Importance of lipolysis in oral cavity for orosensory detection of fat

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Kawai, Takayuki, and Tohru Fushiki. Importance of lipolysis in oral cavity for orosensory detection of fat. Am J Physiol Regul Integr Comp Physiol 285: R447–R454, 2003—Lingual lipase is usually secreted from von Ebner’s glands, although there is great variation between species. Lingual lipase is thought to be an auxiliary enzyme for fat digestion and absorption in mammals; however, the reason for lipolysis in the oral cavity is not known. We focused on the gustatory sense and investigated the significance of lingual lipase in the perception of a fat taste by using orlistat, a potent lipase inhibitor. Five-minute two-bottle preference tests demonstrated that the addition of orlistat diminished the preference for triacylglycerides but not for free fatty acids. Radioactive triolein applied on rats’ circumvallate papilla revealed that lingual lipase was released continuously to generate significant amounts of fatty acids and other lipolytic products within 1–5 s, which was enough time to taste fat. These findings suggest that lingual lipase is released to perceive the taste of triacylglycerides and to find nutritive lipids in food.

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housed individually in large wire-mesh cages in a vivarium maintained at 20 ± 2°C on a 12:12-h light-dark cycle (light onset at 0600). A commercial standard diet (MF; Oriental Yeast, Tokyo, Japan) and water were available ad libitum except where noted. The rats were maintained for 1 wk after arrival to permit them to acclimatize to their surroundings before being tested.

Chemicals and materials. Ols, tetradecylp-ristatin, was obtained from Hoffmann-La Roche (Basel, Switzerland). Sucrose was obtained from Nacalai Tesque (Kyoto, Japan). Oleic acid, triolein, linoleic acid, trilinolein, mineral oil, gum xanthen, and BSA were purchased from Sigma Chemical (St. Louis, MO). Corn oil was obtained from Ajinomoto (Tokyo, Japan). All of the fatty acids and glycerides were >99% pure and were stored at −20°C until used. Radioactive [carboxyl-14C]triolein was obtained from American Radiolabeled Chemicals (St. Louis, MO). Sucrose was obtained from Ohtsuka Seiyaku (Tokyo, Japan). Water-resistant 20 × 20-cm silica thin-layer plates on an aluminum support were purchased from Merck (Darmstadt, Germany). The imaging plate for detecting low-energy beta radiation was purchased from Fuji Photo Film (Kanagawa, Japan). The rat’s pancreatic juice was collected from a bile duct with a cannula of Silastic tubing and was stored at −80°C. 

Preparation of substrate filter papers. Radioactive [carboxyl-14C]triolein was added to 2% triolein emulsion in 10% BSA solution, as a substrate emulsion. The 3MM filter papers (Whatman International, Kent, UK) were cut to the same size, 4 mm × 4 mm, and saturated with 5 μl of the substrate emulsion (~1 kBq/paper). Some were saturated with 5 μl of another substrate in which 0.5% Ols was added.

Lipolytic assay on rat tongue. The rats were anesthetized by an intraperitoneal injection of pentobarbital sodium (50 mg/kg body wt), and their tracheas were cannulated. The rats were fixed with a headholder, and then their oral cavities were split off by a thermal cautery knife (Geiger Medical Technologies, Monarch Beach, CA). The tongues were pulled forward to expose their circumvallate papillae. The 3MM filter papers saturated with substrate solutions were placed on the circumvallate papillae for 1, 5, 10, or 30 s. The surface of the tongue was rinsed two times with surgical swabs saturated with fresh Ringer solution in between each session. The detached filter papers were thrown into diethylether immediately and vortexed to stop enzyme reactions.

TLC. Fatty compounds were extracted from filter papers with a chloroform-diethylether solvent more than one time and dried completely at room temperature. Residues were resuspended with a small amount of hexane, applied to TLC plates, and then developed at room temperature in a solvent containing hexane-diethylether-acetic acid (50:50:1 vol/vol/vol) until the solvent front had risen ~15 cm from the origin (after ~30 min). After development, the TLC plate was placed in contact with the Fuji BAS imaging plate for 3 h; the radioactive lipolytic products of triolein were then detected by fluorography. Radioactive intensity of each spot was digitized by Image Gauge software.

Training procedure. Oils were emulsified into 0.3% gum xanthen solution. For training of two-bottle choice tests, sucrose was suspended in 0.3% gum xanthen solution. All rats were trained in advance of short-term two-bottle choice tests. The food and tap water were taken away from the cage at 1700 every day. The rats were given sucrose solution and vehicle control (0.3% gum xanthen solution) simultaneously from 2100. Two bottles, which had silicon stoppers with straight stainless-steel sipper tubes, were protruded in a cage ~10 cm from the cage floor, 9–12 cm apart. In the first day, 5% sucrose solution and vehicle control were offered to the rats for 30 min to train selective intake of the favorable fluid, and the food and tap water were returned to the cage at 2200. In the subsequent 4 days, 2% sucrose solution and control were offered for 5 min to learn short-term two-bottle choice tests. The left-right positions of the fluids were alternated daily. After removal of the training fluids, oil emulsions containing 2% triolein (2nd and 4th day) or 2% mineral oil (3rd and 5th day) were offered overnight so that the rats could learn to distinguish nutritive from nonnutritive oil. The food and tap water were returned to the cage at 0900. On the 6th day, no procedure was performed. Preference value to the sucrose solution was calculated as described in Statistical analysis. The rat that had not been trained well was excluded from the later tests.

Procedure for a short-term two-bottle choice test. Triolein and trilinolein were used as the TAGs, oleic acid and linoleic acid as the FFAs, and mineral oil as the inedible oil. Oils were emulsified into 0.5% gum xanthen solution, as test fluids. Ols-mixed emulsions were dissolved in oils and then emulsified in 0.5% gum xanthen solution. During the test phase, food and tap water were taken away from the cage at 1700 every day. The choice tests were performed for 5 min from 2100. Two bottles were placed in the front of the cages 9–12 cm apart. The left-right positions of the emulsions were alternated daily at random to avoid any side preference. The food and tap water were returned to the cage at 2200. In the first session of the test phase, triolein emulsion (for group I) or trilinolein emulsion (for group II) was offered together with vehicle control to verify that the rats preferred TAGs. In the second session, oleic acid emulsion (for group I) or linoleic acid emulsion (for group II) was offered together with vehicle control to verify that the rats preferred FFAs. In the third session, triolein emulsion (both for groups I and II) was offered together with mineral oil emulsion. In the fourth session, oleic acid emulsion (for group I) or trilinolein emulsion (for group II) was offered together with mineral oil emulsion to verify that the rats had learned a cue of nutritive oil completely. In the fifth session, triolein (for group I) or trilinolein (for group II) emulsion, with and without Ols, was offered simultaneously. In the sixth session, oleic acid (for group I) or linoleic acid (for group II) emulsion, with and without Ols, was offered simultaneously. After these sessions, additional choice tests were performed only to the rats of group I. One-tenth pseudohydrolyzed triolein emulsion, half pseudo-hydrolyzed triolein emulsion, and corn oil emulsion, with and without Ols, were tested in this order. The animal care and experimental procedures were approved by the Animal Care and Use Committee of the Kyoto University Division of Applied Life Science.

Preparation of pseudohydrolyzed triolein. Nine volumes of triolein and one volume of oleic acid were mixed to prepare the one-tenth pseudohydrolyzed triolein. The same volumes of triolein and oleic acid were mixed to prepare the half-pseudohydrolyzed triolein.

Statistical analysis. The intakes of each fluid in all experiments were measured, and their differences were statistically evaluated by the paired t-test. The preference values for test fluids were obtained as the value of intake of each fluid divided by total intake and multiplied by 100. All differences with a P value < 0.05 were considered statistically significant. Intakes given in Figs. 2–4 are means ± SE. Preference values given in Tables 1–3 are means ± SD.
RESULTS

Lipolytic activity in rat oral cavity. To analyze the extent of lipolysis of the TAG by rat lingual lipase in the oral cavity, the 3MM filter papers saturated with triolein-BSA emulsion were placed on the rat’s circumvallate papilla, the end of von Ebner’s glandular duct, and the lipolytic products were separated by TLC. Conditions were optimized with respect to BSA concentration for triolein emulsion, lipid extraction from 3MM filter paper, and clearance of residual substrates on the tongue. As a result, we selected the conditions described in MATERIALS AND METHODS.

Four spots, triolein (retardation factor (Rf) value \(0.45\)), oleic acid (Rf value \(0.45\)), dioleyl glycerol (Rf value \(0.27\)), and monooleyl glycerol (Rf value \(0.07\)), were detected in all of the samples contacted with the circumvallate papilla for \(1\) s (Fig. 1A). Because the radioactive compound used in this experiment had been synthesized by the esterification of \([1-14C]\)oleic acid with glycerol by the method of Wheeler et al. (45), all side chains had identical specific radioactivity; the ratio of each lipolytic product could be calculated by the radio intensity of the spot on the TLC plate. Within 1 s, 4% of triolein was hydrolyzed, and then the oleic acid content increased to \(1.5\%\). As lipolysis proceeded, \(>10\%\) of triolein was hydrolyzed, and the oleic acid content reached \(5\%\) within an additional 10 s. Table 1 shows the abundance ratios of triolein and the lipolytic products. Because our method evaluated the limited lipolysis on the tongue surface but not total lipolysis, more advanced lipolysis in the cleft of the circumvallate papilla by the concentrated lipase is predicted. Consequently, it is simple to predict that a great amount of FFAs will be generated near the apical part of taste buds when food containing TAGs enters the oral cavity. The other rats also showed similar lipolytic activities although they have some variations (Fig. 1B).

Table 1. Abundance ratio (%) of the hydrolyzed products by rat lingual lipase

<table>
<thead>
<tr>
<th>Reaction Time, s</th>
<th>Triolein</th>
<th>Oleic Acid</th>
<th>Dioleoyl Glycerol</th>
<th>Monooleoyl Glycerol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial</td>
<td>99.70</td>
<td>0.12</td>
<td>0.03</td>
<td>0.00</td>
</tr>
<tr>
<td>1</td>
<td>96.02</td>
<td>1.48</td>
<td>1.94</td>
<td>0.31</td>
</tr>
<tr>
<td>5</td>
<td>94.87</td>
<td>2.17</td>
<td>2.25</td>
<td>0.36</td>
</tr>
<tr>
<td>10</td>
<td>88.30</td>
<td>5.44</td>
<td>4.66</td>
<td>0.93</td>
</tr>
</tbody>
</table>

In contrast, no lipolysis was observed when the sample containing 0.5% Ols, the drug developed as a pan-

Table 2. Abundance ratio (%) of the hydrolyzed products generated for 5 s

<table>
<thead>
<tr>
<th>Rat No.</th>
<th>Triolein</th>
<th>Oleic Acid</th>
<th>Dioleoyl Glycerol</th>
<th>Monooleoyl Glycerol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial</td>
<td>99.14</td>
<td>0.13</td>
<td>0.02</td>
<td>0.00</td>
</tr>
<tr>
<td>1</td>
<td>94.49</td>
<td>2.28</td>
<td>1.78</td>
<td>0.51</td>
</tr>
<tr>
<td>2</td>
<td>96.83</td>
<td>0.93</td>
<td>0.94</td>
<td>0.15</td>
</tr>
<tr>
<td>3</td>
<td>95.93</td>
<td>1.81</td>
<td>1.50</td>
<td>0.34</td>
</tr>
<tr>
<td>4</td>
<td>98.00</td>
<td>0.59</td>
<td>0.38</td>
<td>0.10</td>
</tr>
<tr>
<td>5</td>
<td>96.98</td>
<td>1.25</td>
<td>0.72</td>
<td>0.23</td>
</tr>
</tbody>
</table>
creatic lipase inhibitor, was in contact with the circumvallate papilla (Fig. 1C, right). This result demonstrates that the addition of Ols at a concentration of 0.5% can completely inhibit lipolysis of 2% triolein by lingual lipase.

A small amount of lipolysis was observed in the sample placed several centimeters away from the valvate and foliate papillae for 30 s (Fig. 1C, left). This lipolysis would be caused by the saliva running over the tongue from the foliate or vallate papillae.

Next, the plain samples and the Ols-mixed samples were placed in turns on the circumvallate papilla for 30 s. Before each session, the tongue was wiped mildly by surgical swabs saturated with fresh Ringer solution. Similar levels of lipolysis were observed in both the plain samples before and after the Ols session (Fig. 1D). The Ols did not affect plain samples at all, even though it binds covalently to the active site of lipase (17, 27). This suggests that the basal level of lingual lipase is secreted from von Ebner’s glands continuously and that some flows over to the surface of the tongue, intravitam. In fact, no lipolysis was observed in the plain sample in contact with a circumvallate papilla of a removed tongue (data not shown).

Two-bottle preference tests. The lipolysis of TAGs and the generation of FFAs in the shortest time by lingual lipase in rats suggest that the lipolysis is somewhat related to the gustatory sense. Therefore, we employed short-term two-bottle choice tests as a behavioral study.

During the training phase, 1 of 21 rats in group I consumed more water than sucrose solution more than once; we considered that this rat was unsuitable for the two-bottle tests and excluded it from the following tests.

In the first session, the rats of group I consumed significantly more dietary oil emulsions, 2% triolein emulsion, and 2% oleic acid emulsion than the vehicle solution (Fig. 2A). In the second session, they consumed significantly more 2% triolein emulsion than the 2% mineral oil emulsion (Fig. 2B, left). Furthermore, they consumed significantly more 2% oleic acid emulsion than the 2% mineral oil emulsion (Fig. 2B, right). Similar results were also obtained from the rats of group II (Fig. 2, C and D). The preference values (means ± SD) of these tests are summarized in Table 3. These results suggest that these rats had learned the cue of nutritive oil well, even though the mineral oil emulsion has a similar textural property.

After the preliminary tests, we employed the choice test of TAG emulsion with and without Ols to examine whether the lipolysis by lingual lipase exerted an influence on the perception of the cue of nutritive oil. The lipolytic assay described above suggests that Ols inhibits the lingual lipase activity, but it can be rinsed out thoroughly by a small amount of saliva from the tongue, and then the lipolytic activity is recovered immediately. Therefore, we expected that rats could discriminate the taste of plain TAG emulsion from that of Ols-mixed emulsion without each residual effect, even when they were offered simultaneously. The rats of group I consumed significantly more plain triolein emulsion than Ols-mixed triolein emulsion (Fig. 3A, left). This appears to show that Ols obscured the cue of nutritive oil included in the triolein emulsion and that oleic acid or another lipolytic product of triolein is the cue. However, there was still the possibility that Ols itself gave an unpleasant flavor. Consequently, we employed a similar test using the oleic acid emulsions to examine whether the additive Ols was aversive. The
rats consumed the Ols-mixed oleic acid emulsion as much as the plain emulsion (Fig. 3A, right). This result shows that Ols did not give an unpleasant flavor or inhibit the fluid intake. We have also obtained similar results and Ols effects from the rats of group II. The rats consumed significantly more plain trilinolein emulsion than Ols-mixed trilinolein emulsion (Fig 3B, left). They consumed the Ols-mixed linoleic acid emulsion as much as the plain emulsion (Fig. 3B, right). The preference values of these tests are summarized in Table 3. Oil components and concentrations were equalized in a pair of emulsions to make all other sensory ratings similar in the all tests of this section. Therefore, it is suggested that the cue of nutritive oil is perceived by a gustatory sense and the cue in TAGs generated only after they are hydrolyzed by a lingual lipase.

Additionally, we generated pseudohydrolyzed trilinolein by replacing part of the trilinolein with the oleic acid and employed the choice tests of plain and Ols-mixed emulsions to examine whether FFA played a role in the gustatory cue of nutritive oil in TAG. The rats showed a strong preference for plain one-tenth pseudohydrolyzed trilinolein over Ols mixture but no preference between plain and Ols-mixed half-pseudohydrolyzed trilinolein (Fig. 4A). These results suggest that some level of FFA is necessary to perceive the cue of nutritive oil. They also suggest that gustatory perception of the cue in TAG requires a certain degree of lipolysis by a lingual lipase. The lipolytic assay described above supports the proposal that such a level of lipolysis might occur in the cleft of circumvallate papillae within a few seconds. The latter result that the rats did not refuse Ols-mixed emulsion suggests that the rats have not learned gustatory information associated with the postigestive effects of Ols, such as a fat malabsorption, despite repeated tests.

Finally, we employed a similar test using corn oil as the common dietary oil. The Ols-mixed corn oil emulsion was consumed at the same levels as plain corn oil emulsion (Fig. 4B, right). This result differs from that for the pure trilinolein emulsion. The quality of commercial corn oil is well controlled, with FFA concentrations being <1% to protect the oil from deterioration result-

Table 3. Preference values in 5-min two-bottle choice tests

<table>
<thead>
<tr>
<th>Preference Value for Plain Emulsion</th>
<th>P Value</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Plain Oil emulsion</th>
<th>Control Solution</th>
<th>Group I (n = 20)</th>
<th>Group II (n = 21)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triolein (2%)</td>
<td>vs. Vehicle</td>
<td>0.72 ± 0.32*</td>
<td>0.87 ± 0.15*</td>
</tr>
<tr>
<td>Oleic acid (2%)</td>
<td>vs. Vehicle</td>
<td>0.79 ± 0.21*</td>
<td>0.66 ± 0.19*</td>
</tr>
<tr>
<td>Triolein (2%)</td>
<td>vs. Mineral oil (2%)</td>
<td>0.91 ± 0.14*</td>
<td>0.85 ± 0.14*</td>
</tr>
<tr>
<td>Oleic acid (2%)</td>
<td>vs. Mineral oil (2%)</td>
<td>0.89 ± 0.13*</td>
<td>0.75 ± 0.18*</td>
</tr>
<tr>
<td>Triolein (2%)</td>
<td>vs. 0.5% Ols mixture</td>
<td>0.73 ± 0.29*</td>
<td>0.67 ± 0.29*</td>
</tr>
<tr>
<td>Oleic acid (2%)</td>
<td>vs. 0.5% Ols mixture</td>
<td>0.39 ± 0.35</td>
<td>0.57 ± 0.31*</td>
</tr>
<tr>
<td>One-tenth pseudo-hydrolyzed triolein (2%)</td>
<td>vs. 0.5% Ols mixture</td>
<td>0.73 ± 0.31*</td>
<td>0.35 ± 0.106</td>
</tr>
<tr>
<td>Half pseudo-hydrolyzed triolein (2%)</td>
<td>vs. 0.5% Ols mixture</td>
<td>0.43 ± 0.42</td>
<td>0.31 ± 0.15*</td>
</tr>
<tr>
<td>Corn oil (2%)</td>
<td>vs. Vehicle</td>
<td>0.95 ± 0.06*</td>
<td>0.35 ± 0.06*</td>
</tr>
<tr>
<td>Corn oil (2%)</td>
<td>vs. 0.5% Ols mixture</td>
<td>0.56 ± 0.39</td>
<td>0.19 ± 0.018</td>
</tr>
</tbody>
</table>

Preference values are means ± SD; n, no. of rats. Vehicle is 0.3% gum xanthan solution. ND, no data. *P < 0.05, significant difference by paired t-test.
DISCUSSION

We first focused on the novel role of lingual lipase, which has been researched only in terms of dietary fat digestion and absorbance.

In this study, rat lingual lipase activity intravitam was measured by a novel method that evaluates the spontaneous secretion, which is different from the conventional assay that uses homogenates of von Ebner’s glands. Our experiments show that the rat lingual lipase could hydrolyze ~10% of triolein within several seconds, even on the surface of the tongue and that it was secreted from von Ebner’s glands continuously intravitam. The lipolysis of TAGs and the rapid generation of FFAs by lingual lipase suggest that lingual lipase exists for the perception of taste of TAGs.

The short-term two-bottle choice tests, based on rats consuming more of a preferred fluid when two fluids are offered simultaneously, showed that TAGs were preferred over both gum xanthan solution and mineral oil, although the preference to TAGs was reduced strongly by lingual lipase inhibition. FFAs were also preferred over both gum xanthan solution and mineral oil, but lingual lipase inhibition had little effect on the preference for FFAs. These results strongly suggest that TAGs are perceived as possessing a preferred taste only after FFAs are generated by lingual lipase, although the possibility cannot be denied that TAGs directly bind to some receptor. In other words, it is possible that the palatability of fat is sensed by chemoreception of FFAs on the putative receptors, such as fatty acid translocase, expressed on taste bud cells. The result of the test using pseudohydrolyzed triolein suggests that the presence of some FFAs, which were generated or already present, make a fat more preferable.

Lingual lipase has been regarded as a supplemental enzyme of pancreatic lipase (18, 37), and research has focused almost exclusively on its role in fat digestion and absorption. It has been reported that lingual lipase activity is higher in infancy when the pancreas is undeveloped, and in cystic fibrosis patients with pancreatic insufficiency, compared with controls (1, 13, 18, 36). However, because of the small amount of lipase secreted, lingual lipase is thought to be less important to fat digestion and absorption in healthy adult mammals with well-developed pancreatic lipase (18, 37). Our results suggest that the few seconds are sufficient time to generate enough FFAs to perceive a preferred taste of fat in the oral cavity from pure TAGs. Because TAGs are the main fat component in food, lingual lipase would be an important enzyme for finding TAGs in food, although the physiological role of this lipase has not been explained yet.

The release of lingual lipase from von Ebner’s glands in the cleft of the vallate and foliate papillae suggests that generated FFAs are perceived by apical regions of the taste bud cells directly exposed to high concentrations of lingual lipase. The presence of chemical reception of FFAs in the tongue has already been proposed on the basis of the response of delayed-rectifying K+ channels on taste receptor cells to cis-polysaturated fatty acid (16) and the immunoreactivity of a putative membrane fatty acid translocase localized in the apical part of taste bud cells in the circumvallate papillae (14). Our results also support this proposition and are consistent with the experimental results that the glossopharyngeal nerve responds to FFA stimuli, not to TAG stimuli (38). Thus the palatability in dietary fat seems to be influenced greatly by chemical stimuli such as the binding of FFAs to taste receptors.

To investigate the relationship between lingual lipase and the behavioral response to the taste of fat, training conditions, presenting times, fat concentrations, and fluid pairs were modified and optimized for our two-bottle choice test.

The fat taste involves both textural and chemical properties. Because mineral oil is similar in texture to dietary fat, a rat usually likes mineral oil (3, 12). Therefore, we had to develop the rats that could discriminate dietary oil from mineral oil by some gustatory cue to research a preferred taste in dietary fat. Rats have orosensory mechanisms for detecting and discriminating corn oil and mineral oil by receiving postigestive effects (30). Animals reduce the intake of a substance after they know it is nonnutritive (40). In this study, we offered dietary oil and mineral oil alter-
nately to rats for long periods during the training phase so that they learned that TAG had energy but mineral oil did not.

To examine only gustatory effects, we attempted to minimize the effects of texture and olfaction. All test substances were suspended in 0.3% gum xanthan solution, as a vehicle, to obscure the textural effects of fat and to avoid the separation of fat from water (33). In the two-bottle choice tests of plain and Ols-mixed emulsions, fat components and concentrations were equalized in a pair of emulsions to make all other sensory ratings similar. Moreover, we fixed the presentation time at 5 min to minimize postigestive effects of Ols, such as a fat malabsorption. It may be impossible to foreclose the possibility that rats would learn some orosensory factors paired with postigestive effects of Ols within repeated tests (2). To confirm that the rats did not learn such factors, we scheduled the choice test of Ols-mixed FFAs on the day after the choice test of Ols-mixed TAGs.

In recent years, a better method has been developed that is based on the lick rate associated with tantant concentration (8, 22, 39). It can measure semiquantitatively the intensity of tantant in multiple samples. Moreover, it offers advantages that each test is finished within 10–30 s and multiple sessions can be performed before the postigestive effects occur. This method would give us more detailed information about Ols effects on the recognition of fat taste. It would be worth conducting in the future, even though special instruments are needed.

Rats have the greatest preference for oil at concentrations of 25%. When the intakes are plotted against oil concentration, the graph shows an inverted U shape, with a peak at 25% (30). A detectable threshold by anosmic rats is ~0.5% oleic acid emulsion (unpublished observation). To evaluate the effects of generated oleic acid as a gustatory cue with high sensitivity, all emulsions in our tests were prepared with 2% oil. The additive Ols had a strong effect on intake of the one-tenth pseudohydrolyzed triolein emulsion, which contained 0.2% oleic acid; however, it had little effect on that of the half-pseudohydrolyzed triolein emulsion, which contained 1.0% oleic acid. These results suggest that the taste of triolein is perceived only when the generated oleic acid reaches a sufficient level. A linoleic acid concentration of 10 μmol/l is sufficient for taste receptor cell depolarization in rats, but the same concentration of oleic acid was not sufficient (16). Consequently, it is easy to expect that the threshold of linoleic acid is also lower than that of oleic acid in vivo. However, we do not have sufficient data to discuss the difference in the effects of the lingual lipase on TAG varieties, because no pseudohydrolyzed trilinolein was employed in our tests.

In general, the major factors determining the attractive quality of fat are thought to be its viscosity, its high-calorie content, and its odor, including the odor of impurities. Furthermore, it seems difficult to extrapolate the result obtained from an animal experiment directly to a model for humans, because human indi-

viduals have a great variety of meal experiences. However, the fact that rats with the same background clearly show a relationship between orosensory pleasure of fat and generated FFAs seems very significant; it suggests that the quality and quantity of FFAs are important factors determining the attractive taste of fat, even though few people think that fat provides some gustatory cues in itself.

In another study, we have confirmed that the basal level of lingual lipase is secreted from human circumvallate papillae. It is possible that humans have an orosensory mechanism to detect FFAs and to perceive them as attractive ingredients. If so, the control of FFAs might be important to make more attractive oil.

Today, various kinds of low-calorie fat replacers have been developed; for example, carbohydrate-based Palselli, protein-based Simplesse, and fat-based Olestra (29). Although all of the currently available replacers are designed to have similar mouthfeel to fat, most have limited impact in terms of body or thickness and are not highly palatable. Their inadequate orosensory impact may be because of the absence of tasty ingredients. Surely, the low-calorie replacers are not expected to generate FFAs. Most manufacturers are now focusing on the development of low-melting TAG imitations that provide similar mouthfeel to real fat (4). However, if low-calorie FFA analogs were developed, a delicious low-caloric food with an impact close to real fat could be manufactured by adding them to conventional low-caloric fat replacers.

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REFERENCES