of liquids in the mouth. It has been argued that during consumption of a liquid, in most individuals, closure of the velopharynx inhibits significant retronasal transport of odorant until the swallowing event.[37−39] In some cases subjects, such as wine judges, have been specifically trained to perform conscious "buccal-pumping" or "velum-opening"[22] to allow opening of the velum and retronasal transport. In a larger study of 14 subjects, approximately half were shown to release volatiles from soft gels before swallowing.[23,24] In the current study the majority of subjects (five of six subjects) released a greater proportion of total volatiles in the preswallow phase with thin aqueous solution. Although based only on a limited number of subjects, it was the case that without any specific training in conscious velum opening or buccal pumping, some normal subjects were able to easily release a substantial proportion of volatiles in an extended preswallow phase, with mouth movement. It has been shown that the normal up and down movement of the jaw results in intermittent opening of the velum−tongue border;[37] this then seemed to apply to most of the subjects using the current oral processing protocol.

The orthonasal odor thresholds of ethyl butanoate and ethyl hexanoate have been reported as 1.71 and 3.3 μg/L, respectively,[40] in a deodorized food matrix. The higher threshold of ethyl hexanoate and the lower volatile release (AUC) compared to ethyl butanoate was not reflected in the TI data for the same. A lower TI AUC was expected for ethyl hexanoate. Similarly, the relationship between volatile release and perceived sensory stimulus was less clear for ethyl butanoate, suggesting that subjects may

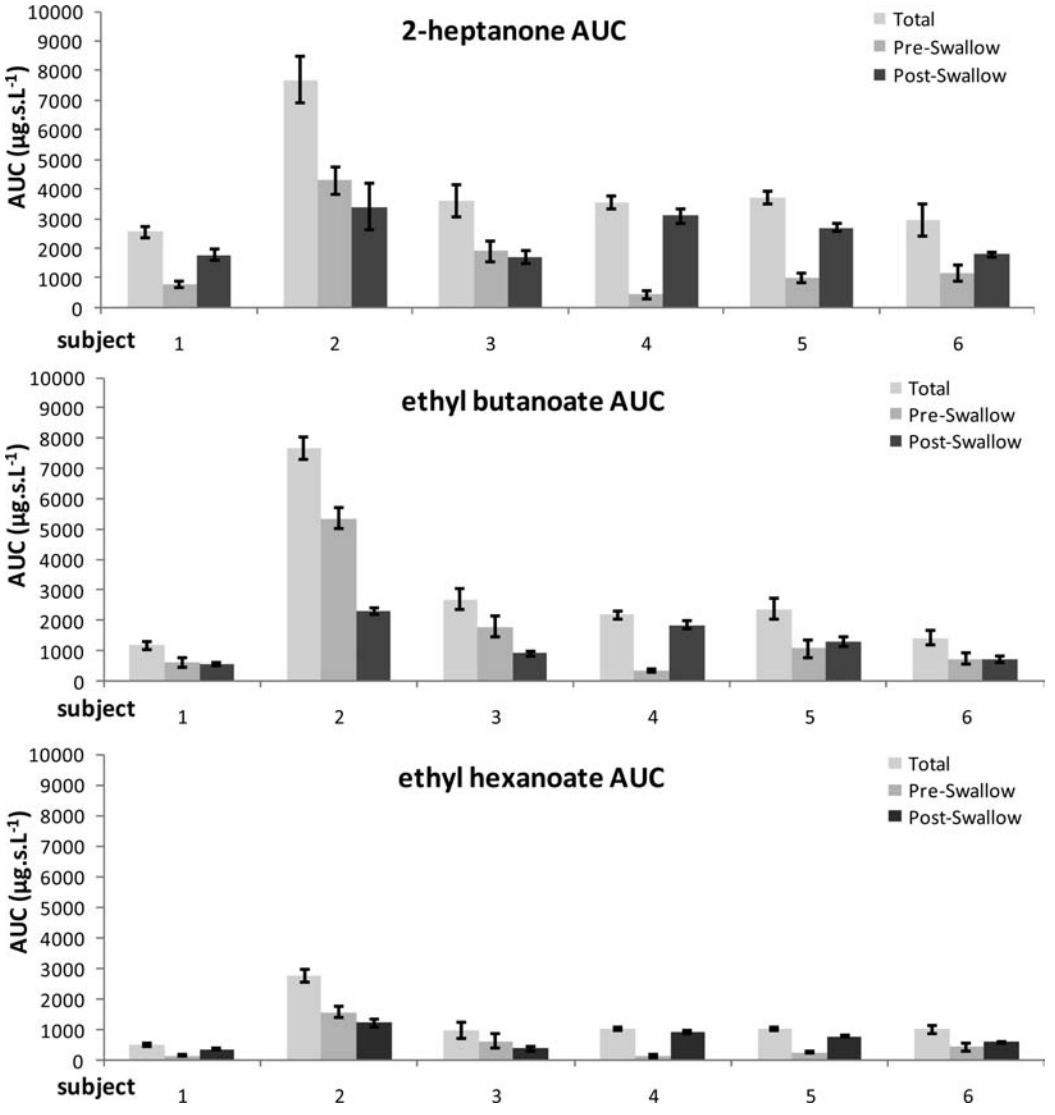

Figure 5. Graphical representation of mean subject ($n = 20$) total pre- and postswallow volatile AUC for 2-heptanone (top), ethyl butanoate (middle), and ethyl hexanoate (bottom) averaged across all fat levels (0, 2, 5, 10, and 20%). The bars represent the standard error. The overall release and relative pre- and postswallow ratios of release remained very constant for each subject.

have had greater difficulty clearly identifying the odor quality of ethyl butanoate in the emulsion matrix compared to that of ethyl hexanoate. The discrepancy from the expected behavior also suggests that the sensitivity of the time intensity method employed may require further refinement and training to be able to make comparative ratings across related volatiles in lipid matrices. Sensory perception is a dynamic process; temporal changes in a stimulus (flavor, odor, texture) are important and probably are likely to be more characteristic than static attribute evaluations;[41] dynamic methods of sensory analysis such as TI, however, require more training. In any case, correlations between volatile release and perceived intensity were obtained for 2-heptanone and ethyl hexanoate, with overall less release and sensory stimulus demonstrated with increased fat addition.

Effect of Fat on Pre- and Postswallow Release. With the addition of fat, the ratio of pre- to postswallow release shifted significantly; however, in all cases a large amount of the total volatile release still occurred in the preswallow phase. As fat was added to the emulsions, the ratio of volatile release changed in the direction of postswallow; this effect became more apparent with increasing $K_{o/w}$ for the volatile compound and may have important implications for the influence of fat generally on perception. For compounds with limited fat solubility (2-butanone and 2-pentanone), the total amount of volatile released did not substantially change with fat addition, but the relative distribution of release into pre- and postswallow phases changed. Removal of fat from products often leads to harsh unbalanced aroma perception. The current data support the idea of an initial preswallow "burst" of aroma in the absence of fat. Under normal conditions, upon ingestion of a liquid bolus, the residence time in the mouth preswallow is on a per-second time scale. After swallowing, the time until the next bolus may vary, but would typically also be on a per-second time scale. It is unknown whether the same observed relative partitioning of volatiles, pre- and postswallow, as a consequence of fat addition, would occur on the shorter time scales occurring during typical oral processing of liquids and whether the brain is able to consciously or unconsciously detect such differences. It is hypothesized that if such differences do occur during

normal consumption of liquids, and assuming that the difference can be perceived, the pre- and postswallow release ratio may be responsible for some of the fundamental differences in flavor perception between low- and full-fat-containing systems and, may, at least in part, be responsible for the "smoother", more "balanced", perception often reported in foods with added fat.[2]

In general, the relationship between preswallow release and perceived stimulus was stronger than postswallow, suggesting that this phase of the eating experience in liquid foods may be quite important for some people. Further experiments with a greater number of subjects are required before generalizations on the effects of fat in liquid foods can be made. It also remains to be proven that these pre- and postswallow differences occur on time scales relevant to normal consumption of liquid foods.

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