The fatty acid translocase gene CD36 and lingual lipase influence oral sensitivity to fat in obese subjects

Marta Yanina Pepino,[1] Latisha Love-Gregory, Samuel Klein, and Nada A. Abumrad

Center for Human Nutrition, Washington University School of Medicine, St. Louis, MO 63110

Abstract The precise orosensory inputs engaged for dietary lipids detection in humans are unknown. We evaluated whether a common single nucleotide polymorphism (rs1761667) in the CD36 gene that reduces CD36 expression and the addition of orlistat, a lipase inhibitor, to reduce FA release from triacylglycerols (TGs), the main component of dietary fats, would attenuate fat orosensory sensitivity in humans. Twenty-one obese subjects with different rs1761667 genotypes (6 AA, 7 AG, and 8 GG) were studied on two occasions in which oleic acid and triolein orosensory detection thresholds were measured using emulsions prepared with and without orlistat. Subjects homozygous for the G-allele had 8-fold lower oral detection thresholds for oleic acid and triolein than subjects homozygous for the A allele, which associates with lower CD36 expression ($P = 0.03$). Thresholds for heterozygous subjects were intermediate. The addition of orlistat increased detection thresholds for triolein (log threshold = -0.3 ± 0.2 vs. 0.3 ± 0.1; $P < 0.001$) but not oleic acid (log threshold = -1.0 ± 0.2 vs. -0.8 ± 0.2; $P > 0.2$). In conclusion, this is the first experimental evidence for a role of CD36 in fat gustatory perception in humans. The data also support involvement of lingual lipase and are consistent with the concept that FA and not TG is the sensed stimulus.—Pepino, M. Y., L. Love-Gregory, S. Klein, and N. A. Abumrad. **The fatty acid translocase gene CD36 and lingual lipase influence oral sensitivity to fat in obese subjects. *J. Lipid Res.* 2012.** 53: **561–566.**

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Obesity is caused by ingesting more energy than is expended over a long period of time. Dietary fat is the most energy-dense macronutrient, and its overconsumption has been linked to obesity (1–4). Obese people prefer foods with higher fat content (5), crave high-fat foods more frequently (6, 7), and consume more fat than lean individuals (8).

Traditionally, perception of fat in the oral cavity is thought to rely almost entirely on textural and aromatic cues activating the somatosensory and olfactory systems. However, there is now increasing evidence to support an important role of the gustatory system in fat perception (9–20) as well as in intestinal lipid metabolism (10, 21). Oral and gastrointestinal fat sensory sensitivity appear to be associated (16) and there is similarity in the chemosensory reception events and their signaling transduction pathways in the tongue and gastrointestinal tract (21). An important requirement for the involvement of a gustatory component in dietary lipid detection is the hydrolysis of triacylglycerols (TGs) to release free FAs, the signaling stimulus, as was demonstrated through the use of the lipase inhibitor orlistat (22). In rodents, lingual lipase is essential for the gustatory perception of dietary fats (22) and the addition of orlistat to fat emulsions diminishes the rat's preference for TG, but not FA (22). Although it is not known whether lingual lipase is important for oral fat perception in humans, data from a recent study suggests that lingual lipase lipolytic activity can produce FA within the concentration range required to activate oral sensors (18).

Several putative fat taste receptor classes have been identified in rodents (12, 23, 24), including the glycoprotein CD36 (25). The presence of CD36, a scavenger receptor that mediates uptake and trafficking of lipids in diverse cell types (26), has been documented in the gustatory papillae of rodents (25, 27), pigs, and humans (28). In rodents, the interaction between CD36 and FA results in signaling events that depend on an intact neuronal gustatory pathway (15, 29). CD36 gene knockout impedes fat detection in mice without affecting sweet or bitter perception

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Abbreviations: BMI, body mass index; CD36, cluster of differentiation 36; SNP, single nucleotide polymorphism; TG, triacylglycerol.

[1] To whom correspondence should be addressed.
e-mail: ypepino@dom.wustl.edu

and blunts the cephalic phase of pancreatobiliary secretions that are triggered by exposure of specific areas of the tongue to fat (25).

The primary goal of this study was to advance our understanding of fat orosensory perception in humans by evaluating the role of lingual lipase and CD36 on fat detection thresholds. We studied only obese subjects because of their documented preference and consumption of more high-fat foods than lean subjects (5–8), which would tend to neutralize the effect of dietary fat and diminish individual variability. The following two hypotheses were investigated: *1*) whether a common variant in the CD36 gene that reduces CD36 expression [i.e., single nucleotide polymorphism (SNP) rs1761667-A allele (30, 31)] will associate with higher oral fat detection thresholds (i.e., lower oral sensitivity to fat) and *2*) whether addition of orlistat to a fat emulsion increases the oral detection threshold for TG more than those for FA. Oleic acid and triolein orosensory detection thresholds were measured in obese subjects who were either carriers or noncarriers of the rs1761667-A allele by having subjects taste emulsions prepared with and without orlistat.

MATERIALS AND METHODS

Subjects

Three groups of obese subjects [body mass index (BMI) $\geqslant$30 kg/m^2] participated in this study (Table 1). Two groups were carriers of the rs1761667-A allele (AA, n = 6 and AG, n = 7) and one group was a noncarrier (GG, n = 8). The three groups were matched on age because there is a generic decline in taste perception with age (32). Potential subjects who smoked cigarettes in the last 6 months, had chronic sinus problems, previous malabsorptive or restrictive intestinal surgery, diabetes, or who were pregnant, breastfeeding, or taking any medication that might affect taste perception were excluded.

Ethics

All procedures were approved by the Human Research Protection Office at Washington University in St. Louis and each subject gave informed written consent before participation.

Study protocol

CD36 genotyping. Genomic DNA was isolated from blood (Gentra Puregene Blood Kit, Qiagen) and genotyped for CD36 SNP rs1761667 using Applied Biosystems predeveloped TaqMan SNP Genotyping Assay (Assay ID: C___8314999_10) (33). For each sample, 20 ng of DNA was genotyped in triplicate with negative and positive genotype controls included on the plate (controls were 100% concordant).

Taste testing. Participants completed taste testing studies on 2 separate days (day 1 and day 2) approximately 1 week apart. For 10 participants, fat taste perception was assessed in the presence of orlistat on day 1 and without orlistat, control day, on day 2. The remaining participants were assessed in the reverse order (i.e., control on day 1 and orlistat on day 2). The type of fat used as the first taste stimuli to measure detection thresholds (i.e., oleic acid or triolein) was counterbalanced within the groups. In addition to the sensory test, all participants but one (from the GG group) were interviewed by a nutritionist to estimate daily fat and energy intake and completed validated questionnaires to assess fat preferences (34) and food cravings (6).

Preparation of oleic acid and triolein emulsions. Preparation of emulsions followed Chalé-Rush et al. (9) with some modifications. Food grade oleic acid (Sigma Aldrich, St Louis, MO) and food grade triolein (Abitec Corporation; Janesville, WI) were stored in opaque bottles below 4°C. Triolein and oleic acid were added at varying concentrations to double distilled water. Concentrations used ranged from 0.0009 w/v% to 5 w/v% for oleic acid and from 0.006 w/v % to 31.7w/v % for triolein and were prepared in quarter-log dilution steps. All preparations were mixed with 5% (w/v) Gum Arabica (AEP Colloids, Hadley, NY) and white food colorant was added to produce perceptually identical viscosity and color between oil and control samples. For the testing session that used orlistat, emulsions were mixed with 0.5% w/v of orlistat (Glakosmithkline, Parsippany, NJ). All samples were sonicated for 6–9 min using a Branson 250 digital sonicator (Branson Ultrasonic Corporation, Danbury, CT) at 50% power with 30 s on, 60 s off. An ice bath was used during sonication to control for temperature. Samples were stored in opaque polypropylene cylinders and used for testing within 48 h of preparation. Control samples were prepared in the same way but without added oil. To ensure that the emulsions did not alter the nature of the food grade fats used and that no changes occurred that could affect the taste profile of the emulsion, headspace GC analyses were performed on the samples and free FAs were measured with the iodomethane method (35). The values and composition of oils measured on the emulsion samples were within those described in the original food grade product; no product of oxidation was detected, and 100% of the original concentration of oleic acid and triolein was recovered.

Determination of detection thresholds. Triolein and oleic acid taste detection thresholds were separately assessed using a staircase method (36) implemented in a three-alternative, forced choice paradigm (37). On each trial, subjects were presented with three samples: two were "blank" control and one contained the fat stimulus under evaluation. Subjects were instructed to taste the three samples, without swallowing, and to choose the sample that was different (i.e., the one with fat). The subjects rinsed their mouth with deionized water before and after tasting each sample. The concentration of oleic acid or triolein in the emulsion presented was increased after a single incorrect response and decreased after two correct responses in a row. A reversal was considered to have occurred at points where the concentration sequence changed direction. The procedure was terminated when four reversals that met the following two criteria occurred. First, there were no more than two dilution steps between the two successive reversals. Second, the series of reversals could not form an ascending pattern (i.e., one in which positive and negative reversals are achieved at successively higher concentrations). These additional criteria ensure a more stable measure of the threshold attained (36). The threshold concentration was then calculated as the mean of the log values of the last four reversals. To control for visual and olfactory cues, testing was conducted under red light and participants wore nose clips. Personnel involved in the sensory test were blinded to the genotype groups.

Food consumption, fat preferences, and food-specific cravings

Subjects' dietary intakes were evaluated by a trained dietitian on each testing day. Dietary intake data was collected and analyzed using Nutrition Data System for Research (NDSR) software version 2009, developed by the Nutrition Coordinating Center, University of Minnesota, Minneapolis, MN. The NDSR software

utilizes the 24-h diet recall with a multiple-pass system. The 24-h recall is an in-depth interview that collects detailed information on all foods and beverages consumed by a participant during the previous 24 h. Mean energy intake, total fat consumed (in grams), and macronutrient distribution (% energy from carbohydrate, protein, and fat) were quantified. In addition, subjects completed the Fat Preference Questionnaire (34) and the Food Craving Inventory (6). The Fat Preference Questionnaire is a validated self-administered test that assesses preference for dietary fat. Subjects selected the food that tastes better and is eaten more frequently from 19 sets of food. Each set is comprised of related foods differing in fat content. The percentage of food sets in which high-fat foods are reported to "taste better" (TASTE score) and to be "eaten more often" (FREQ score) is then determined and a measure of dietary fat restriction (DIFF) is created by subtracting TASTE from FREQ. The Food Craving Inventory is a 28-item validated questionnaire designed to measure the frequency of overall food cravings as well as cravings for specific types of foods. Cravings for specific types of foods (i.e., an intense desire for a specific food that is difficult to resist) are measured by four independent subscales, each consisting of four to eight items within the food category: high fats, sweets, carbohydrates/starches, and fast-food fats. Participants rated how often they experienced a craving for each of the foods over the past month using a 5-point Likert scale (1 = never, 5 = always/almost every day) (6).

Statistical analyses

To determine the acute effects of orlistat and CD36 genotype on fat detection thresholds, a mixed ANOVA was conducted with type of fat (oleic acid vs. triolein) and experimental condition (orlistat day vs. control day) as the within-subjects factor and CD36 genotype (AA, AG, and GG) as the between-subjects factor. In addition, one-way ANOVAs were used to detect differences in habitual energy, macronutrient intakes, fat preferences, and food cravings as a function of CD36 genotype group. Triolein and oleic acid detection thresholds were positively skewed and required logarithmic transformation to approximate a normal distribution. When the ANOVAs revealed significant effects, post hoc Fisher Least Significant Difference analyses were conducted. Data in the tables and figures are presented as means ± SEM. All analyses were performed with STATISTICA 8.0 (StatSoft, Tulsa OK), and criterion for statistical significance was $P < 0.05$.

RESULTS

Influence of the CD36 common SNP rs1761667 on oleic acid and triolein detection thresholds

CD36 genotype affected orosensory detection of fats ($F_{(2, 18)} = 4.3$; $P = 0.03$). Subjects homozygous for the rs1761667 G-allele had lower detection thresholds for oleic acid and triolein than subjects homozygous for the A allele, which associates with lower CD36 expression **(Fig. 1)**. Detection threshold values for heterozygous subjects were intermediate of the values in homozygous subjects and not statistically different from either group.

Influence of orlistat on oleic acid and triolein detection thresholds

Overall, across all genotype groups, oleic acid was detected at significantly lower concentrations than triolein ($F_{(1,18)}$=53.4; $P < 0.00001$; **Fig. 2**). The presence of orlistat in the emulsion increased fat detection thresholds ($F_{(1,18)} = 17.0$; $P < 0.001$). However, this effect was tempered by an almost significant interaction between the effect of orlistat and type of fat (i.e., oleic acid or triolein) ($P = 0.10$). Based on previous data on animal models (22) and our a priori hypothesis, we further explored whether orlistat had a greater effect on triolein than on oleic acid detection thresholds with simple *t*-tests corrected for multiple comparisons. As shown in **Fig. 3**, orosensory detection thresholds for triolein ($t_{(20)} = 3.86$; $P < 0.001$) but not for oleic acid ($t_{(20)} = 1.24$; $P > 0.2$) were significantly higher in the orlistat day than in the control day.

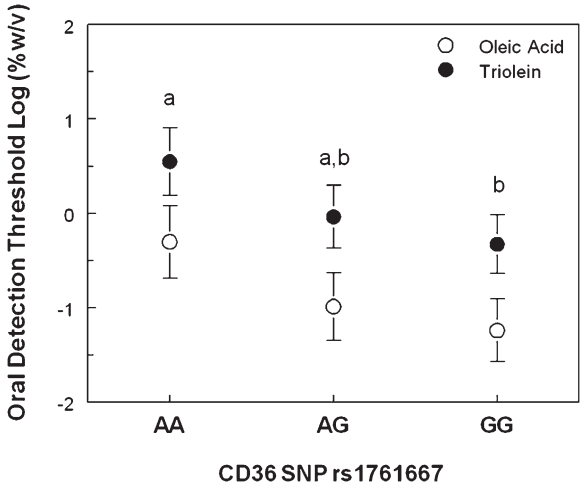

Fig. 1. Oleic acid (open symbol) and triolein (closed symbol) detection thresholds in individuals who are homozygous for the allele associated with low (AA, n = 6) or high (GG, n = 8) CD36 expression levels and in heterozygous subjects (AG, n = 7). Note that the lower the detection threshold, the higher the sensitivity. Different letters signify significant differences at $P < 0.05$ between groups.

Food consumption, fat preferences, and food cravings

Total energy, fat consumption, fat preference scores, and food cravings were similar among AA, AG, and GG subjects (all *P*-values > 0.20) (**Table 1**).

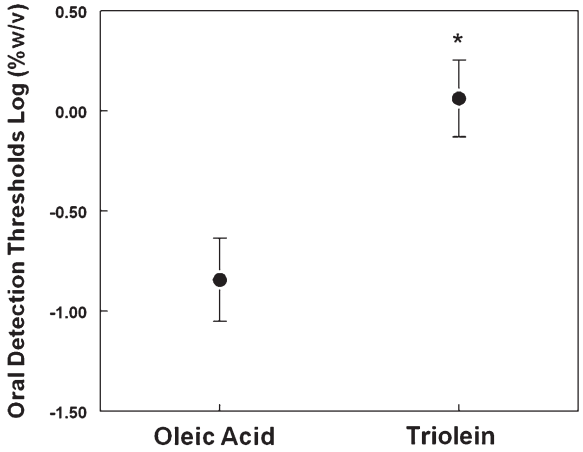

Fig. 2. Triolein and oleic acid oral detection thresholds measured in 21 obese subjects. Lower detection thresholds indicate higher sensitivity.

TABLE 1. Subject characteristics

	AA	AG	GG
Age (yrs)	38.7 ± 3.8	39.6 ± 3.5	39.1 ± 3.3
Gender			
Female	6	6	6
Male	0	1	2
Race			
African American	4	7	8
Caucasian	2	0	0
BMI (kg/m2)	34.9 ± 2.3	38.3 ± 2.1	41.5 ± 2.0
Fat Preference Questionnaire			
TASTE	68.5 ± 7.0	67.0 ± 6.5	70.1 ± 6.5
FREQ	48.8 ± 10.5	55.5 ± 9.7	59.1 ± 9.7
DIFF	19.7 ± 6.9	11.9 ± 6.4	11.1 ± 6.4
Food Craving Inventory			
High fats	2.3 ± 0.3	2.3 ± 0.3	2.4 ± 0.3
Starches	2.4 ± 0.4	2.1 ± 0.3	2.4 ± 0.3
Sweets	2.2 ± 0.2	2.8 ± 0.2	2.5 ± 0.2
Fast food fats	2.8 ± 0.4	2.8 ± 0.3	2.7 ± 0.3
General food cravings	2.4 ± 0.3	2.5 ± 0.2	2.5 ± 0.2
Dietary Intake			
Fat (g/d)	82 ± 12	77 ± 11	85 ± 11
Fat (% Kcal)	37 ± 3	37 ± 3	39 ± 3
Energy intake (Kcal/d)	1951 ± 231	1882 ± 214	1868 ± 214
Number of subjects	6	7	8

DISCUSSION

Dietary fat generates textural and aromatic cues that activate somatosensory and olfactory systems, but it is not known whether fat is perceived as a basic taste in humans (17). This issue is particularly important in obesity because obese subjects prefer foods with higher fat content (5) and crave more high-fat foods (6, 7) as compared with lean subjects (8). The data from the present study provide strong support that there is a taste component in the orosensory perception of dietary fat in obese subjects. First, we found that a genetic variant that associates with expression level of CD36, a putative lipid taste receptor, affected fat orosensory detection thresholds. Second, the presence of orlistat, a tasteless substance that is a potent lipase inhibitor, decreased the orosensory detection thresholds of triolein (a TG) more than those of oleic acid (an FA). Third, differences in subjects' thresholds for detecting triolein and oleic acid were observed under conditions where nongustatory cues were minimized.

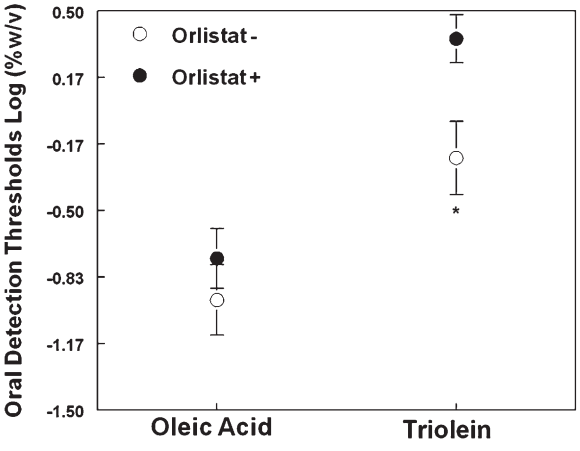

Fig. 3. Triolein and oleic acid oral detection thresholds measured in 21 obese subjects using emulsions with (Orlistat day; closed symbol) and without (Control day, open symbol) 0.5%w/v orlistat. Lower detection thresholds indicate higher sensitivity.

A major finding from the present study is that subjects homozygous for the rs1761667 G-allele were more sensitive in detecting oleic acid and triolein than subjects homozygous for the A-allele, which associates with lower CD36 expression levels, whereas subjects heterozygous for this allele were intermediate. These results are consistent with recent data from studies conducted in mice showing an association between CD36 expression level and oral fat detection (38). Mice heterozygous for CD36 deficiency ($CD36^{+/-}$) have 50% lower CD36 expression in circumvallate taste papillae than wild-type animals ($CD36^{+/+}$), and like CD36 knockout mice, they fail to exhibit spontaneous preference for fat, suggesting impaired ability to detect FA (38). The current study provides the first experimental evidence to demonstrate that CD36 is involved in fat gustatory perception in humans as observed previously in rodents (25, 27, 38). Although we did not measure CD36 expression in tongue tissue, CD36 has been identified in human taste bud cells (28).

The CD36 gene on human chromosome 7 is located close to the GNAT3 gene, which encodes α-gustducin, the primary G-protein involved in signal transduction of taste for bitter, sweet, and savory. However, it is unlikely that the altered fat detection thresholds we observed in carriers of CD36 rs1761667-A reflect alterations in *GNAT3*. Rs1761667, which associates with reduced CD36 expression (30, 31), lies between two alternative CD36 promoters, 1C and 1A and is 103.6-kb away from GNAT3, which is transcribed opposite to the direction of CD36 [UCSC gene track (GRCh37/h19)]. It has been shown previously that alterations in CD36 expression do not associate with changes in gustducin expression. Alpha-gustducin expression levels in taste buds are unaffected under conditions of lower CD36 expression or with complete CD36 deletion in mice (38). More importantly, α-gustducin is not involved in fat taste signaling. Alpha-gustducin knockout mice have robust fat preferences that are identical to those of wild-type mice (39). In addition, the signaling mechanisms involved in CD36-mediated fat perception involve pathways distinct from those involving α-gustducin (40). In humans, there does not appear to be any cross-interaction between the effects of CD36 and GNAT3 on taste perception. Detection thresholds for FA are unrelated to the sensitivity to prototypical tastants, such as sweet, sour, or umami (18) where GNAT3 plays a critical role in taste transduction signaling. Conversely, polymorphisms in GNAT3 but not those in CD36, including rs1761667 that we evaluated in our study, affect taste responses to sugar in humans (41).

Addition of orlistat to fat emulsions diminished orosensory sensitivity (i.e., increased detection thresholds) to triolein but not to oleic acid, which is consistent with earlier findings in rodents indicating that the FA is the signaling stimulus (22). These data also suggest that lingual lipase plays a functional role in the gustatory perception of dietary fat in humans. Accordingly, prolonged chewing of food that contains fat before swallowing it should allow

greater interaction between lingual lipase and dietary fat, which would increase FA concentration and thereby enhance oral fat perception. The concentration of orlistat used in our study (i.e., 0.5%w/v), which was selected based on its effectiveness in inhibiting lingual lipase in rodents (22), decreased our subjects' oral sensitivity in detecting triolein, even though it did not annul their capability in detecting it. Additional dose-response studies with lipase inhibitors and chewing time examining how decreasing or increasing oral fat sensitivity affects cephalic phases of fat digestion are needed. In addition, future studies should consider the possibility that orlistat and other lipase inhibitors could interfere with both intestinal fat absorption and gut FA sensing (42, 43).

Variations of several orders of magnitude have been reported for fat orosensory detection thresholds in humans (9, 11, 16, 18, 19). Our data concur with this and identify CD36 genotype as one of the factors contributing to the large individual differences. Other putative fat taste receptors for long chain FA, such as GPR120, have been identified in rodent and human lingual tissue (23, 24, 44) and variation in these fat taste receptors could impact human oral fat perception contributing to further variability.

Our study was conducted in subjects selected for both obesity (presumably with high fat consumption) and the CD36 genotype. BMI affects fat orosensory detection thresholds; the higher the BMI, the lower the oral sensitivity in detecting oleic acid (16, 18), although it remains unknown whether the effect of BMI involves altered expression of CD36 and other putative fat taste receptors. Oral and gastrointestinal sensitivities to oleic acid are related to each other and inversely associated with dietary fat consumption (16). However, whether the decreased oral and gastrointestinal sensory sensitivity to fats is a cause or a consequence of obesity cannot be determined from association studies. Data from recent work in human subjects show that dietary fat manipulations alter oral (20) and gastrointestinal (45) sensitivity to fat. In lean subjects, oral sensitivity to detect the taste of oleic acid is decreased after 4 weeks on a high-fat diet and increased after 4 weeks on a low-fat diet (20). These findings are consistent with studies conducted in rodents showing that a high-fat diet decreases CD36 expression in taste buds cells (38, 46) and reduces intestinal sensory sensitivity to the presence of fat (47). In obese subjects, oral sensitivity to oleic acid is unchanged after 4 weeks on a high-fat diet (20). On the other hand, it is increased after 4 weeks on a low-fat diet (20). Similarly, acute dietary restriction in obese subjects enhances gastrointestinal sensitivity to fat, which is associated with an increased effect of fat on satiation (45). Although we demonstrated the existence of a relationship between fat perception sensitivity and genotype, our study was not able to determine whether oral fat perception sensitivity affects fat intake or body weight. Future studies are needed to answer this important question.

To our knowledge, this is the first study to measure orosensory detection thresholds of a TG (i.e., triolein) and its constituent FA (i.e oleic acid) in the same subjects, which permits a robust comparison of the relative orosensory sensitivity. We could effectively measure FA and TG orosensory sensitivity in our subjects when visual and olfactory cues were eliminated and textural cues minimized. Subjects were less sensitive in detecting triolein than oleic acid, despite triolein having higher viscosity (48), which supports the notion that taste rather than texture is the primary detection mechanism in our threshold measurements. However, interactions between the gustatory and trigeminal pathways might occur in the oral cavity and contribute to the detection thresholds we measured, analogous to the documented olfactory/trigeminal interactions in nasal chemoreception (49). CD36 and other putative fat receptors are present in trigeminal neurons (50), so the potential contribution of the trigeminal pathway, i.e., via sensations of pungency or oral burn, on fat oral perception needs further study.

In summary, our findings support the existence of a taste component in orosensory perception of dietary fat in humans. We found that a genetic variant in the FA translocase gene CD36 and lipase inhibition affect oral taste sensitivity to oleic acid and triolein in obese subjects. These findings have important implications in understanding factors involved in the regulation of food intake. A better understanding of the sensory mechanisms underlying oral and gastrointestinal fat sensing could lead to new strategies in food design and dietary therapy for obesity.

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