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Title

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Journal

Cell Metabolism, 8(4)

ISSN

1550-4131

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Publication Date

2008-10-01

DOI

10.1016/j.cmet.2008.08.005

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Peer reviewed



Published in final edited form as:

Cell Metab. 2008 October ; 8(4): 281–288. doi:10.1016/j.cmet.2008.08.005.

The lipid messenger OEA links dietary fat intake to satiety

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Summary

The association between fat consumption and obesity underscores the need to identify physiological signals that control fat intake. Previous studies have shown that feeding stimulates small-intestinal mucosal cells to produce the lipid messenger oleoylethanolamide (OEA) which, when administered as a drug, decreases meal frequency by engaging peroxisome proliferator-activated receptors- α (PPAR- α). Here we report that duodenal infusion of fat stimulates OEA mobilization in the proximal small intestine, whereas infusion of protein or carbohydrate does not. OEA production utilizes dietary oleic acid as a substrate and is disrupted in mutant mice lacking the membrane fatty-acid transporter CD36. Targeted disruption of CD36 or PPAR- α abrogates the satiety response induced by fat. The results suggest that activation of small-intestinal OEA mobilization, enabled by CD36-mediated uptake of dietary oleic acid, serves as a molecular sensor linking fat ingestion to satiety.

Introduction

Feeding stimulates cells in the mucosal layer of duodenum and jejunum to produce OEA (Fu et al., 2007; Fu et al., 2008), suggesting that this lipid messenger participates in the induction of satiety. Consistent with this idea, administration of OEA causes in rodents a marked reduction in food intake, which is both pharmacologically and behaviorally selective (Gaetani et al., 2003; Proulx et al., 2005; Rodríguez de Fonseca et al., 2001). This anorexic action is accounted for by the ability of OEA engages PPAR- α (Fu et al., 2003), a lipid-activated nuclear receptor implicated in regulating the absorption, storage and utilization of dietary fat (Bookout et al., 2006; Evans et al., 2004; Lefebvre et al., 2006). Indeed, the hypophagic effects of exogenous OEA are abolished by genetic deletion of PPAR- α (Fu et al., 2003), are closely mimicked by administration of synthetic PPAR- α agonists (Astarita et al., 2006a; Fu et al., 2003), and are accompanied by changes in the expression of PPAR- α target genes such as the membrane lipid transporter CD36 (Fu et al., 2003). Furthermore, the small-intestinal concentrations reached by OEA after feeding (100–400 nM) (Fu et al., 2007) are sufficient to fully activate PPAR- α (median effective concentration, EC₅₀ \approx 100 nM) (Astarita et al., 2006a; Fu et al., 2003), but not GPR119 (EC₅₀ \approx 3 μ M) or TRPV1 (EC₅₀ \approx 2 μ M), two receptors

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that have been also implicated in the pharmacological actions of OEA (Ahern, 2003; Overton et al., 2006).

The molecular steps leading from OEA-dependent activation of PPAR- α to feeding inhibition are still incompletely understood. PPAR- α may act by influencing expression of satiety-inducing proteins such as apolipoprotein A-IV (Nagasawa et al., 2007; Tso and Liu, 2004; Whited et al., 2005). However, the rapid onset of the OEA response (<30 min) and its reliance on an intact vagal sensory innervation suggest the initial involvement of a transcription-independent signal that recruits sensory vagal afferents in the gut (Rodríguez de Fonseca et al., 2001). This signal remains unidentified, though the ability of PPAR- α and other nuclear receptors to elicit rapid non-genomic responses has been documented (Bravo et al., 2006; Kumar et al., 2007; LoVerme et al., 2006; Zanello and Norman, 2004).

Exogenous OEA modulates feeding by prolonging the time interval between meals (Fu et al., 2003; Gaetani et al., 2003; Oveisi et al., 2004). This mode of action is noteworthy for two reasons. First, it distinguishes OEA from gut-derived peptides such as cholecystokinin (CCK), which inhibit food intake by influencing meal size rather than meal timing (Moran, 2006; Wren and Bloom, 2007). Second, it is reminiscent of the effects exerted by duodenal infusion of dietary fat which, similar to OEA, lowers meal frequency (Woltman and Reidelberger, 1995) through an as-yet-uncharacterized mechanism that requires activation of the sensory vagus (Randich et al., 2000; Tamura and Ritter, 1994; Woltman et al., 1995). This similarity led us to hypothesize that OEA mobilization in the proximal small intestine might provide a functional link between fat ingestion and across-meal satiety.

Results

Dietary fat stimulates small-intestinal OEA mobilization

Infusion of a lipid emulsion (Intralipid, 2 kcal/ml) into the duodenum of awake catheterized rats, at a rate that mimicked gastric emptying (Kaplan et al., 1997), caused a rapid increase in jejunal OEA levels, whereas infusion of glucose or amino acid solutions of equal caloric and osmotic value had no such effect (Fig. 1A). Moreover, control infusions of physiological or hyperosmotic saline did not alter jejunal OEA content (Fig. 1A). To determine whether free fatty acids generated during lipid digestion promote OEA formation, we administered emulsions of monounsaturated oleic acid (18:1 Δ^9) or saturated palmitic acid (16:0) (each at 2 kcal/ml). Oleic acid infusion increased jejunal OEA levels (Fig. 1B), but not those of its saturated analog palmitoylethanolamide (PEA) (Supplementary Fig. 1A). By contrast, palmitic acid infusion had no effect on either OEA (Fig. 1B) or PEA content (Supplementary Fig. 1B), even though it significantly increased palmitic acid levels in jejunal tissue (Supplementary Fig. 1C).

Feeding regulates three distinct steps of OEA metabolism (Fu et al., 2007): (i) it increases biosynthesis of the OEA precursor, *N*-oleoyl-phosphatidylethanolamine (NOPE), catalyzed by an as-yet-uncharacterized *N*-acyltransferase (NAT) activity; (ii) it activates the OEA-producing enzyme *N*-acyl-phosphatidylethanolamine phospholipase D (NAPE-PLD); and (iii) it inhibits the OEA-degrading enzyme fatty-acid amide hydrolase (FAAH). Oleic acid infusion mimicked all these effects, as it raised jejunal levels of the representative NOPE species, 1-stearoyl-2-arachidonoyl-*sn*-glycero-3-phosphoethanolamine-*N*-oleoyl (likely resulting from enhanced NAT activity) (Fig. 1C), it stimulated NAPE-PLD activity (Fig. 1D), and it suppressed FAAH activity (Fig. 1E). The results suggest that ingestion of fat, but not protein or carbohydrate, initiates OEA mobilization in rat duodenum and jejunum. Notably, duodenal infusion of the unsaturated constituent of dietary fat, oleic acid, is sufficient to recapitulate this response.

Postingestive control of OEA mobilization

The infusion of lipids into the duodenum bypasses oral sensory mechanisms that monitor fat availability (Greenberg and Smith, 1996), implying that small-intestinal OEA mobilization is not critically dependent on cephalic sensors. To further evaluate this possibility, we used a sham-feeding procedure that gauges the impact of orosensory stimulation on food intake in the absence of postingestive feedback (Sclafani and Ackroff, 2004). Sham feeding rats with either a lipid emulsion (Intralipid) or a nutritionally complete liquid diet (Ensure[®]) had no effect on duodenal and jejunal OEA levels (Supplementary Fig. 2), supporting the suggestion that fat stimulates OEA mobilization through a postingestive mechanism localized in the upper gut.

Role of the lipid transporter CD36 in OEA production

How do cells in small-intestinal mucosa detect ingested lipids to mobilize OEA? We hypothesized that the membrane glycoprotein CD36 (Febbraio et al., 2001) may participate in this process because of its localization to the apical surface of the absorptive epithelium (Poirier et al., 1996) and its ability to bind long-chain fatty acids, translocate them across cell membranes (Ehehalt et al., 2006), and initiate intracellular signaling responses (Huang et al., 1991). To test this idea, we food deprived wild-type C57/B16 mice and CD36-null mice generated on the same background (Febbraio et al., 1999), and measured small-intestinal OEA and NOPE contents before and 30 min after re-exposure to food. As expected from previous data (Fu et al., 2003), wild-type mice responded to feeding with an increase in OEA and NOPE levels (Fig. 2A,C). This response was completely absent in mice lacking CD36 (Fig. 2B,D), indicating that this protein plays an obligatory function in food-stimulated OEA production.

Food-derived oleic acid is a substrate for OEA production

Despite initial uncertainty (Chen et al., 2001; Drover et al., 2005), the role of CD36 in small-intestinal fatty acid transport is now firmly established (Nassir et al., 2007). Consistent with such role, we found that CD36 deletion suppresses oleic-acid uptake into the duodenum and reduces it in the jejunum of mice that were fasted for 6h and then refed for 30 min (Fig. 3A,B). This prompted us to examine whether transport of exogenous oleic acid into mucosal cells contributes to OEA biosynthesis. We infused the duodenum of rats with 10Z-heptadecenoic acid (HDA, 17:1 Δ^{10}), which chemically resembles oleic acid but is not naturally found in mammalian tissues, and determined the fate of this tracer in mucosal lipid extracts by liquid chromatography coupled to ion-trap mass spectrometry (LC/MS) (Astarita et al., 2006b; Fu et al., 2007). HDA was primarily recovered either as non-esterified fatty acid (Fig. 3C) or as glycerol ester in two essential constituents of chylomicrons: triacylglycerol and phosphatidylcholine (Supplementary Fig. 3; for identifications see Supplementary Fig. 4). Importantly, HDA infusion was also accompanied by significant formation of the OEA analog heptadecenylethanolamide and its precursor *N*-10-heptadecenoyl-PE (Fig. 3D,E; for identifications see Supplementary Fig. 5). Oral administration of HDA in wild-type mice yielded similar results (Fig. 3F,G). By contrast, intestinal HDA absorption and heptadecenylethanolamide formation were both suppressed in CD36-null mice (Fig. 3F,G). The results indicate that exogenous HDA is internalized by small-intestinal cells through a CD36-dependent pathway, and is then targeted to the production of NOPE (catalyzed by NAT) and the subsequent conversion of NOPE into OEA (catalyzed by NAPE-PLD). These reactions likely occur in enterocytes, the main mucosal cell type that expresses both CD36 (Poirier et al., 1996) and NAPE-PLD (Fu et al., 2007). We conclude, therefore, that dietary oleic acid, in addition to its ability to regulate enzyme activities involved in the formation and degradation of OEA (Fig. 1C-E), serves also as metabolic precursor for enterocyte OEA biosynthesis.

Plasma-derived oleic acid is not converted to OEA

Enterocytes internalize diet-derived fatty acids at their apical cell membrane and plasma-derived fatty acids at their basolateral membrane. This process is functionally polarized, so that fatty acids crossing the apical surface are channeled to different metabolic pathways than those crossing the basolateral surface: the former are routed to chylomicron formation, while the latter are utilized for intracellular β -oxidation and phospholipid remodeling (Gangl and Ockner, 1975; Levin et al., 1992). The finding that dietary oleic acid is a precursor for OEA prompted us to ask whether plasma oleic acid may be used for the same purpose. To address this question, we injected HDA in the peritoneum of rats, thus allowing the tracer to access the intestinal mucosa *via* the mesenteric circulation rather than the intestinal lumen. Intraperitoneal HDA administration markedly increased HDA levels in duodenal mucosa (Fig. 4A), yet failed to produce any detectable change in mucosal heptadecenoyl ethanolamide content (Fig. 4B). The results confirm that food-derived, rather than plasma-derived oleic acid serves as substrate for OEA production.

OEA signaling mediates fat-induced satiety

The entry of ingested fat into the duodenum triggers a feedback mechanism that modulates feeding through activation of the sensory vagus (Greenberg et al., 1990; Woltman and Reidelberger, 1995). Although release of CCK from enteroendocrine cells may participate in this response, other as-yet-unidentified mediators are also likely to be involved. Indeed, CCK receptor antagonists only partially inhibit the anorexic effects elicited by lipid infusion, particularly when higher lipid loads are administered, implying the existence of a CCK-independent pathway by which dietary fat regulates feeding (Meyer et al., 1998; Whited et al., 2005; Woltman et al., 1995). To test whether OEA signaling contributes to the satiating properties of fat, we assessed the impact of duodenal Intralipid infusion on food intake in mutant C57/B16 mice lacking either CD36, which enables OEA mobilization, or PPAR- α (Gonzalez et al., 1995), which mediates the anorexic effect of OEA. The lipid emulsion markedly reduced feeding in wild-type CD57/B16 mice, but failed to do so in CD36-null (Fig. 5A) or PPAR- α -null mutants (Fig. 5B). The possibility that free fatty acids derived from Intralipid digestion directly activate intestinal PPAR- α (Bocos et al., 1995; Götlicher et al., 1992) appears to be unlikely for three reasons: firstly, common dietary fatty acids are approximately 1000-fold less potent than OEA at activating PPAR- α ($EC_{50} \approx 100 \mu\text{M}$ for fatty acids *vs* $\approx 100 \text{nM}$ for OEA) (Fu et al., 2003; Götlicher et al., 1992); secondly, fatty acids are rapidly metabolized in enterocytes (Mansbach and Gorelick, 2007), which would limit their ability to act as signaling molecules; finally, and most importantly, intraperitoneal fatty acid injections exert no anorexic effect in rats (Rodríguez de Fonseca et al., 2001), even though they increase mucosal fatty acid content (Fig. 3 D,E), suggesting that elevations in mucosal fatty acid levels are not sufficient to evoke satiety. Thus, a more plausible explanation of our results is that lipid infusions inhibit food intake by stimulating OEA production. This interpretation views the conversion of dietary oleic acid to OEA as an activating process that maximizes the fatty acid's ability to interact with PPAR- α .

OEA signaling at PPAR- α contributes to physiological satiety

The insensitivity of PPAR- α -null mice to the anorexic effects of fat infusions suggests that OEA signaling at PPAR- α may contribute to the physiological induction of satiety. To address this possibility, we studied meal patterns (Gaetani et al., 2003) in wild-type and PPAR- α -null mice freely feeding on a standard lab chow. PPAR- α -null mice started their first nocturnal meal significantly earlier than did wild-type controls (Fig. 6A,B). In contrast, mice of the two genotypes consumed similar amounts of food (PPAR- α -null: $14.0 \pm 3.3 \text{ g/kg}$; wild-type: $9.8 \pm 1.9 \text{ g/kg}$; $P > 0.05$, $n = 10-12$), over equivalent time periods (PPAR- α -null: $2.1 \pm 0.7 \text{ min}$; wild-type: $1.4 \pm 0.4 \text{ min}$; $P > 0.05$, $n = 10-12$). In addition, their blood glucose levels at dark onset

were identical (PPAR- α -null: 142.8 ± 9.8 mg/dl; wild-type: 128.1 ± 7.8 mg/dl; $P > 0.05$; $n = 4$). To gain further insight into the hyperphagic phenotype of PPAR- α -null mice, we measured satiety ratio (intermeal interval/meal size), a parameter that gauges the level of satiety experienced by an animal per unit of food energy ingested. During the first 3 hours of the night, PPAR- α -null mice showed markedly lower satiety ratio than did either wild-type C57/B16 mice (Fig. 6C) or mice of two different genetic strains (CD1, Sv/129) (Supplementary Table). Moreover, PPAR- α -null mice fed more often than did wild-type controls (Fig. 6D), while eating comparable amounts of food at each meal (PPAR- α -null, 12.8 ± 0.8 g/kg; wild-type, 9.7 ± 1.1 g/kg; $P > 0.05$, $n = 12$). These differences dissipated after the fourth nocturnal meal, but resulted nonetheless in significant changes in daily food intake (Fig. 6E). Importantly, PPAR- α deletion had no significant influence on either feeding-induced OEA mobilization (Fig. 6F) or general behavior. Various aspects of feeding behavior (food probing, eating rate), motor and exploratory activity (open-field locomotion, rearing) and fear-related responses (open-field thigmotaxis and number of fecal boli) were similar in wild-type and PPAR- α -null mice (Fig. 6G-H and Supplementary Fig. 6). We interpret these findings to indicate that disruption of food-stimulated OEA signaling at PPAR- α deactivates a physiological satiety mechanism that controls meal timing. Notably, this mechanism primarily operates during the first hours of nocturnal feeding, when rodents eat the first and largest of their daily meals.

Discussion

Our results suggest that dietary fat stimulates small-intestinal enterocytes to produce OEA, which initiates in turn a local PPAR- α -dependent response that delays the start of a subsequent meal (Fig. 7). Three features of this model stand out. The first centers on the mechanism governing OEA mobilization. We found that this process is selectively elicited by fat, not protein or carbohydrate, and requires CD36, a membrane glycoprotein that binds fatty acids and facilitates their transmembrane movements (Ehehalt et al., 2006). A role for CD36 as small-intestinal lipid sensor is consistent with three known properties of this protein: (i) its function as a taste receptor for unsaturated fatty acids in the mouse oral cavity (Laugerette et al., 2005); (ii) its localization to the apical surface of enterocytes in rodent duodenum and jejunum (Poirier et al., 1996); and (iii) its association with intracellular protein kinases involved in signal transduction (Huang et al., 1991). Our experiments, showing that CD36 deletion abolishes OEA mobilization and that dietary oleic acid serves both as stimulus and substrate for this reaction, indicate that CD36 may act in the dual capacity as receptor and transporter for diet-derived oleic acid. Through this two-fold action, CD36 may enable the rapid conversion, hitherto unreported, of a lipid nutrient into a cellular lipid signal.

A second feature of our model relates to the signaling modality through which OEA regulates feeding. The still limited data available do not allow us to distinguish between two possible scenarios. In one, OEA may act within small-intestinal enterocytes to induce the release of a diffusible anorexic mediator (for example, apolipoprotein A-IV, a secreted PPAR- α -regulated protein that affects vagal activity and inhibits feeding) (Nagasawa et al., 2007; Tso and Liu, 2004; Whited et al., 2005). Alternatively, OEA may diffuse away from enterocytes to recruit neighboring vagal afferents in the mucosal and submucosal layers of the gut (Berthoud et al., 2004). Despite their differences, both scenarios posit that endogenous OEA targets a subpopulation of PPAR- α located in close proximity to its sites of production. The small-intestinal localization of this signaling mechanism, suggested by our duodenal infusion and sham-feeding experiments, does not exclude the possibility that cephalic signals might modulate its function, for example by influencing autonomic outflow to the gut (Lo Verme et al., 2005). The existence of feed-back mechanisms regulating OEA mobilization is further suggested by the observation that prolonged exposure to a diet enriched in oleic acid reduces small-intestinal OEA levels in rats (Artmann et al., 2008).

A third noteworthy point of the model pertains to the physiological consequences of fat-stimulated OEA mobilization. We found that targeted deletion of PPAR- α not only suppresses the satiety response elicited by fat infusion, but also dramatically shortens the interval between subsequent meals during the first hours of normal feeding. These deficits are consistent with those expected from disruption of OEA signaling at PPAR- α , and suggest that endogenous OEA, by prolonging the intermeal interval, may help minimize the metabolic challenge created by the arrival of a fat-rich meal into the duodenum (Strubbe and Woods, 2004; Woods, 1991). Such a protective mechanism might cooperate with premeal insulin release and other cephalic anticipatory responses (Strubbe and Woods, 2004) to optimize lipid absorption and prepare the body for a subsequent meal. Indeed, PPAR- α belongs to a cluster of nuclear receptors that control the utilization of diet-derived lipid nutrients as fuels (Bookout et al., 2006), and OEA activation of PPAR- α enhances expression of lipid transporters and lipid-metabolizing enzymes in enterocytes (Bünger et al., 2007; Fu et al., 2003; Yang et al., 2007).

In conclusion, our studies identify OEA as the first physiological signal that specifically links dietary fat ingestion to across-meal satiety. Nutritional and pharmacological strategies aimed at magnifying this lipid-sensing mechanism, such as inhibitors of OEA degradation, might be useful in the treatment of obesity and other eating disorders.

Experimental Procedures

Chemicals

Reagent grade chemicals were used in all experiments. Peptone, sodium oleate, sodium palmitate, and glucose were purchased from Sigma Aldrich (St. Louis, MO). Intralipid 10% was from Pharmacia-Upjohn (Bridgewater, NJ). Vanilla Ensure[®] liquid diet was obtained from Ross Product Division (Abbott Laboratories, Abbott Park, IL). Anandamide [ethanolamine-³H] was from American Radiolabeled Chemicals (ARC, St. Louis, MO). Syntheses of OEA, PEA and other chemicals are described in Supplementary Methods.

Animals

Adult male Wistar rats (300 g) and adult male Sprague Dawley rats (300 g) were purchased from Charles River (Wilmington, MA). CD36-null mice and wild-type controls were a kind gift of Dr. Maria Febraio (Lerner Research Institute, Cleveland, OH). Male PPAR- α -null mice (B6.129S4-*Ppara*^{tm1GonzN12}) and wild-type controls (C57/B16) were purchased from Taconic (Germantown, NY). Rats and mice were individually housed in temperature and humidity controlled rooms on a 12-hour light/dark cycle, set with lights on at 5:30 a.m. Water and standard chow pellets (Prolab RMH 2500, PMI Nutrition International, Brentwood, MO) were available *ad libitum*, except when animals were food deprived. Food deprivation was conducted for 24 h in bottom-wired cages to prevent coprophagia. All procedures met the 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 and of the Albert Einstein College of Medicine of Yeshiva University.

Surgery and duodenal infusions

Duodenal chronic in-dwelling silicone catheters were implanted in rats or mice under ketamine/xylazine anesthesia (Azzara et al., 2002). After recovery, infusions were conducted for 10 min at a rate of 0.5 ml/min (rats) or 0.01 ml/min (mice). Nutrient concentrations were equicaloric at 2 kcal/ml. Additional surgical procedures are described in Supplementary Methods.

Lipid analyses

Animals were sacrificed with halothane and tissues were rapidly collected and snap-frozen in liquid N₂. Frozen tissues were weighed and homogenized in methanol (1 ml/100 mg tissue) containing the following internal standards: [²H₄]-OEA, [²H₄]-PEA, 1,2-dipalmitoyl-*sn*-glycero-3-phosphoethanolamine-*N*-heptadecanoyl (prepared as described in Supplementary Methods), heptadecanoic acid, monoheptadecanoin, diheptadecanoin, trionadecenoin (Nu-Chek Prep, Elysian, MN) and 1,2-diheptadecanoyl-*sn*-glycero-3-phosphocholine (Avanti Polar Lipids, Alabaster, AL). Lipids were extracted with chloroform (2 vol) and washed with water (1 vol). Organic phases were collected and dried under liquid N₂. Lipids were reconstituted in chloroform/methanol (1:4, vol/vol, 0.1 ml) for LC/MS analyses. Lipid identification and quantification are described in Supplementary Methods.

Enzyme Assays

NAPE-PLD activity—Duodenal and jejunal tissues, or the corresponding mucosal layers, were removed and homogenized in ice-cold Tris-HCl (20 mM, pH 7.4, 10 vol) containing 0.32 M sucrose. Tissue homogenates were centrifuged at 1,000×g for 10 min. NAPE-PLD activity was measured at 37°C for 30 min in Tris-HCl buffer (50 mM, pH 7.4) containing 0.1% Triton X-100, phenylmethylsulphonyl fluoride (1 mM), protein (100 µg) and 1,2-dipalmitoyl-*sn*-glycero-3-phosphoethanolamine-*N*-heptadecanoyl (100 µM) as substrate. The reactions were stopped by adding chloroform/methanol (2:1, vol/vol) containing [²H₄]-OEA. After centrifugation at 1,500×g at 4°C for 5 min, the organic layers were collected and dried under liquid N₂. The residues were suspended in chloroform/methanol (1:3, vol/vol, 50 µl) and analyzed by LC/MS. For quantification purposes, we monitored the [M+Na]⁺ ions of heptadecanoylethanolamide (mass-to-charge ratio, *m/z*, 334) and [²H₄]-OEA (*m/z*, 352).

FAAH activity—Tissues homogenates were centrifuged at 27,000×g for 30 min, and pellets suspended in phosphate-buffered saline (PBS, pH 7.4). Reactions were conducted at 37°C for 30 min in Tris-HCl buffer (50 mM, pH 8.0, 0.5 ml) containing fatty acid-free bovine serum albumin (0.05%), protein (50 µg) and anandamide[ethanolamine-³H] (10,000 dpm, specific activity 20 Ci/mmol). After stopping the reactions with chloroform/methanol (1:1, vol/vol, 1 ml), we measured radioactivity in the aqueous layers by liquid scintillation counting.

Feeding behavior

Food intake in free-feeding mice was recorded using an automated monitoring system, as previously described (Gaetani et al., 2003) with individual meals defined using a minimum meal size criterion of 200 mg and a minimum intermeal interval criterion of 1 min. Liquid diet (Ensure[®]) intake in mice was measured after a 6-h daytime fast. Duodenal preloads were administered at a rate of 0.01 ml/min for 10 min (2.0 kcal/ml) immediately prior to diet access. Intake was measured at 15-min intervals for 30 min. Additional behavioral analyses are described in Supplementary Methods.

Statistical analyses

Results are expressed as the mean±s.e.m of n separate experiments. The significance of differences between groups was evaluated by one-way analysis of variance (ANOVA) followed by a Dunnett's test for multiple comparisons, or Student's *t*-test with or without Welch's correction. Analyses were done with GraphPad Prism software (GraphPad Software, San Diego, CA), and differences were considered significant if *P* < 0.05.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

We thank Dr. Viviana Trezza for help with the behavioral characterization of PPAR- α -null mice and Ms. Fariba Oveisi for help with other animal experiments. This study was supported by NIH DK073955 (DP), NIH DK047208 (GJS), the Skirball Institute, the NY Obesity Research Center (NIH 5P30DK026687), and Italian Ministry of Research (PRIN) (VC).

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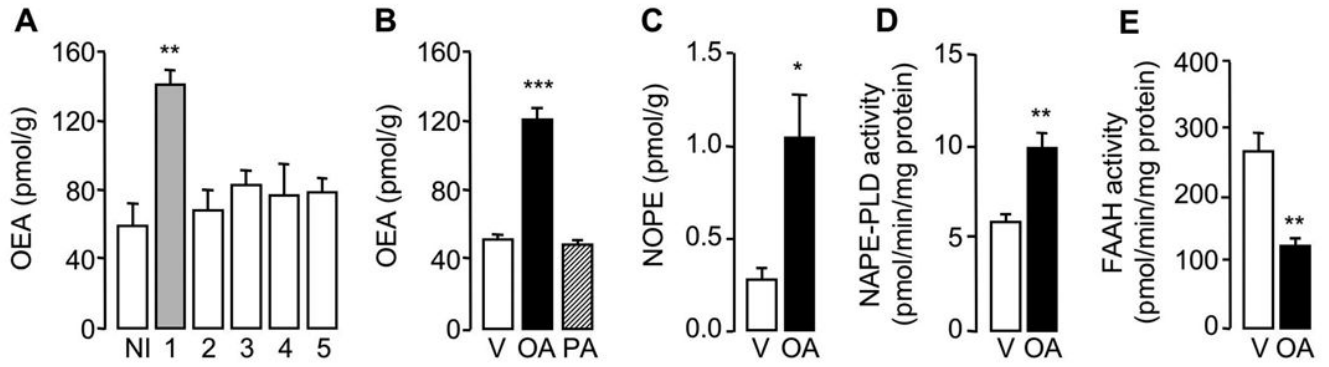


Figure 1. Dietary fat stimulates OEA mobilization in proximal small intestine

(A) Effects of duodenal nutrient infusions on jejunal OEA mobilization. *NI*, No infusion; *1*, Intralipid; *2*, glucose; *3*, peptone; *4*, hypertonic saline; *5*, isotonic saline. (B-E) Duodenal infusion of sodium oleate (OA), but not palmitate (PA), regulates jejunal levels of (B) OEA, (C) 1-stearoyl-2-arachidonoyl-*sn*-glycerol-3-phosphoethanolamine-*N*-oleyl (NOPE), (D) NAPE-PLD activity, and (E) FAAH activity. Infusions were conducted at 0.5 ml/min for 10 min with nutrients equicaloric at 2 kcal/ml. Jejunal tissue was harvested 30 min after starting the infusion; Values are expressed in pmol/g of wet tissue; one asterisk, $p < 0.05$; two asterisks, $p < 0.01$; three asterisks, $p < 0.001$; $n = 5-6$ per group.

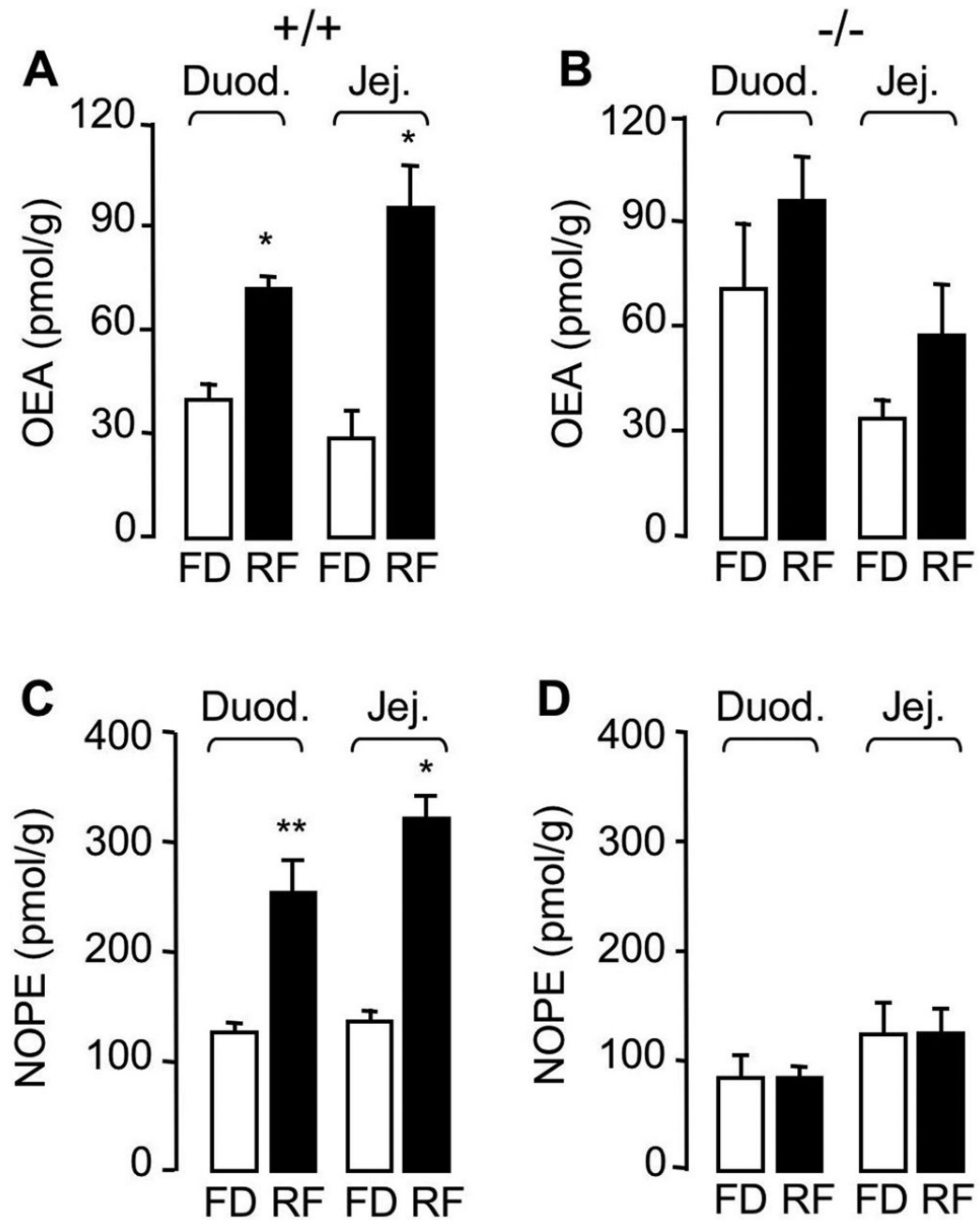


Figure 2. The fatty-acid transporter CD36 is required for small-intestinal OEA production
 Refeeding mice (RF) after a 6-h daytime fast (FD) increases levels of (A) OEA and (C) 1-stearoyl-2-arachidonoyl-*sn*-glycerol-3-phosphoethanolamine-*N*-oleyl (NOPE) in duodenum (Duod.) and jejunum (Jej.) of wild-type mice ($+/+$). No such effect is observed in CD36-null mice ($-/-$) (B,D). FD, food-deprived; RF, 30-min refeed; one asterisk, $p < 0.05$; two asterisks, $p < 0.01$; n.s., not significantly different at $p < 0.05$; $n = 3-4$ per group.

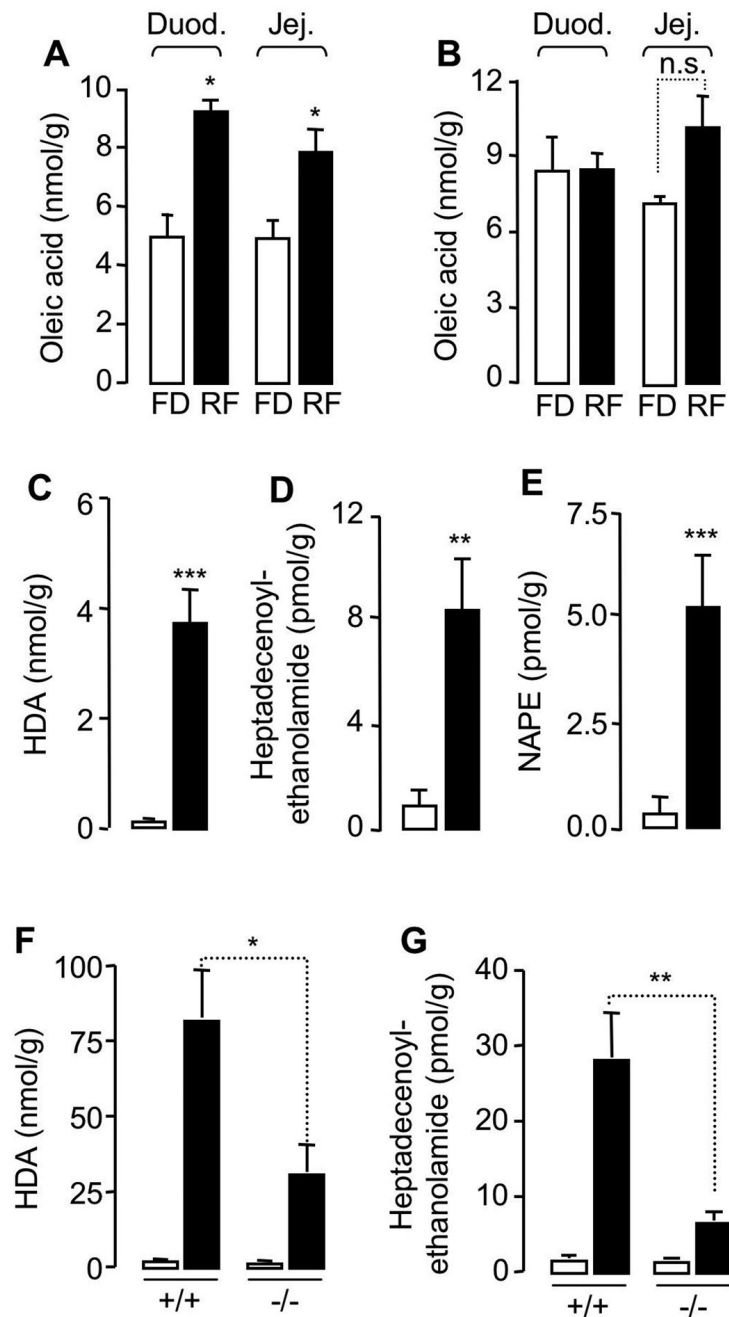


Figure 3. Internalization of diet-derived oleic acid by CD36 enables OEA mobilization
 Refeeding mice (RF) after a 6-h daytime fast (FD) increase oleic acid levels in duodenum (Duod.) and jejunum (Jej.) of wild-type (A), but not CD36-null mice (B). (C-E) duodenal infusion of the unnatural oleic acid analog 10Z-heptadecenoic acid (HAD) increases jejunal levels of (C) HDA, (D) heptadecenylethanolamide (HDE), and (E) 1-stearoyl-2-arachidonoyl-*sn*-glycero-3-phosphoethanolamine-*N*-heptadecenoyl (NAPE), open bars, Vehicle (50 mM Tris buffer); closed bars, HAD infusion. Infusions were conducted at 0.5 ml/min for 10 min and jejunal tissue was harvested 30 min after starting the infusion. (F,G) Oral gavage of HDA (200 mg/kg in 1 ml of saline/polyethylene glycol/Tween 80, 90/5/5, vol/vol; closed bars) increases jejunal levels of HDA (F) and HDE (G) in wild-type (+/+), but not in

CD36-null (-/-) mice, open bars, Vehicle (saline/polyethylene glycol/Tween 80, 90/5/5, vol/vol). One asterisk, $p < 0.05$; two asterisks, $p < 0.01$; three asterisks, $p < 0.001$; $n = 5-6$ per group.

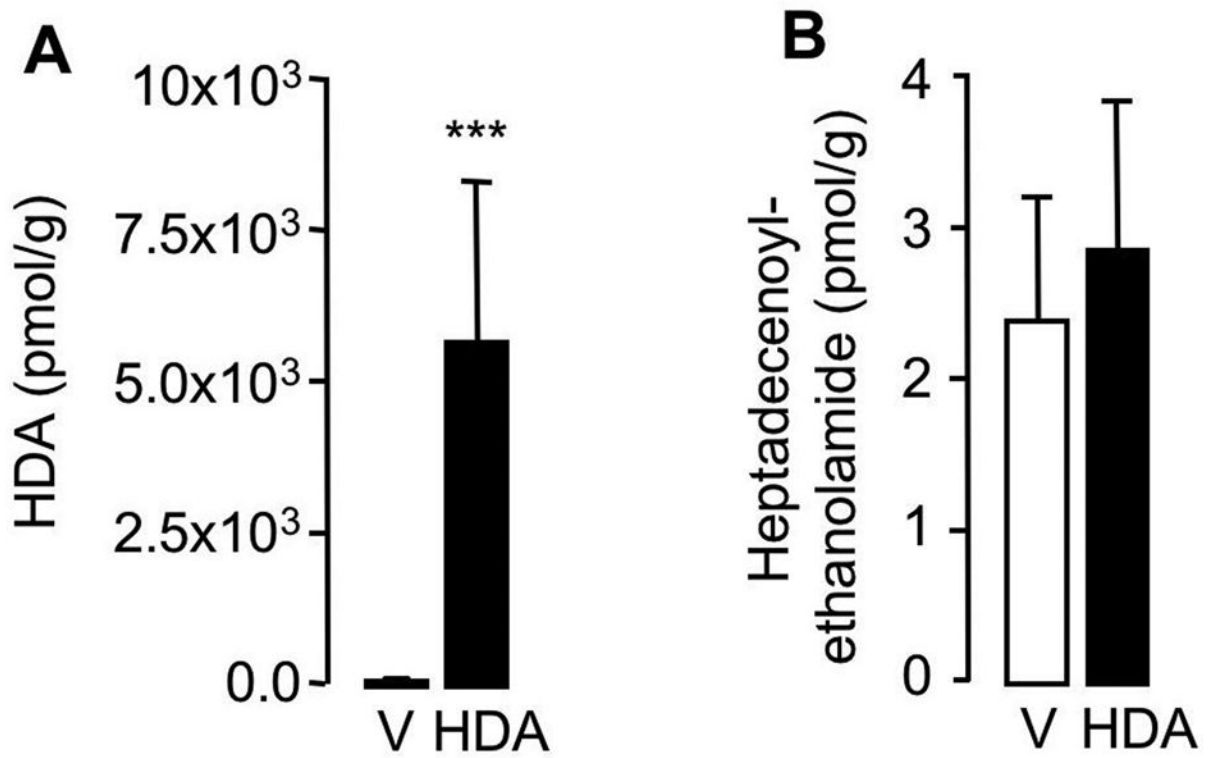


Figure 4. Plasma-derived oleic acid is not utilized for OEA mobilization

Intraperitoneal administration of the oleic acid analog 10Z-heptadecenoic acid (HDA) (10 mg/kg) increases jejunal HDA levels (A) without affecting jejunal heptadecenoyl-ethanolamide (HDE) levels (B) V, vehicle (saline/polyethylene glycol/Tween 80, 90/5/5, vol/vol, 2 ml/kg). Three asterisks, $p < 0.001$; $n = 6$ per group. Values are expressed as pmol/g of wet tissue.

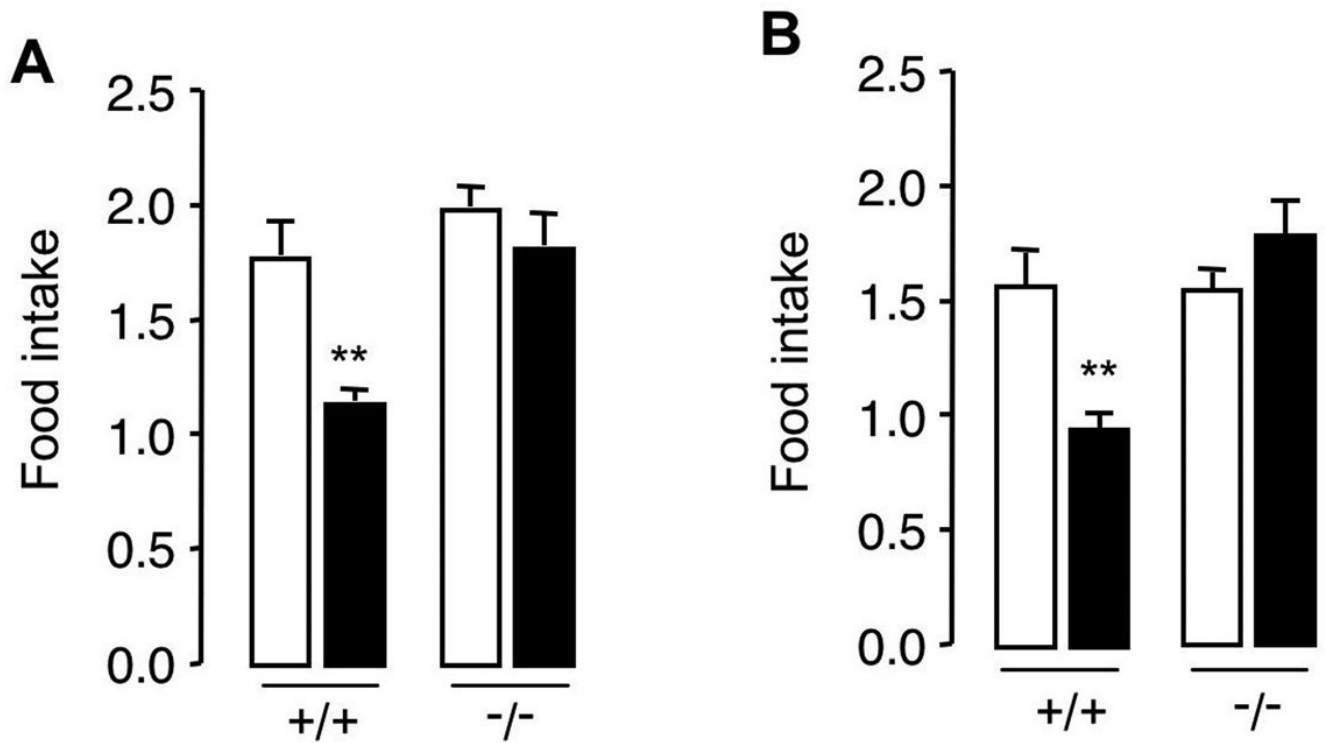


Figure 5. CD36 and PPAR- α mediate fat-induced satiety

Duodenal infusion of the lipid emulsion Intralipid reduces intake of a liquid diet (Ensure[®]) in wild-type mice (+/+), but not CD36-null mice (-/-) (A) or PPAR- α -null mice (-/-) (B). Closed bars, Intralipid; open bars, saline. Intake is expressed in ml/30min. Two asterisks, $p < 0.01$; $n = 6-7$.

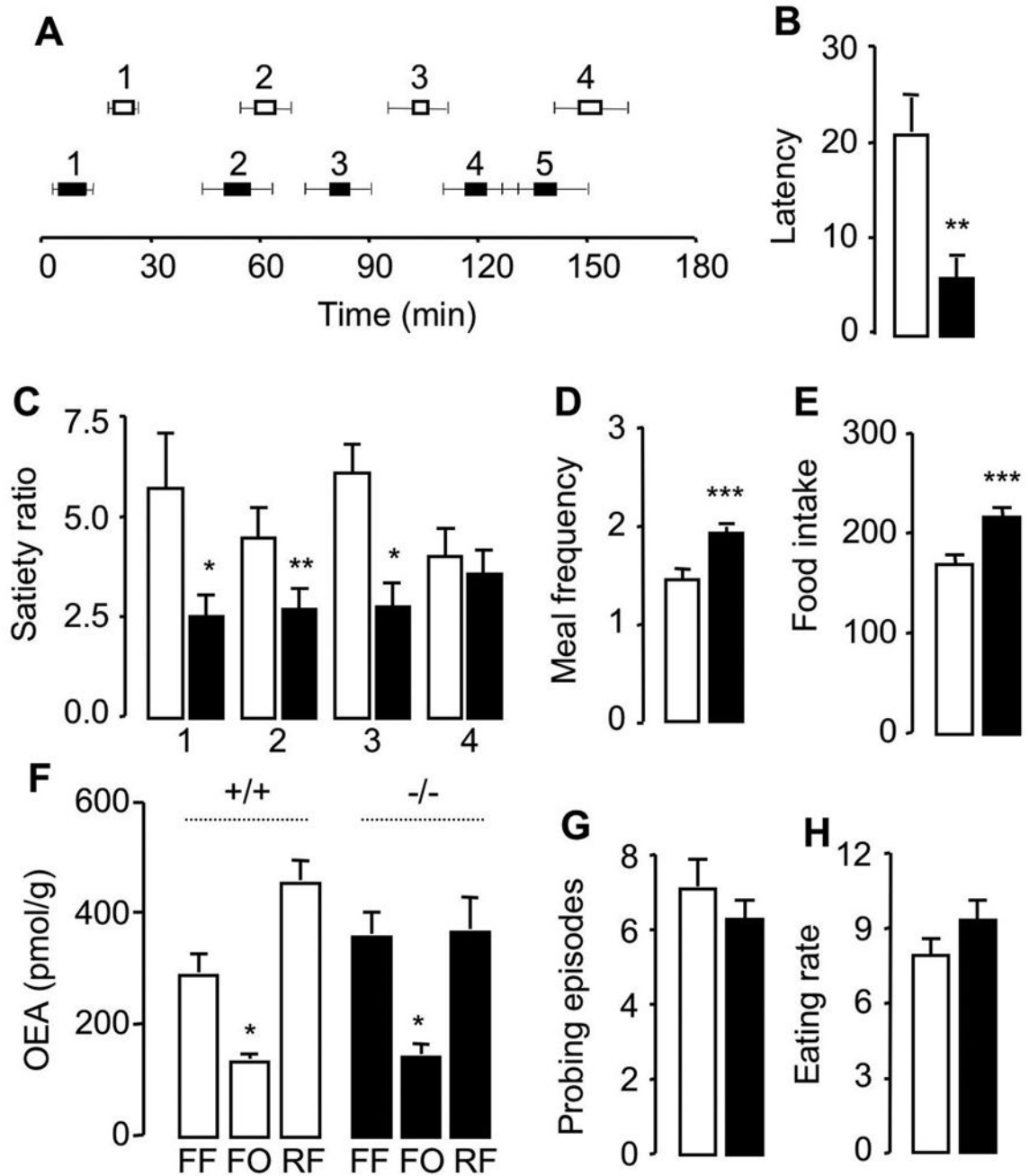


Figure 6. OEA signaling at PPAR- α regulates normal feeding

(A) Meal patterns in free-feeding wild-type mice (open bars) and PPAR- α -null mice (closed bars) during the first 3 hours after dark onset (5:30 pm); bar lengths symbolize the duration (\pm s.e.m.) of each meal. No difference in meal pattern was observed between wild-type and PPAR- α -null mice after the fourth nocturnal meal (data not shown). (B) Latency for the first meal (min). (C) Satiety ratio values (min/g/kg) for the first 4 meals. (D) Meal frequency (meals/h) in the first 3 hours of nocturnal feeding. (E) Total food intake in 24 h (g/kg). (F) No difference in feeding-induced jejunal OEA mobilization in wild-type mice (+/+) and PPAR- α -null mice (-/-). FF, free-fed; FO, 24 hr food deprivation; RF, 30 min refed after 24 hr food deprivation. (G) Number of food-probing episodes (non-eating contacts with the food basket). (H) Average

eating rate (g/min) for the 24-h observation period. One asterisk, $p < 0.05$; two asterisks, $p < 0.01$; three asterisks, $p < 0.001$; $n = 8-12$.

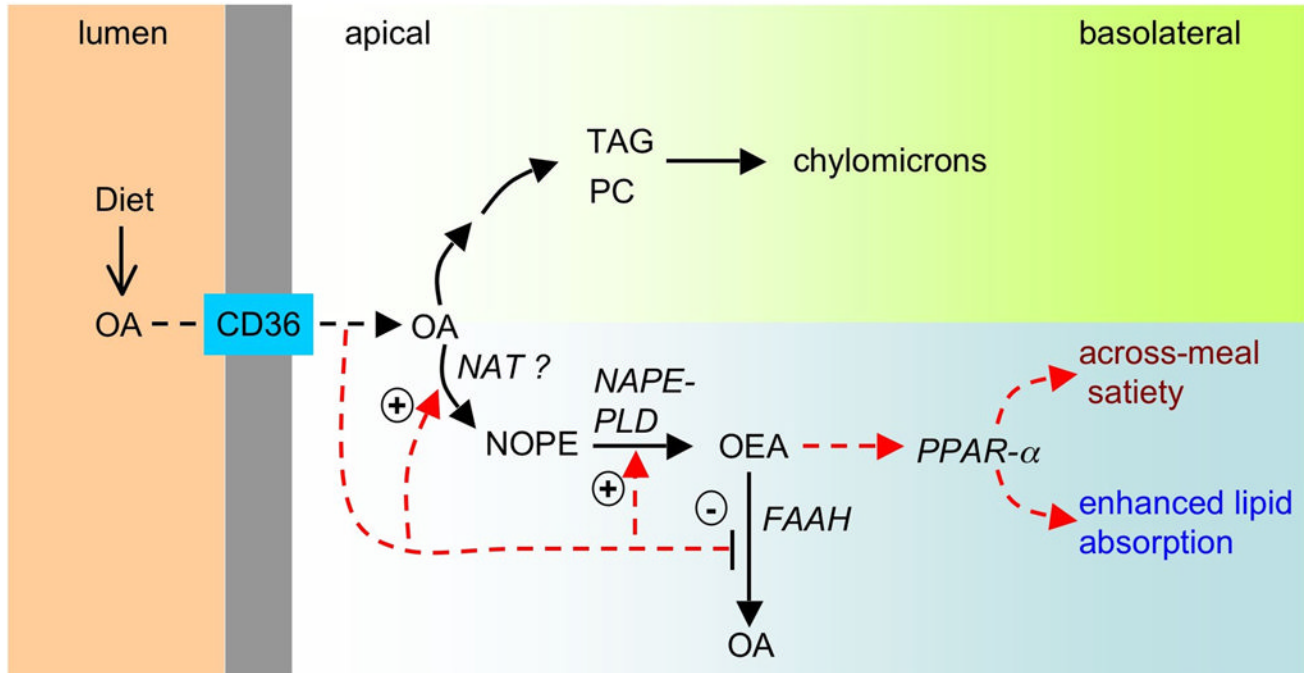


Figure 7. OEA mobilization links dietary fat intake to across-meal satiety

According to this hypothetical model, CD36 localized to the apical surface of small-intestinal enterocytes recognizes luminal oleic acid (OA) derived from the digestion of dietary fat and internalizes it. (Top) Most newly absorbed OA is channeled to the biosynthesis of triacylglycerol (TAG) and phosphatidylcholine (PC) *en route* to chylomicron formation. (Bottom) A small fraction of fatty acid is converted into OEA, which activates PPAR- α to prolong across-meal satiety and increase expression of lipid-metabolizing genes. Dashed arrows show that CD36 may further facilitate OEA mobilization (*i*) by stimulating NOPE production, presumably catalyzed by NAT activity; (*ii*) by enhancing NAPE-PLD activity, which converts NOPE to OEA; and (*iii*) by inhibiting FAAH activity, which hydrolyzes OEA and terminates its actions.