Oral fat exposure alters postprandial lipid metabolism in humans\textsuperscript{1-3}

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ABSTRACT  Accumulating evidence indicates that oronasal sensory stimulation influences nutrient metabolism. This work examined the effects of oral exposure to dietary fat on postprandial plasma triacylglycerol (triglyceride) concentrations. Fifteen (six male, nine female) healthy adults were exposed to each of four treatments presented in random order. After ingestion of a 50-g load of safflower oil in capsules (to preclude oral exposure to the fat), they masticated and expectorated 1) crackers with cream cheese, 2) crackers with nonfat cream cheese, 3) crackers alone, or 4) nothing. Blood samples were collected at baseline and 2, 4, and 6 h after load ingestion. Sensory discrimination tests were conducted with the cream cheese samples after these sessions. Oral exposure to the full-fat cream cheese led to a significantly greater area under the plasma triacylglycerol curve than did the other treatments ($P < 0.05$). The increment was attributable to both a significantly higher peak concentration and a more enduring elevation ($P < 0.05$). The oral stimuli were not ingested (so did not add to the load), subjects were not aware of the macronutrient composition of the cream cheese samples (thereby eliminating cognitive effects), and subjects could not distinguish between the cream cheese samples in sensory tests (minimizing a sensory influence). Consequently, these data suggest that there is a chemosensory or tactile mechanism in the oronasal region of humans for detecting some aspect of the chemical composition of dietary fat, or a component derived from or carried in fat, that elicits a change in postprandial lipid metabolism. Am J Clin Nutr 1996;63:911–7.

KEY WORDS  Dietary fat, oral sense, cephalic phase, lipid, triglyceride, triacylglycerol, humans, postprandial period

INTRODUCTION

Oronasal sensory stimulation elicits an array of rapid, neurally mediated, digestive, endocrine, thermogenic, cardiovascular, and renal responses (1–6). The health implications of these "cephalic phase" responses have not been established, but accumulating evidence suggests the sensory properties of foods that trigger them may exert influences beyond their contribution to food selection and quality of life (7, 8). The increasing use of fast-modified products by the population is of interest in this regard because such items disassociate the sensory attributes commonly associated with dietary fats from their metabolic effects. Thus, to the extent that sensory stimulation with fats in the oronasal region primes the body to respond to an anticipated lipid load, low-fat products containing fat mimics may elicit metabolic responses disproportionate to the actual fat content of the items. The principal issue addressed in this study was whether oral exposure to dietary fats influences postprandial lipid metabolism in humans.

Our primary focus was on plasma triacylglycerols (triglycerides) because previous work with rats suggests this plasma constituent is markedly influenced by oronasal stimulation. Intragastric feeding with oil leads to a more rapid rise and fall of triacylglycerol relative to the pattern observed with oral ingestion of the same load (9). Further, oronasal exposure to a nutritionally inconsequential amount of corn oil (0.3 mL) just before intragastric lipid loading results in a prolonged elevation of triacylglycerol compared with concentrations after oral stimulation with water (10). The attributes of dietary lipid stimuli responsible for the observed effects on triacylglycerol metabolism are not known. The mouthfeel imparted by dietary fat is one potential feature addressed in the present work by comparing responses to oral stimuli differing along this dimension (cream cheese on a cracker compared with the cracker alone). The presence of triacylglycerol or other components of complex dietary fat sources may also be critical. This was assessed by comparing responses to full-fat and nonfat cream cheese. These same comparisons permit a partial determination of a cognitive influence because study participants were not aware of the fat content of the samples. Constituents carried by fats and the chemical nature of the fats are other possible signals (11), but were not addressed in this work.

An improved understanding of the potential for dietary oronasal fat exposure to enhance postprandial lipemia is clinically important. Elevated triacylglycerol concentrations are associated with increased very-low-density-lipoprotein and intermediate-density-lipoprotein concentrations, both of which have atherogenic and cytogenic potential (12, 13). The elevation of plasma triacylglycerol in previous animal studies was marked and persisted for hours postprandially. Whether oronasal factors promote concentrations in plasma of the forms of lipoproteins that increase atherogenic risk in humans warrants consideration.

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This work also has methodologic implications for conducting and interpreting oral fat challenge tests. Studies in rats show that the degree of oral exposure to fat alters the postprandial lipid profile (11, 14). Thus, because the variety of vehicles (eg, capsules, milkshakes) and routes (eg, oral, intravenous) of lipid delivery used in human studies would result in different levels of oronasal stimulation, results across studies may not be comparable. Further, sensory interactions could be important. Recent studies indicate that the addition of sucrose (15, 16) or fructose (15, 17) to an experimental high-fat load leads to an augmented rise in triacylglycerol concentration relative to that noted without the sweetener. The effect has been attributed to the fructose moiety because similar results are not obtained with glucose (15). However, on a molar basis, glucose is substantially less sweet than either fructose or sucrose (18) and studies with rats reveal that oral exposure to saccharin will promote a prolonged elevation of triacylglycerol (10). Thus, it is possible that the sweet taste alone may account, at least in part, for the phenomenon. These findings indicate that the sensory properties (taste, texture, hedonic appeal) of foods and beverages used in fat-challenge tests should be standardized across subjects and studies to permit appropriate interpretation of the findings. This work begins to explore the sensory attributes of high-fat oral stimuli that may influence the metabolic response they elicit.

SUBJECTS AND METHODS

General protocol

Participants were informed that the purpose of the study was to evaluate how the digestion of fat is influenced by the amount ingested. They reported to a private area in the hospital at 0800 after fasting from 2200 the previous day. A baseline blood sample was drawn from an antecubital vein followed by ingestion of a lipid load. The load was provided as 50 1-g capsules of safflower oil (Nature’s Plus, Melville, NY) with 150 mL water (consumed in its entirety within 10 min). The use of capsules permitted delivery of the gastric load without intubation or exposure of the oral cavity to dietary lipid. Subjects remained seated throughout the 6-h test session and no additional fluids or foods were ingested during that time. Additional blood samples were drawn 2, 4, and 6 h postingestion.

Four experimental conditions were administered in random order to all participants: 1) no oral stimulation, 2) oral stimulation with cracker pieces (Original Premium Saltine Crackers; Nabisco Foods, East Hanover, NJ), 3) oral stimulation with nonfat cream cheese (Philadelphia Free, Kraft Foods, Glenview, IL) on crackers, and 4) oral stimulation with full-fat cream cheese (Philadelphia brand; Kraft Foods) on crackers. The crackers weighed ≈0.75 g and the cream cheese portions were ≈3.25 g/cracker. Each sample of the full-fat cream cheese on a cracker contained 62 kJ, 1.2 g fat, 0.3 g protein, and 0.7 g carbohydrate, whereas the nonfat cream cheese samples contained 26 kJ, 0.1 g fat, 0.8 g protein, and 0.5 g carbohydrate. The nonfat cream cheese did not contain a fat substitute. The samples were presented every 5 min during the first hour and every 15 min for the second hour (16 samples, total). Samples were masticated for ≈1 min and expectorated. Weights of each stimulus were obtained before and after mastication. Test sessions were conducted ≥ 3 d apart. After completion of the four test sessions, participants returned to the research center twice for sensory testing to determine whether the nonfat and full-fat cream cheese stimuli were distinguishable by their sensory properties.

This protocol was approved by the Committee on Studies Involving Human Beings at the University of Pennsylvania.

Subjects

Participants were recruited by public advertisement. They were 15 (6 male, 9 female) healthy adults who were taking no medications and not adhering to any special diet. Their mean (± SD) age was 27.8 ± 4.7 years and body mass index (BMI; in kg/m²) was 27.3 ± 9.6 (range: 16.8–48.6).

Sensory testing

During the first sensory test session, a triangle test (19) was administered with the nonfat and full-fat cream cheeses on crackers (as they were presented in the study). Ten sets of three stimuli were presented, two alike and one different. Participants were required to masticate and expectorate each stimulus and identify the odd sample. Subjects rinsed with deionized water between samples. Testing was conducted under red light to mask subtle color differences between the samples. Room lighting was used during the fat-challenge trials because dissimilar samples were never presented together. A modified Harris-Kalmus procedure (20) was used during the second sensory test session. Fourteen samples, seven full-fat and seven nonfat, were presented in random order a single time and participants were requested to assign like samples to one of two unique groups. They also assigned hedonic ratings to each of these samples using a nine-point category scale (21).

Blood testing

Plasma triacylglycerol and serum cholesterol concentrations were determined at baseline and 2, 4, and 6 h after load ingestion by an enzymatic procedure using a Boehringer Mannheim Hitachi 747 analyzer (Indianapolis). The precision of both assays was 2%.

Statistical analyses

The effects of the different forms of sensory stimulation on the primary dependent variable, plasma triacylglycerol concentration, were assessed by repeated-measures analysis of variance (ANOVA). The within-subjects factors were form of stimulation (four levels) and time (four levels). Sex was included as a between-subjects factor. The Newman-Kuels test was used for post hoc comparisons. Area under the curves (AUC) for triacylglycerol clearance was computed by the trapzoidal method. Comparisons between two conditions were conducted by independent-sample t tests and paired-sample t tests where appropriate. To evaluate whether findings were robust (ie, subjects maintained their rank order), Wilcoxon rank-order tests were also conducted on the AUC data. The associations between postprandial triacylglycerol concentration and sensory test performance, BMI, and baseline triacylglycerol concentration were determined by computing Pearson correlation coefficients. Given the probabilities of random correct responses on the taste tests, the criterion for assessing nonchance performance was a correct response to 7 of 10 trials in the triangle test (P < 0.05) and 12 of 14 samples for the
RESULTS

Repeated-measures ANOVA revealed a significant treatment-by-time interaction on plasma triacylglycerol concentrations ($F_{19,126} = 1.92$, $P = 0.05$). Although baseline triacylglycerol concentrations did not differ across conditions, the rise of plasma triacylglycerol was particularly sharp during the session in which full-fat cream cheese was sampled. This is clearly apparent in a plot of difference (relative to baseline) scores (Figure 1). Only values associated with exposure to the full-fat cream cheese were significantly ($P < 0.05$) different from baseline at all time points, indicating that this treatment led to the most prolonged elevation of triacylglycerol concentration.

A significant effect of the oronasal stimuli on peak triacylglycerol concentration was also observed ($F_{3,42} = 3.47$, $P = 0.024$). Mean ($\pm$ SE) peak changes of triacylglycerol concentration were as follows: full-fat cream cheese, $0.82 \pm 0.12$ mmol/L (73.0 $\pm$ 11.1 mg/dL); nonfat cream cheese, $0.49 \pm 0.10$ mmol/L (43.3 $\pm$ 8.5 mg/dL); cracker, $0.43 \pm 0.15$ mmol/L (38.5 $\pm$ 13.2 mg/dL); and no oral stimulation, $0.63 \pm 0.14$ mmol/L (55.5 $\pm$ 12.7 mg/dL). The peak response to full-fat cream cheese was significantly greater than that to nonfat cream cheese ($P < 0.02$), to cracker alone ($P < 0.02$), and almost so relative to no oral stimulation ($P < 0.07$). Differences among other treatments were not significant. Rank-order tests indicated that the differences were robust—peak responses to full-fat cream cheese exceeded those to all other conditions in 12 of 15 subjects (all $P < 0.05$).

An evaluation of the triacylglycerol AUCs (Figure 2) indicated that oronasal stimulation with full-fat cream cheese led to triacylglycerol concentrations that were significantly greater than those noted after exposure to the nonfat cream cheese, the cracker alone, or no oronasal stimulation (all $P < 0.05$). The AUC value for the full-fat cream cheese exposure was 77% higher than that for the nonfat cream cheese exposure, 109% greater than concentrations associated with exposure to the cracker alone, and the increment was 34% compared with no oronasal stimulation. The latter difference would have been 60% if a single high outlier value at the 2-h time point of the no-stimulation treatment had been excluded from the analysis.

Triacylglycerol AUC values after exposure to full-fat cream cheese were higher than those after exposure to nonfat cream cheese in 13 of 15 subjects ($P < 0.05$). Values were higher after exposure to full-fat cream cheese relative to the cracker alone in 10 of 15 subjects ($P < 0.05$), and a comparison between the full-fat cream cheese and no oral stimulation revealed that the former led to a higher triacylglycerol concentration in 11 of 15 subjects, with one tie ($P < 0.05$).

There was a significant sex effect for triacylglycerol AUC values ($F_{11,131} = 8.23$, $P = 0.013$), according to which males had significantly higher triacylglycerol responses than females after exposure to the full-fat cream cheese, nonfat cream cheese, and the no-stimulation condition (Figure 3). Overall, peak triacylglycerol concentrations for males were significantly higher than those of females ($F_{1,131} = 5.74$, $P = 0.032$). However, the only condition for which this was significant was after exposure to the full-fat cream cheese [1.14 $\pm$ 0.22 mmol/L (101.2 $\pm$ 19.4 mg/dL) for males and 0.62 $\pm$ 0.11 mmol/L (54.2 $\pm$ 9.7 mg/dL) for females; $P < 0.05$].

No significant treatment effects on serum cholesterol concentrations were observed. Mean ($\pm$ SE) AUC of the change of serum cholesterol values for the full-fat, nonfat, cracker, and no oral stimulus conditions were $-0.03 \pm 0.05$, $-0.03 \pm 0.04$, $-0.06 \pm 0.09$, and $0.05 \pm 0.06$ mmol·h/L, respectively. Note that the lipid load was safflower oil, and nothing else was ingested over the 6-h test session.

BMI was not significantly associated with the triacylglycerol response to treatments. All correlation coefficients were < 0.15.

**FIGURE 1.** Mean ($\pm$ SE) change from baseline of plasma triacylglycerol concentration after ingestion of a lipid load and various forms of oral stimulation.
The associations between baseline triacylglycerol concentration and AUC values for change of triacylglycerol concentration were also not significant for any treatment condition. The Pearson correlation coefficient for the full-fat cream cheese condition, for which the response was greatest, was $r = 0.32$, whereas all others were $< 0.25$. A nonparametric test did reveal a significant association for the full-fat cream cheese condition (Spearman $r_s = 0.66$, $P < 0.02$), suggesting that with a larger population sample some relation may become apparent.

Ten of the 15 participants returned for the sensory tests. Only one individual was able to distinguish reliably between the samples during the Harris-Kalmus procedure. Neither of these two subjects met the identification criterion for the triangle test. Subjects also rated the two forms of cream cheese as equally palatable. The mean ratings for the full-fat and nonfat samples were $5.7 \pm 0.4$ and $5.2 \pm 0.4$ on a nine-point category scale, respectively. These ratings were not significantly different. Further, no significant correlation was observed between the triacylglycerol and hedonic responses to any of the sensory stimuli. Thus, under the conditions of this protocol, in which stimuli were sampled blindly, the nonfat and full-fat cream cheeses were well-matched on sensory properties.

All taste stimuli were masticated and expectorated under the supervision of a research technician. There was no instance in
which samples were swallowed intentionally. To document the extent to which portions were inadvertently ingested, weights of the stimuli were obtained before and after mastication. Because of variable saliva contributions, the data provide only a qualitative index of compliance. The mean increases in weights of the full-fat cream cheese, nonfat cream cheese, and cracker-alone samples were 37.8 ± 10.3, 30.3 ± 9.7, and 24.0 ± 5.3 g, respectively. Preweights of masticated nonfat cream cheese exceeded masticated weights for three participants, indicating consumption. Postmastication weights exceeded preweights for all other participants and stimuli.

**DISCUSSION**

Consistent with earlier findings in rats (10), these data demonstrate that oronasal exposure to dietary fat can influence postprandial lipid metabolism in humans. The postprandial rise of plasma triacylglycerol was significantly higher and of longer duration when associated with mastication of the full-fat stimulus compared with each of the control conditions. Although the ecological validity of these findings must be established because variations in the oronasal stimulus and lipid load will undoubtedly elicit different physiologic responses, these findings are based on conditions in which the form of dietary fat exposure (e.g., cream cheese on a cracker) and size of the lipid load (50 g) are not remarkable.

It is unlikely that the effect is attributable to ingestion of the sensory stimuli. All samples were masticated and expectorated in the presence of a research technician who repeatedly instructed participants not to swallow. In addition, weights, albeit crude, of the stimuli before and after exposure indicate that little was consumed. Even if some fat from the cream cheese was ingested, the increment to the load would be trivial and unlikely to account for the noted effect with this stimulus. Still, ingestion of a small amount of fat cannot be eliminated as an explanation. Rather than contributing to the pool of ingested triacylglycerol, it is plausible that fat swallowed inadvertently from the cream cheese was detected by a visceral sensory system and initiated processes that influence triacylglycerol handling. This hypothesis is bolstered by findings that gastric emptying is a highly regulated process in primates whereby the energy content of a load is insensibly monitored and determines the rate of ingesta delivery to the duodenum (23) as well as evidence for an extremely sensitive system of detecting carbohydrates in the gut of rats (24).

Excluding the possibility of stimulus ingestion, the mechanism underlying the differential treatment effects on plasma triacylglycerol concentration appears to require at least a biphasic response wherein some salient attribute of the full-fat stimulus was detected before or during oronasal exposure and elicited a change in the processing of the lipid load. Cognitive influences have been documented for several cephalic phase responses in humans (25–27). Whether knowledge of the fat content of a food or beverage can influence its digestion and utilization has not been tested, but this cannot explain the present findings. The triacylglycerol responses to the full-fat and nonfat cream cheese were significantly different, yet participants were not aware of the fat contents of the two stimuli. Further, similar responses were observed to the nonfat cream cheese and to cracker alone, for which differences in fat content should have been anticipated.

The hedonic appeal of oronasal stimuli (28–30) or anticipated effects they may have on the consumer stemming from prior experiences with the items (31) may also alter the cephalic phase response they elicit. Plasma triacylglycerol reportedly rises more quickly after ingestion of a palatable meal than after one that is less appealing (32). However, our subjects were not able to discriminate reliably between the samples and gave them comparable hedonic ratings. This suggests that sensory factors did not contribute to the differential triacylglycerol response, but evidence that subthreshold olfactory stimulation can elicit electroencephalographic, mood, and performance responses precludes eliminating this as a potential mechanism (33). It is possible that the effective stimulus for cephalic phase elicitation is not the same as that used by subjects to discriminate between test samples.

The explanation for the data that is most parsimonious, but that challenges prevailing views regarding oral fat perception, is that there is a mechanism in the oronasal region for detecting fats per se, or compounds present in or derived from fats. The preponderance of data indicates that fats are perceived by textural cues (34, 35). However, humans can discriminate between dairy products modified to have the same viscosity (36) and rats can easily discriminate between dilute suspensions with comparable tactile characteristics (11). There has been speculation about the presence of an oral receptor mechanism (11) or possibly a role for the gustatory system (35), but this has never been documented. The principle objection to a receptor-mediated mechanism for fat perception is the size of the lipid droplets. Whether digestion by lingual lipase in the oral cavity can generate particles small enough for effective receptor interactions is not known. The products generated by lingual lipase include polar free fatty acids and monoacylglycerols (37) that could translocate to the surface of fat globules and interact with receptors. A more direct role for lingual lipase in producing the triacylglycerol elevation with the full-fat stimulus is unlikely. Whereas lipase activity can be induced by feeding a high-fat diet for 2 wk (38), there is no evidence for an acute effect and the tongue reportedly accounts for only 0.015% of the lipolytic activity of the gastrointestinal tract of human adults (39). Further, free fatty acid concentrations are similar in the stomachs of rats 1 h after an intragastric lipid meal regardless of whether concomitant oral stimulation was provided (10). Thus, the contribution of lingual lipase to the digestion of the lipid load was probably trivial.

Whatever the signal may be from the oral cavity, it must elicit responses that increase load absorption, enhance endogenous triacylglycerol synthesis, and/or decrease lipid clearance to account for the elevation of plasma triacylglycerol with oral fat exposure. The present study was not designed to address this issue, but an explanation based solely on increased triacylglycerol absorption can be rejected. Because absorption of safflower oil (the load used in this study) in healthy adults is nearly complete (40), the fat challenge represents a finite load. Given this constraint, the observed combination of a higher peak and longer duration of elevated triacylglycerol would not be expected with a shift in absorption.

Endogenous triacylglycerol synthesis and secretion by enterocytes and hepatocytes could account for the noted treatment effects only if this activity is particularly enhanced by a fat-containing oronasal stimulus. The fat load administered in all conditions was identical, so it provided a constant form and
amount of substrate and metabolic challenge across treatments. One mechanism whereby a differential synthetic and secretory response could occur is through a neurally mediated endocrine response stemming from the oronasal exposure. This could take many forms, for example, a cephalic phase release of pancreatic polypeptide has been documented in humans (41, 42). Fat may be a particularly effective elicit of pancreatic polypeptide secretion directly or through cholecystokinin (43), and this gut peptide significantly enhances release of triacylglycerol from rat hepatocytes (44). However, even if such a mechanism is functional, the relative importance of endogenously derived triacylglycerol in postprandial lipemia remains controversial (17, 45–48).

Decreased triacylglycerol clearance, alone or in conjunction with enhanced absorption or endogenous triacylglycerol synthesis, could also result in an elevated postprandial plasma triacylglycerol concentration. Suppression of lipoprotein lipase (LPL) activity would be one potential mechanism, although it is not clear that the activity of this enzyme would be rate-limiting under the conditions of this study (32, 49). Given that a signal from the oronasal region appears to be required for any explanation of the present findings, once again, a cephalic phase response warrants consideration. Several hormones capable of suppressing adipose LPL activity are, in part, under neural control. Glucagon, recently shown to be released by a high-fat oral stimulus (50), directly diminishes adipose LPL activity (51). Insulin normally stimulates adipose LPL, but an influx of dietary lipid decreases adipose tissue LPL responsiveness to insulin (52). An effect on skeletal muscle LPL activity is also possible, but its contribution during the early postprandial period when plasma triacylglycerol concentrations peaked may be limited. Oral, but not intravenous administration of triacylglycerol increases the plasma insulin concentration, which could reduce skeletal muscle LPL activity (53). Whether the fat or a substance carried in or derived from the fat in the cream cheese used in this study can differentially (eg, through hormonal regulation of enzyme activity or alterations of lipoprotein composition or structure) elicit a direct or indirect reduction in triacylglycerol clearance will require additional study.

Our observation of a significantly greater elevation of plasma triacylglycerol in males than in females was unexpected. Other work has not revealed a sex difference in adipose tissue LPL activity (52) and if the postprandial lipemia is attributable to suppressed LPL clearance of triacylglycerol, one might hypothesize a greater response in females who typically have a higher percentage of body weight as fat. Indeed, all the obese subjects (BMI > 30) in this study were female and females had a higher mean BMI than the males.

Our failure to note a treatment effect on cholesterol concentrations is consistent with findings from other fat-challenge studies in which the lipid load and oronasal stimulation did not involve an added nutritive sweetener (16, 54). Cholesterol concentrations do rise when sucrose is added to the test load (16). The extent to which this is attributable to metabolism of fructose or a cephalic phase response to the sweet taste is not known.

Given the evidence that postprandial lipemia is related to atherogenesis (12, 13), these data raise the question as to whether oronasal exposure to dietary fats contributes to this problem. Future work will be required to determine whether the elevated lipemia associated with oral fat exposure consists of increased concentrations of atherogenic lipoprotein particles. This has been shown in other work involving fat-challenge tests (16, 48), but those loads contained other macronutrients so it is not possible to attribute the elevation of atherogenic lipoproteins to the fat itself.

Without knowledge of the mechanism by which the full-fat cream cheese augmented plasma triacylglycerol concentrations, responses to foods containing various fat mimics or substitutes cannot be predicted. Protein, carbohydrate, and fat each serve as the basis for ingredients designed to replace the sensory properties normally contributed by fats to foods (55). Thus, if the effective stimulus is a macronutrient, different fat substitutes may elicit dissimilar responses. In the present study, the nonfat cream cheese was higher in protein and lower in fat than the full-fat version, suggesting that protein may not be a salient stimulus. However, this requires further verification because the form of the protein may be important.

In summary, these data demonstrate that oronasal exposure to dietary fat can influence the postprandial metabolism of lipid in humans. The effect is large and robust. Its health implications are presently unclear, but given that ≈80% of the adult US population consumes fat-modified foods and beverages (56) that are specifically designed to impart a particular sensory impression, an improved understanding of the issue is warranted.

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REFERENCES