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Endocannabinoid signaling in the gut mediates preference for dietary unsaturated fats

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ABSTRACT **Dietary fat exerts a potent stimulatory effect on feeding. This effect is mediated, at least in part, by a cephalic mechanism that involves recruitment of the vagus nerve and subsequent activation of endocannabinoid signaling in the gut. Here, we used a sham-feeding protocol in rats to identify fatty-acid constituents of dietary fat that might be responsible for triggering small-intestinal endocannabinoid signaling. Sham feeding rats with a corn oil emulsion increased endocannabinoid levels in jejunum, relative to animals that received either mineral oil (which contains no fatty acids) or no oil. Sham-feeding emulsions containing oleic acid (18:1) or linoleic acid (18:2) caused, on average, a nearly 2-fold accumulation of jejunal endocannabinoids, whereas emulsions containing stearic acid (18:0) or linolenic acid (18:3) had no such effect. In a 2-bottle-choice sham-feeding test, rats displayed strong preference for emulsions containing 18:2, which was blocked by pretreatment with the peripherally restricted CB_1 cannabinoid receptor antagonists, AM6546 and URB447. Our results suggest that oral exposure to the monoenoic and dienoic fatty acid component of dietary fat selectively initiates endocannabinoid mobilization in the gut, and that this local signaling event is essential for fat preference.—DiPatrizio, N. V., Joslin, A., Jung, K.-M., Piomelli, D. Endocannabinoid signaling in the gut mediates preference for dietary unsaturated fats. *FASEB J.* 27, 2513–2520 (2013). www.fasebj.org**

*Key Words: 2-arachidonoyl-*sn*-glycerol · anandamide · taste · lipids*

Fat-rich foods are inherently palatable to mammals, and evidence indicates that this innate attraction is primarily due to the orosensory detection of dietary lipids. Several putative receptors on taste buds have been proposed to detect fat "taste" and are required for the preferences displayed by rodents for fatty foods (1–3). Signals activated by these receptors travel through cranial nerves to the brain, where they elicit dopamine outflow in the ventral striatum (4), a key forebrain region involved in processing the "liking" and "wanting" of pleasurable sensory stimuli (5, 6), and stimulate further fat intake (7).

The endocannabinoid system, which comprises endogenous signaling molecules (endocannabinoids) and receptors responsible for their actions [cannabinoid 1 and 2 receptors (CB_1Rs and CB_2Rs)] and enzyme systems that catalyze their biosynthesis and degradation, is a key regulator of palatable food intake (for review, see refs. 8, 9), and pharmacological activation of this system increases the consumption of fatty foods in rats (10). Gustatory signals from food are transmitted from the oral cavity along branches of the cranial nerves VII (chorda tympani of the facial nerve), IX (lingual branch of glossopharyngeal), and X (branches of the vagus nerve) to the nucleus of the solitary tract (NST) in the brain stem (11). Sensory information provided by these afferent nerves is integrated with satiation and satiety signals coming from the viscera and is projected to the pontine parabrachial nucleus (PBN), which serves as a sensory relay node that communicates bidirectionally with forebrain structures (12, 13). Microinfusions of the endocannabinoid 2-arachidonoyl-*sn*-glycerol (2-AG) into the PBN stimulate intake of diets containing high levels of fat or sugar, but not standard rodent chow (14). Similarly, indirect activation of CB_1Rs in the PBN with an inhibitor of endocannabinoid degradation increases the intake of fatty foods (15). Furthermore, infusions of another endocannabinoid, anandamide [*N*-arachidonoylethanolamine (AEA)], into the ventral striatum, which communicates with the PBN, heighten hedonic responses to sweeteners in rats (16).

In addition to these centrally mediated actions, the endocannabinoid system may also regulate hedonically

Abbreviations: 2-AG, 2-arachidonoyl-*sn*-glycerol; 18:0 FA, 18:0 fatty acid (stearic acid); 18:1 FA, 18:1 fatty acid (oleic acid); 18:2 FA, 18:2 fatty acid (linoleic acid); 18:3 FA, 18:3 fatty acid (linolenic acid); AEA, *N*-arachidonoylethanolamine (anandamide); ANOVA, analysis of variance; CB_1R, cannabinoid 1 receptor; i.p., intraperitoneal; MO, mineral oil; NST, nucleus of the solitary tract; PBN, parabrachial nucleus

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positive taste stimuli through peripheral mechanisms. Systemic administration of endocannabinoids in mice enhances gustatory nerve responses to sweeteners, but not to salt, sour, bitter, or umami flavors (17). This effect is mimicked by stimulating isolated taste buds with endocannabinoids, and is mediated by CB_1Rs located on type 2 taste cells. A functionally similar response may occur in the proximal gut: we recently reported that sham feeding rats with liquid diets rich in fats, but not sugar or protein, markedly increases endocannabinoid accumulation in the jejunum (18). This effect is confined to the upper small intestine, requires an intact vagus nerve, and results from a coordinated modification of endocannabinoid production and degradation. Notably, local pharmacological blockade of intestinal CB_1Rs interrupts fat sham feeding, suggesting that the endocannabinoid system in the gut may be a critical component of the positive feedback mechanism required for maintaining the intake of palatable fatty diets.

Dietary fats primarily comprise complex lipids, such as triglycerides and phospholipids. Lingual lipase cleaves a small fraction of these lipids while they are still in the mouth, releasing their constituent fatty acids (19–21). In the present report, we used a sham-feeding procedure that isolates the orosensory component of feeding from its postingestive influences (22) to examine whether the textural properties of complex lipids drive gut endocannabinoid signaling or, rather, it is their fatty acid composition that is responsible.

MATERIALS AND METHODS

Animals

Adult male Sprague-Dawley rats (250–300 g) were purchased from Charles River (Wilmington, MA, USA) and were housed at room temperature (22°C) in individual stainless-steel suspension cages with wire bottoms to prevent coprophagia. Rats were maintained on a 12-h light-dark cycle (on at 6:30 AM and off at 6:30 PM) and had free access to water and standard chow pellets (Harlan Teklad 2020; Harlan Bioproducts, Indianapolis, IN, USA) throughout the study, until 7 d prior to testing (see below for more details). All procedures met the U.S. National Institutes of Health guidelines for the care and use of laboratory animals and were approved by the Institutional Animal Care and Use Committee of the University of California–Irvine.

Chemicals

The peripherally restricted CB_1 receptor antagonists, AM6545 (Sigma-Aldrich, St. Louis, MO, USA) and URB447 (Cayman Chemical, Ann Arbor, MI, USA), were dissolved in 100% DMSO (Sigma-Aldrich) and administered by intraperitoneal (i.p.) injection in a volume of 1 ml/kg. All fatty acids were purchased from Nu-Chek Prep (Elysian, MN, USA) and were >99% pure. These included stearic acid [18:0 fatty acid (FA)], oleic acid (18:1 FA), linoleic acid (18:2 FA), and linolenic acid (18:3 FA).

Sham-feeding procedure

The sham-feeding protocol used in this investigation was based on the procedure outlined by G. P. Smith (22) and was performed as described previously (18). The sham-feeding paradigm allows nutrients to pass through the mouth and esophagus, then subsequently drain out of the stomach *via* an implanted gastric cannulae. Animals were placed in individual plastic suspension cages (with a 2-cm-wide slit running the entire length of the floor of the cage) on test days 1 h (9:00 AM) prior to testing, and returned to their metal suspension cages following testing (11:00 AM). After a 1-h daily acclimation period to the test cages, the stainless-steel plugs were removed from the gastric cannulae, and the stomachs were flushed (20 ml) until the water flowed free of any particles. A stainless-steel tube (length 1.5 cm) was fitted to Silastic tubing (length 25 cm, inner diameter 0.040 inch, outer diameter 0.085 inch) and threaded into the gastric cannulae, and the tubing was then placed through a slit in the bottom of the cage to allow for the free movement of animals while feeding. Animals were given access to the liquid test diets in small sipper tubes (40 ml) and were allowed to sham feed for 30 min. The liquid diets drained into a plastic container placed beneath the test cages. The drainage tubes were removed immediately after sham feeding, and the stainless-steel plugs were threaded back into the gastric cannulae. Animals were returned to their home cages and given free access to standard chow for 6 h.

Test diets and feeding schedule

Animals were given free access to standard rodent chow for 6 h (12:00–6:00 PM) daily throughout the duration of testing. Separate groups of animals were sham fed for 30 min (10:30–11:00 AM) separate equicaloric [except for the mineral oil (MO) group] lipid emulsions (10 or 40 ml): MO (25% v/v); MO plus 18:0 FA (25% v/v MO plus 5% w/v 18:0 FA); MO plus 18:1 FA (20% v/v MO plus 5% v/v 18:1 FA); MO plus 18:2 FA (20% v/v MO plus 5% v/v 18:2 FA); and MO plus 18:3 FA (20% v/v MO plus 5% v/v 18:3 FA). The lipid emulsions were prepared in distilled water (75% v/v), as described previously (18).

Experimental design

Role of fatty acid taste in stimulating gut endocannabinoid signaling

Separate groups of animals were sham fed (30 min, 10:00–10:30 AM) for 4 consecutive days: on d 1 and d 2, animals received a MO emulsion (10 ml) in order to acclimate to the testing procedure; on d 3 and 4, animals received MO containing the appropriate fatty acid. Control animals received no test diet. Immediately following the final 30-min sham-feeding session on d 4, animals were anesthetized with isoflurane; then, the jejunum was rapidly removed and rinsed with phosphate-buffered saline (PBS) and snap-frozen in liquid N_2. All tissues were subsequently stored at −80°C until time of processing.

Role of gut endocannabinoids in mediating dietary fat intake and preference

The 2-bottle choice test in sham-feeding rats was adapted from previous studies (7, 23, 24). See **Table 1** for complete details.

TABLE 1. *Two-bottle choice test in sham-feeding rats*

Test and day	Subgroup 1	Subgroup 2	Both
One-bottle sham training			
T1	MO (10 ml)	MO + FA (10 ml)	
T2	MO + FA (10 ml)	MO (10 ml)	
T3	MO (10 ml)	MO + FA (10 ml)	
T4	MO + FA (10 ml)	MO (10 ml)	
Two-bottle sham choice test			
C1			Vehicle + both (40 ml, L/R)
C2			Vehicle + both (40 ml, R/L)
C3			CB_1R antagonist + both (40 ml each)

Each group was split into 2 subgroups during 30-min 1-bottle sham training (days T1–T4), and test diets [MO alone or MO + fatty acid (FA); 10 ml] were alternated between the left (L) and right (R) side daily for 4 d in order to ensure that no side preferences developed. For 2-bottle sham choice testing (days C1 and C2), all animals received a vehicle injection (i.p.) 20 min prior to testing. Animals were presented with 2 bottles for 30 min (30 ml each of MO or one that also contained an FA; left and right positions of the test diets were alternated), and intakes for these 2 sessions were recorded and averaged for statistical comparison *vs.* drug condition. On C3, animals received injection of the CB_1R antagonists, AM6545 or URB447, 20 min prior to sham feeding in the 2-bottle choice test.

Gastric cannulae

The surgical implantation of stainless-steel gastric cannulae into the stomach was performed as described previously (18, 22).

Tissue processing

Lipid extractions

Frozen tissues were weighed and homogenized in 1.0 ml of methanol containing [$^2H_4$]-AEA and [$^2H_8$]-2-AG (Cayman Chemical). Lipids were extracted with chloroform (2 vol), and the extracts were washed with water (1 vol). Organic phases were collected and fractionated by open-bed silica gel column chromatography, as described previously (25). Eluted fractions were dried under N_2 and reconstituted in 0.1 ml of methanol for lipid analyses.

Measurement of fatty acid ethanolamides and 2-AG

We used an 1100 liquid chromatography system coupled to a 1946D mass spectrometer detector (Agilent Technologies, Palo Alto, CA, USA) equipped with an electrospray ionization (ESI) interface. Lipids were separated on a XDB Eclipse C_{18} column (50×4.6 mm i.d., 1.8 μm, Zorbax), eluted by a gradient of methanol in water (from 85 to 90% methanol in 2.5 min) at a flow rate of 1.5 ml/min. Column temperature was kept at 40°C. Mass spectrometry detection was in the positive ionization mode, capillary voltage was set at 3 kV, and fragmentor voltage at 120 V. Lipids were quantified with an isotope-dilution method (26) monitoring sodium adducts of the molecular ions [$M+Na^+$] in the selected ion monitoring (SIM) mode.

Statistical analyses

Results are expressed as means ± SE. The significance of differences between groups was evaluated by Student's *t* test or 2-way analysis of variance (ANOVA) followed by a Tukey *post hoc* evaluation for comparison of means when significant differences were found. Analyses were made using Sigma Plot 11 (Systat Software, San Jose, CA, USA), and differences were considered significant at values of $P < 0.05$.

RESULTS

Oral exposure to nutritive, but not nonnutritive, oil stimulates jejunal 2-AG mobilization

Our previous studies showed that sham-feeding liquid diets containing fat, but not carbohydrate or protein, stimulates endocannabinoid production in the jejunum, but not other central or peripheral tissues of rats (18). Confirming those results, we found that sham feeding a suspension of corn oil in water (25% v/v) for 30 min increased the accumulation of the endocannabinoid 2-AG (8, 27) in the jejunum, when compared to controls that received no diet (corn oil; $P=0.03$; $n=4/4$; **Fig. 1**). To evaluate the role of the textural properties of dietary fat in this response, we allowed rats to sham feed a liquid diet that contained only MO, which has a texture similar to that of nutritive oils but contains no fatty acids (24). Sham intake of MO did not modify jejunal 2-AG levels (MO; $P=0.71$; $n=4/4$; Fig. 1), suggesting that the texture of dietary fat alone is not sufficient to trigger jejunal 2-AG mobilization.

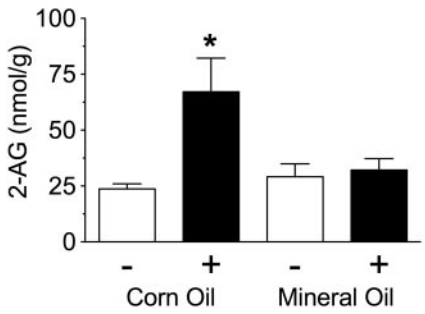

Figure 1. Sham feeding a corn oil emulsion, but not a MO emulsion, mobilizes jejunal 2-AG. Effects of 30 min sham feeding on levels of jejunal 2-AG. –, no diet presented; +, diet presented. Results are expressed as means ± SE; $n = 4$/condition. Unpaired Student's *t* tests, 2-tailed, between no-diet and diet conditions. $^*P < 0.05$ *vs.* corresponding no-diet group.

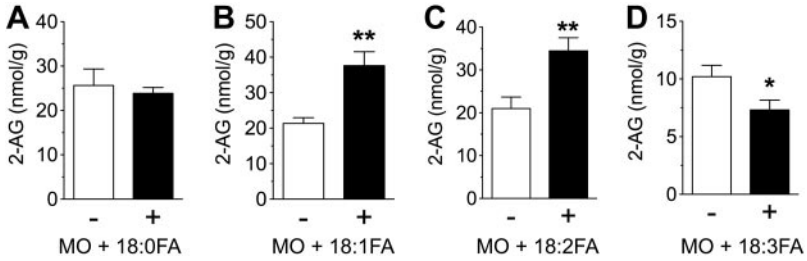

Figure 2. Sham feeding emulsions containing monoenoic or dienoic fatty acids, but not saturated or polyunsaturated fatty acids, mobilizes jejunal 2-AG. Effects of 30 min sham feeding of MO + 18:0 FA (*A*), MO + 18:1 FA (*B*), MO + 18:2 FA (*C*), or MO + 18:3 FA (*D*) on levels of jejunal 2-AG. –, no diet presented; +, diet presented. Results are expressed as means ± SE; n = 4–8/condition. Unpaired Student's t tests, 2-tailed, between no-diet and diet conditions; $^{*}P < 0.05$, $^{**}P < 0.01$ *vs.* corresponding no-diet group.

Monoenoic and dienoic fatty acids increase jejunal 2-AG mobilization

We next evaluated what role a key structural feature of long-chain dietary fatty acids, namely their degree of unsaturation (*i.e.*, number of double bonds) might play in jejunal endocannabinoid mobilization. First, we allowed rats to sham feed a MO suspension that contained the saturated fatty acid 18:0 FA (see Materials and Methods: Test Diets and Feeding Schedule for procedural details). Sham intake of 18:0 FA did not affect jejunal 2-AG content (P=0.67; n=4/4; **Fig. 2*A***). By contrast, sham feeding animals MO containing the monoenoic fatty acid 18:1 FA markedly increased 2-AG accumulation in the jejunum (P=0.002; n=5/4; Fig. 2*B*). A similar effect was obtained when rats were sham-fed MO containing the dienoic fatty acid 18:2 FA (P=0.008; n=6/6; Fig. 2*C*), whereas the polyunsaturated fatty acid 18:3 FA resulted in a modest decrease in jejunal 2-AG content (P=0.048; n=8/7; Fig. 2*D*).

Effects of dietary fatty acid composition on AEA mobilization

Similarly to 2-AG, AEA mobilization in jejunum was increased following sham intake of emulsions containing monoenoic (18:1 FA) and dienoic (18:2 FA) fatty acids (**Fig. 3**). Sham feeding MO suspensions in water containing 18:0 FA failed to modify jejunal AEA content (P=0.46; n=4/4; Fig. 3*A*). However, emulsions containing 18:1 FA or 18:2 FA effectively increased AEA accumulation in the jejunum (18:1 FA, P=0.02, n=5/4; 18:2 FA, P=0.02, n=6/6; Fig. 3*B, C*, respectively). In contrast to monoenoic and dienoic fatty acids, 18:3 FA did not affect AEA levels (P=0.76; n=8/7; Fig. 3*D*).

Postingestive exposure to dienoic fatty acids does not mobilize small-intestinal endocannabinoids

Sham feeding allows for the isolation of oral from postingestive influences of foods, and extensive work has confirmed that the latter are essentially absent in this test (7). Nonetheless, we sought to confirm that the mobilization of jejunal endocannabinoids following sham intake of unsaturated fats (Figs. 2 and 3) resulted solely from oral exposure, rather than postingestive events. To achieve this, we implanted intraduodenal catheters in rats and evaluated jejunal endocannabinoid levels after local infusion of an emulsion that contained 18:2 FA (5% v/v). In contrast to sham feeding (Figs. 2*C* and 3*C*), intraduodenal administration of 18:2 FA exerted no significant effects on the jejunal levels of 2-AG (P=0.41; n=4/3) or AEA (P=0.53) (**Table 2**). These results confirm that the taste of dietary fat selectively drives endocannabinoid mobilization in the jejunum, and the successful isolation of the orosensory components of feeding from postingestive influence *via* the sham-feeding paradigm under our conditions.

Gut endocannabinoid signaling controls unsaturated dietary fat intake and preference

Oral exposure to a corn oil emulsion enhances the accumulation of jejunal endocannabinoids (Fig. 1 and ref. 18), and our previous work has shown that local pharmacological blockade of this signaling event at CB_1Rs reduces the intake of corn oil (18). We utilized a 2-bottle choice test in sham feeding rats (see Table 1 for complete details) to assess the preference for 18:2 FA, the most abundant fatty acid constituent of corn oil (28) and the fatty acid that most robustly causes accumulation of jejunal endocannabinoids (Figs. 2*C* and 3*C*), *vs.* MO; and the role of gut endocannabinoid

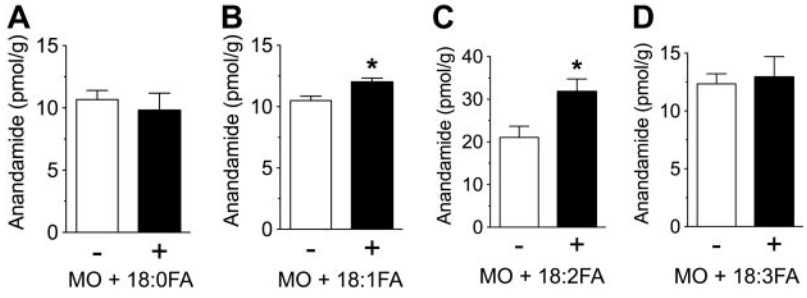

Figure 3. Sham feeding emulsions containing monoenoic or dienoic fatty acids, but not saturated or polyunsaturated fatty acids, mobilizes jejunal AEA. Effects of 30 min sham feeding of MO + 18:0 FA (*A*), MO + 18:1 FA (*B*), MO + 18:2 FA (*C*), or MO + 18:3 FA (*D*) on levels of jejunal AEA. –, no diet presented; +, diet presented. Results are expressed as means ± SE; n = 4–8/condition. Unpaired Student's t tests, 2-tailed, between no-diet and diet conditions. $^{*}P < 0.05$ *vs.* corresponding no-diet group.

TABLE 2. *Postingestive exposure to 18:2 FA fails to modify jejunal endocannabinoid levels*

Endocannabinoid	No diet	Diet
2-AG (nmol/g)	20.5 ± 2.0	17.0 ± 3.7
AEA (pmol/g)	19.7 ± 2.1	17.7 ± 2.0

Intraduodenal infusion of an emulsion containing 5% 18:2 FA did not alter levels of the endocannabinoids in the jejunum. Unpaired Student's *t* tests, 2-tailed, between no-diet (n=4) and diet (n=3) conditions.

signaling in determining the preference for dietary fat. The results showed that rats strongly prefer MO emulsions containing 18:2 FA over those containing only MO (**Fig. 4*A***: vehicle, MO *vs.* 18:2 FA, $P<0.001$; $n=5$). Pretreatment with an effective dose of the peripherally restricted neutral CB_1R antagonist, AM6545 (29), reduced the intake and preference for 18:2 FA, when compared to vehicle treatment (Fig. 4*A*: AM6545 18:2 FA *vs.* vehicle 18:2 FA, $P<0.05$; $n=5$). Similarly to AM6545, pretreatment with an effective dose of URB 447 (18, 30), a structurally different but also peripherally restricted neutral CB_1R antagonist, reduced the intake and preference for 18:2 FA *vs.* vehicle treatment (Fig. 4*B*: URB447 18:2 FA *vs.* vehicle 18:2 FA, $P<0.05$; $n=6$).

We next evaluated whether rats prefer 18:0 FA, the intake of which does not modify endocannabinoid levels in jejunum (Figs. 2*A* and 3*A*), over MO in the 2-bottle choice sham-feeding test. In contrast to 18:2 FA, rats preferred emulsions containing only MO to those containing both MO and 18:0 FA (Fig 4*C*: vehicle, MO *vs.* 18:0 FA, $P<0.05$, $n=5$). Pretreatment with AM6545 failed to affect the intake of MO when compared to vehicle treatment (Fig. 4*C*: AM6545 MO *vs.* vehicle MO, $P=0.7$; $n=5$).

DISCUSSION

In the present investigation, we report that orosensory exposure to the free fatty acid component of dietary fat, rather than fat texture, mobilizes endocannabinoid substances in the rat jejunum; this effect is mediated by monoenoic (18:1 FA) and dienoic (18:2 FA) fatty acids, but not saturated (18:0 FA) or polyunsaturated (18:3 FA) fatty acids; rats display, when given a choice, a strong preference for 18:2 FA *vs.* a nonnutritive MO that contains no fatty acids, but has similar textural properties to that of nutritive oils; and this preference for unsaturated fatty acids is impaired by pharmacological blockade of peripheral CB_1Rs. Collectively, the results suggest that the taste of monoenoic and dienoic fatty acids in the diet initiates endocannabinoid signaling at CB_1Rs of the proximal small intestine, and this signaling event controls the preference displayed by rats for dietary fat.

We recently reported that oral exposure to a corn oil emulsion, but not an emulsion containing only carbohydrate or protein, triggers the accumulation of the endocannabinoids 2-AG and AEA in the jejunum of rats. This response was found to be tissue specific and inhibited by pharmacological blockade of CB_1Rs in the gut (18). These studies provide supporting evidence for the existence of a fat taste, and a critical role of the gut endocannabinoid system in controlling the intake of dietary fats based on their orosensory characteristics.

Fat is becoming recognized as a primary taste quality, and several receptors in the oral cavity are involved in its detection (1–3). For example, when given a choice, rodents display a robust preference for diets rich in fats, including 18:2 FA (31, 32), but such preference is absent in mutant mice that lack CD36 (1, 33), a putative fat receptor expressed in taste buds and other tissues (34). Furthermore, application of 18:2 FA to the tongue of anesthetized CD36-null mice does not elicit the release of digestive secretions found in control mice under the same conditions (1). Together, these results suggest that CD36 is a critical component for the detection of dietary unsaturated fatty acids. Dietary lipids from foods, however, are mostly found in the form of triglycerides and phospholipids, not free fatty acids. Nonetheless, studies suggest that triglycerides are not readily detected in the oral cavity; rather, it is their

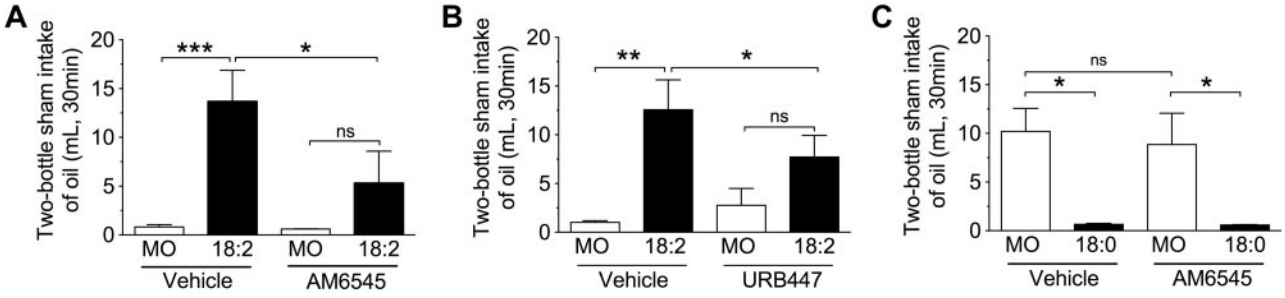

Figure 4. Jejunal endocannabinoid signaling at CB_1Rs controls dienoic fatty acid intake and preference. *A*) Sham-feeding rats prefer an emulsion containing MO + 18:2 FA *vs.* MO alone (vehicle 18:2 *vs.* vehicle MO), an effect reduced by i.p. (10 mg/kg) administration of AM6545 (AM6545 18:2 *vs.* vehicle 18:2). *B*) Intraperitoneal administration of URB447 (20 mg/kg) also reduced the preference for 18:2 *vs.* MO (URB447 18:2 *vs.* vehicle 18:2). *C*) Sham-feeding rats prefer an emulsion containing MO alone *vs.* one that also contained 18:0 FA (vehicle 18:0 *vs.* vehicle MO), and pretreatment with AM6545 failed to affect this response (AM6545 MO *vs.* vehicle MO). Results are expressed as means ± SE; n = 5–7/condition. Two-way repeated-measures ANOVA, with a Tukey *post hoc* evaluation of means. ns, not significant. $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

free fatty acid component that contributes to fat taste (35). Lingual lipases, which liberate free fatty acids from triglycerides, are released from glands located on the tongue of humans (*i.e.*, Ebner's glands; refs. 20, 21) and rodents (19). Pharmacological blockade of triglyceride hydrolysis with an inhibitor of lingual lipase activity (tetrahydrolipstatin) reduced preferences for triglycerides, but not for free fatty acids, in a short-term 2-bottle choice test in rats (35). This observation suggests that mammals detect free fatty acids in the oral cavity, and their release from triglycerides by lingual lipase is required for dietary fat taste and preference. Our present findings are congruent with those results: oral exposure to the unsaturated free fatty acids 18:1 FA or 18:2 FA increases endocannabinoid accumulation in the proximal small intestine (Figs. 2 and 3). Furthermore, the mobilization of gut endocannabinoids is specific for monoenoic and dienoic fatty acids, because sham feeding the saturated fatty acid 18:0 FA or the polyunsaturated fatty acid 18:3 FA failed to drive this biochemical event in the jejunum (Figs. 2 and 3). The specificity of this response to dietary fatty acids with distinct levels of unsaturation provides additional evidence that dietary lipids are detected by a receptor-dependent mechanism in the oral cavity, for which CD36 is a plausible candidate. A role for CD36 and lingual lipase activity in initiating endocannabinoid signaling in the jejunum remains, however, to be determined.

Our results indicate that the texture of dietary fats alone is not sufficient to cause accumulation of endocannabinoids in the jejunum, because, in contrast to corn oil, oral exposure to MO alone failed to elicit this biochemical response. Nonetheless, MO is readily consumed by rodents (24), which might suggest that texture is also important in maintaining fat intake. When given a choice, however, animals reliably prefer corn oil to MO (36). We show here that when sham-feeding rats are given a choice in a 2-bottle choice test between emulsions containing only MO and one also containing 18:2 FA, the most abundant fatty acid found in corn oil (28) and a potent stimulator of intestinal endocannabinoid accumulation (Figs. 2 and 3), animals intensely prefer 18:2 FA over MO (Fig. 4*A*, *B*). We further show that pharmacological inhibition of endocannabinoid signaling in the gut with peripherally restricted neutral CB_1R antagonists, given just prior to the 2-bottle choice test, blocks the intake of and the preference for 18:2 FA (Fig. 4*A*, *B*). In contrast to the clear preference for 18:2 FA *vs.* MO (Fig. 4*A*, *B*), 18:0 FA was not preferred over MO (Fig. 4*C*). Surprisingly, the opposite was true: when given a choice, animals preferred MO to 18:0 FA, and this preference was not modified by CB_1R antagonism (Fig. 4*C*). This observation is consistent with previous results showing that mice exhibit high licking rates to corn oil and long-chain unsaturated fatty acids, including 18:1 FA and 18:2 FA, but low responses to saturated fatty acids (37). Collectively, the available data suggest that endocannabinoid signaling in the small intestine contributes to the preference that rats display for fatty foods rich in unsaturated fatty acids (*i.e.*, 18:2 FA and 18:1 FA), but not saturated or polyunsaturated fatty acids (*i.e.*, 18:0 FA or 18:3 FA, respectively).

The levels of dietary 18:2 FA have increased in Western diets over the 20th century, from 1% of total energy intake to 8%, and this rise positively correlates with a dramatic escalation in the prevalence of obesity during this same time period (38). A seminal study in humans demonstrated that chronic intake of meals enriched with an isocaloric quantity of 18:2 FA, at the expense of 18:0 FA, led to substantial increases in the content of adipose 18:2 FA, which was positively correlated with weight gain when compared to control subjects maintained on control diet (39). The endocannabinoid system is a likely biological contributor to weight gain after chronic exposure to fat-rich foods. Indeed, levels of liver and erythrocyte 2-AG and AEA were increased in mice maintained for 14 wk from weaning on a high-fat diet (60% of total energy) that contained 8% 18:2 FA *vs.* those maintained on a high-fat diet that contained 1% 18:2 FA (38). Furthermore, levels of jejunal 2-AG were elevated in mice maintained on a high-fat diet for 8 wk (40); however, the role that small-intestinal endocannabinoids might play in obesity in response to chronic oral exposure to 18:2 FA has not been determined.

Several studies have linked the peripheral endocannabinoid system to obesity in humans. For example, plasma endocannabinoid levels were found to be positively correlated with several markers of obesity, including body mass index, visceral fat mass, and waist circumference (41–43). A recent report demonstrated elevated endocannabinoid levels in saliva of obese insulin-resistant humans under fasting conditions, when compared to normal weight controls (44). Notably, such levels were positively correlated with body mass index, waist circumference, and plasma insulin concentrations. Furthermore, systemic administration of endocannabinoids in mice was shown to increase gustatory neural activity from the tongue, which contains CB_1Rs on type 2 taste cells, in response to lingual application of sweet tastants (17). Together, these studies suggest that elevated endocannabinoid levels in the saliva of obese subjects might contribute to the overconsumption of carbohydrate-rich foods by enhancing gustatory neurotransmission associated with palatable sweet taste. It would be of interest to evaluate whether activation of lingual CB_1Rs modifies gustatory neural responses to dietary fats, which may also drive fat overconsumption in obese individuals when salivary endocannabinoid content is high. Indeed, gustatory neurotransmission is important in maintaining dietary fat detection and preference in rodents, because surgical transection of the chorda tympani or glossopharyngeal nerves, which transmit gustatory information from the oral cavity to the brainstem, impairs preferences for dietary fats, including 18:2 FA (45–48).

The precise mechanism by which CB_1R signaling in the gut controls the intake and preference for dietary

fats remains to be determined. A recent report identified the expression of CB_1Rs in duodenal enteroendocrine I cells, which release the satiation signal, cholecystokinin (49). Thus, it is plausible that tasting monoenoic and dienoic fatty acids increases endocannabinoid signaling at CB_1Rs located on enteroendocrine I cells, which, in turn, regulates the intake of fatty foods by modifying the expression or release of neurohumoral satiation/satiety signals from the small intestine, such as cholecystokinin. The existence of other mechanisms cannot be excluded, however.

In summary, the results of the present study suggest that overactivity of the endocannabinoid system in the proximal small intestine might contribute to obesity when energy intake exceeds expenditure in modern environments that provide a disproportionate quantity of energy from foods that contain high levels of unsaturated fats. Targeted inhibition of this signaling event in the intestine might provide substantial benefits for the control of compulsive eating and obesity, and resulting pathologies. FJ

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Endocannabinoid signaling in the gut mediates preference for dietary unsaturated fats

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