

# Mechanism of oleoylethanolamide on fatty acid uptake in small intestine after food intake and body weight reduction

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**Yang Y, Chen M, Georgeson KE, Harmon CM.** Mechanism of oleoylethanolamide on fatty acid uptake in small intestine after food intake and body weight reduction. *Am J Physiol Regul Integr Comp Physiol* 292: R235–R241, 2007. First published August 10, 2006; doi:10.1152/ajpregu.00270.2006.—The increase in the prevalence of human obesity highlights the need to identify molecular and cellular mechanisms involved in control of feeding and energy balance. Oleoylethanolamide (OEA), an endogenous lipid produced primarily in the small intestine, has been identified to play an important role in the regulation of animal food intake and body weight. Previous studies indicated that OEA activates peroxisome proliferator-activated receptor- $\alpha$ , which is required to mediate the effects of appetite suppression, reduces blood lipid levels, and enhances peripheral fatty acid catabolism. However, the effect of OEA on enterocyte function is unclear. In this study, we have examined the effect of OEA on intestinal fatty acid uptake and FAT/CD36 expression *in vivo* and *in vitro*. We intraperitoneally administered OEA to rats and examined FAT/CD36 mRNA level and fatty acid uptake in enterocytes isolated from the proximal small intestine, as well as in adipocytes. Our results indicate that OEA treatment significantly increased FAT/CD36 mRNA expression in intestinal mucosa and isolated jejunal enterocytes. In addition, we also found that OEA treatment significantly increases fatty acid uptake in isolated enterocytes *in vitro*. These results suggest that in addition to appetite regulation, OEA may regulate body weight by altered peripheral lipid metabolism, including increased lipolysis in adipocytes and enhanced fatty acid uptake in enterocytes, both in conjunction with increased expression of FAT/CD36. This study may have important implications in understanding the mechanism of OEA in the regulation of fatty acid absorption in human physiological and pathophysiological conditions.

obesity; enterocyte; fatty acid translocase/CD36

OBESITY HAS BECOME one of the most significant public health problems facing the world today (4, 11, 20, 25, 30). In the United States the prevalence of obesity has risen by 32% in adults and 40% in children over last two decades. A better understanding of how energy balance is maintained is critical to developing effective therapeutic strategies for obesity. Oleoylethanolamide (OEA) is a naturally occurring lipid mediator that inhibits food intake and body weight gain and therefore has been a molecule of recent intense scientific interest in the search for therapeutic strategies for the treatment of human obesity (13, 24).

OEA is synthesized in brain, adipocytes, and the small intestine and is structurally similar to the endogenous cannabinoid anandamide (arachidonoyleoylethanolamide), but it does not bind to or activate cannabinoid receptors. Rather, pharmacological and molecular biological experiments have demonstrated that OEA induces satiety (14) and reduces body weight

gain in mice and rats (24) through activation of the nuclear receptor peroxisome proliferator-activated receptor (PPAR)- $\alpha$  (13). This ligand-activated transcription factor is abundantly expressed in liver and intestine and is known to play a pivotal role in the regulation of lipid homeostasis (6, 7). Intestinal OEA has been shown to rise after a meal, suggesting that there is a fairly rapid feedback signal from the gut to the brain, promoting inhibition of further feeding after a meal is consumed. This OEA-mediated event is believed to involve the activation of intestinal PPAR- $\alpha$  and signaling via vagal sensory fibers to the brain stem (17). OEA also has been reported to alter serum triglyceride levels, suggesting a possible participation of OEA in the control of energy expenditure and accumulation (24). However, the effect of OEA on the small intestinal function is not well described.

Fatty acid translocase (FAT/CD36), an 88-kDa integral membrane protein, facilitates free fatty acid uptake in adipocytes and myocytes (1, 9, 18, 19). We have previously shown that intestinal mucosal FAT/CD36 mRNA levels varied by anatomic location along the longitudinal gut axis. FAT/CD36 protein levels also were higher in the proximal compared with distal intestinal mucosa. FAT/CD36 mRNA levels and enterocyte long-chain fatty acid (LCFA) uptake were rapidly down-regulated by intraduodenal oleate infusion (10). In the present study, we investigate the effect of OEA on fatty acid uptake and FAT/CD36 expression in the intestinal cells. Our results indicate that OEA increases fatty acid uptake in intestinal cells, possibly by upregulating FAT/CD36 function.

## MATERIALS AND METHODS

**Materials.** Oleate, bovine serum albumin (BSA; essentially fatty acid free), HEPES, and other chemicals were purchased from Sigma Chemicals (St. Louis, MO). Enhanced chemiluminescence reagents, [ $\alpha$ - $^{32}$ P]dCTP, and random-primer cDNA labeling kits were obtained from Amersham (Arlington Heights, IL). Horseradish peroxidase-conjugated anti-mouse IgG and anti-human CD36 mouse monoclonal antibodies were obtained from Transduction Laboratories (Lexington, KY). Glass fiber filter type A/E was obtained from Gelman Sciences (Ann Arbor, MI). NucTrap Probe purification columns were purchased from Stratagene (La Jolla, CA). Normal mouse IgG was purchased from Santa Cruz Biotechnology (Santa Cruz, CA).

**Animal preparation.** Male Sprague-Dawley rats (240–280 g) were housed at constant temperature (22°C) with a 12:12-h light-dark cycle. The animal protocol was approved by the University of Alabama, Birmingham, Alabama, Animal Care and Use Committee. Before experimentation, rats were allowed 3–5 days to acclimate and given free access to standard rat chow (PMI Nutrition International) and water. Rats were randomly assigned to two groups. Rats fed ad

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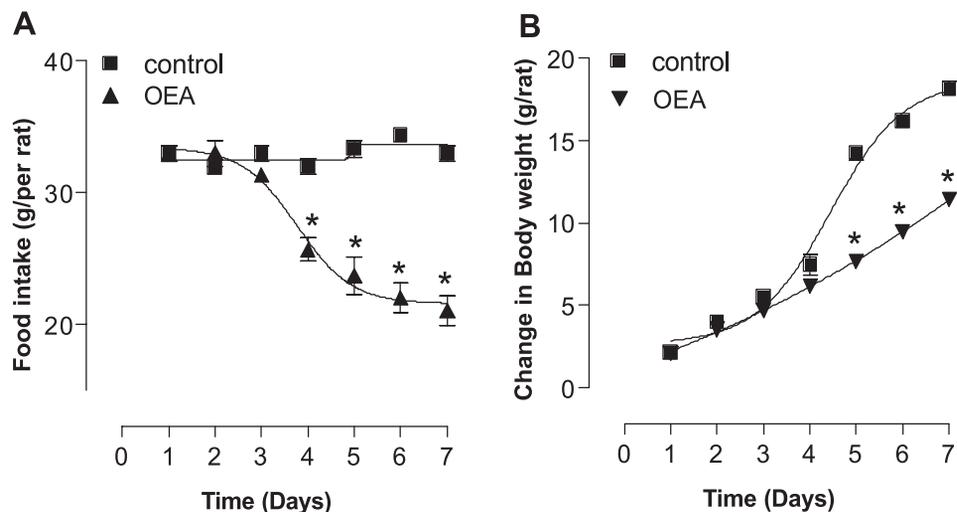


Fig. 1. Effect of oleoylethanolamide (OEA) on food intake and body weight. *A*: average food consumed was decreased by 28% in OEA-treated rats (5 mg/kg each day) compared with that in controls (5% Tween 80 in sterile saline) ( $P < 0.05$ ,  $n = 7$ ). *B*: time course of the effects of OEA or vehicle (control) on body weight increase.

libitum received daily intraperitoneal injections of vehicle or OEA (5 mg/kg at 7:00 AM) for 7 consecutive days, while body weight, food intake, and water intake were measured. The animals were killed by pentobarbital overdose; organ tissues were collected for biomedical analyses.

**Tissue sampling and preparation.** A 5-cm intestinal segment was harvested from the proximal jejunum (5 cm beyond the ligament of Treitz) and immediately rinsed twice with phosphate-buffered saline (PBS) solution. The mucosa was scraped from the proximal jejunum. Half of the mucosal scrapings were frozen in liquid nitrogen and stored at  $-80^{\circ}\text{C}$  for subsequent RNA analyses; the remaining half of the mucosal scrapings were homogenized and assayed for total protein content as described by Zimmerman-Belsing and Feldt-Rasmussen (30).

**Adipose tissue culture and adipocytes isolation.** Adipose tissue and adipocyte preparation followed the methods described in detail by Fried and Moustaid-Moussa (12). In each experiment, two rats were killed and rat epididymal fat pads from each rat were quickly removed. After the blood vessels were carefully dissected away, the adipose tissue was finely minced to obtain sufficiently small fragments. The fragments were rinsed thoroughly with DMEM containing 1% BSA and incubated at  $37^{\circ}\text{C}$  for 1 h. Isolated adipocytes were prepared by digestion of minced fat pad tissue with collagenase IV (Worthington Biochemicals, Freehold, NJ) and 1 mg/ml HBSS in a shaking bath at  $37^{\circ}\text{C}$  for 30–45 min. Before in vitro OEA treatments, adipose tissue or adipocytes were incubated for 2 h in DMEM with

1% BSA at  $37^{\circ}\text{C}$  in an incubator under an atmosphere of 5%  $\text{CO}_2$ -95% air.

**Preparation of intestinal cells.** The method of Gore was used to isolate enterocytes from male rats (15). Briefly, after death, the small bowel segment luminal contents were rinsed with oxygenated solution A (96 mM NaCl, 1.5 mM KCl, 5.6 mM  $\text{KH}_2\text{PO}_4$ , and 27 mM sodium citrate, pH 7.3) at  $37^{\circ}\text{C}$ . The distal duodenum and first 5 cm of jejunum were used in this assay. The isolated intestinal segment was filled with medium A (3 mM phosphate buffer, 136 mM NaCl, 0.6 mM  $\text{CaCl}_2$ , 5.2 mM KCl, 0.8 mM  $\text{MgSO}_4$ , and 5 mM glucose, pH 7.3) and submersed in Hanks' medium for 15 min at  $37^{\circ}\text{C}$  with oxygenation. The intestinal tube was then emptied, filled with solution B (140 mM NaCl, 16 mM  $\text{Na}_2\text{HPO}_4$ , 1.5 mM EDTA, and 0.5 mM dithiothreitol, pH 7.3) and incubated for 3 min at  $37^{\circ}\text{C}$  under oxygenation, after which it was gently palpated with fingers for 2 min. The luminal solution containing mucosal cells was filtered through 0.25-mm mesh gauze and recovered in 100 ml of Hanks' medium at room temperature. The treatment of the intestinal segment with solution B was repeated two times. The cells were washed twice with Hanks' medium at  $22^{\circ}\text{C}$  and recovered by centrifuging at 70  $g$  for 2 min. Light microscopy of the cell suspension showed that  $\sim 90\%$  of the cells displayed typical features of villus tip cells. Cell viability was checked using the Trypan blue dye-exclusion method (29) and was found to be 95% (data not shown).

**Quantitative real-time PCR.** Total RNA was isolated using Trizol reagent (GIBCO BRL, Grand Island, NY). Reverse transcription of

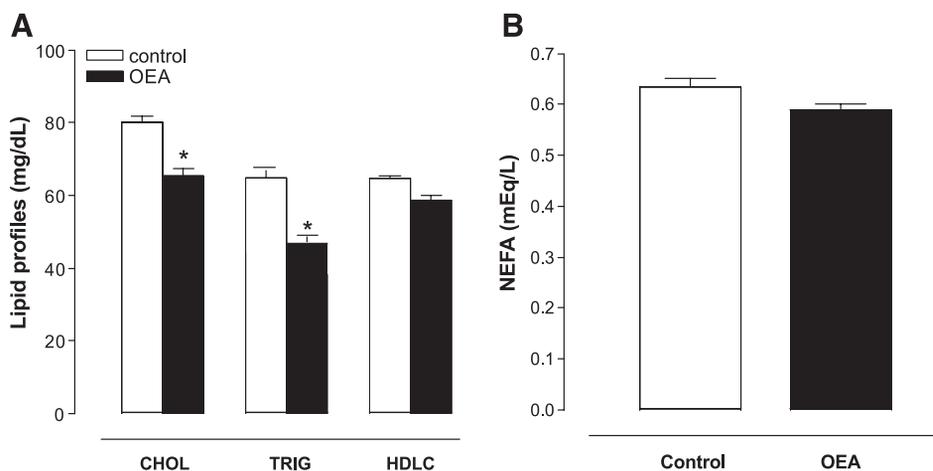


Fig. 2. Effect of OEA on serum lipid profiles. *A*: OEA treatment resulted in the decrease of serum cholesterol (Chol) and triglyceride (Trig) levels but had no effect on HDL cholesterol (HDLc) level. *B*: OEA had no effect on total nonesterified fatty acid (NEFA) release. All values are means  $\pm$  SE ( $n = 5$ ). \* $P < 0.05$  vs. control.

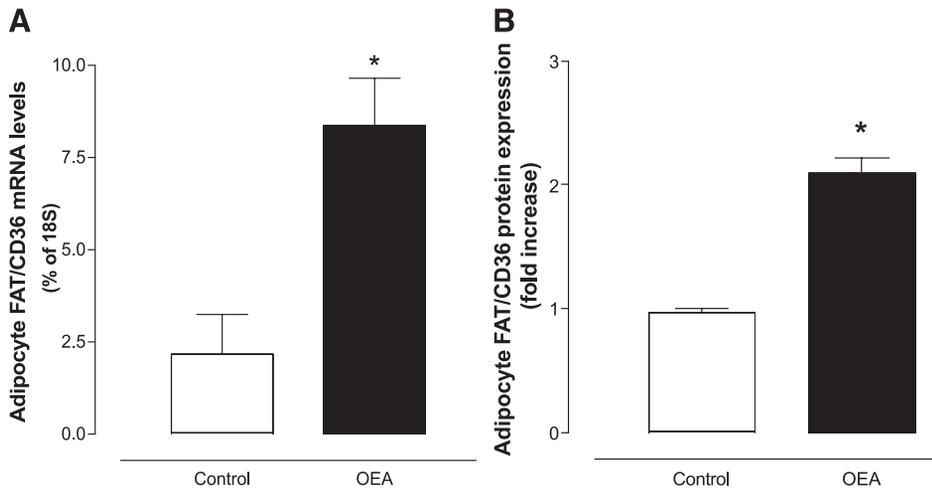


Fig. 3. Effect of OEA on fatty acid translocase (FAT/CD36) mRNA and protein expression in adipose tissue. FAT/CD36 mRNA levels in adipose tissue were increased after 7-day OEA treatment (A). FAT/CD36 protein expression also was increased by OEA treatment (B). All values are means  $\pm$  SE from FAT/CD36 densitometric analysis ( $n = 5$ ). \* $P < 0.05$  vs. control.

total RNA (1  $\mu$ g) was performed using avian myeloblastosis virus reverse transcriptase with the First-Strand cDNA synthesis kit for RT-PCR. The sequences of TaqMan PCR primers and probe were selected for the rat FAT/CD36 gene using Primer Express software (Applied Biosystems, Foster City, CA). Rat FAT/CD36 and 18 S primers were purchased from Applied Biosystems (identification nos. Rn 00580728 and Hs 99999901, respectively). The TaqMan probe was fluorescence labeled at the 5' end with 6-carboxyfluorescein (FAM) as the reporter dye and at the 3' end with 6-carboxytetramethyl-rhodamine (TAMRA) as the quencher dye (5'-FAM-ACAGAGTCAAT-GACATTA-TAMRA). TaqMan PCR was performed with a final volume of 10  $\mu$ l of PCR mixture, containing 5  $\mu$ l of TaqMan Universal PCR master mix (Applied Biosystems), 400 nM (final concentration) of each of the primers, 250 nM TaqMan probe, and 5  $\mu$ l of reverse-transcribed RT reaction. Amplification and detection were performed with an ABI PRISM 7900 HT sequence detection system (Applied Biosystems) using the manufacturer's standard protocols. PCR amplification was performed in separate wells for 2 min at 50°C to eliminate carryover contamination, for 10 min at 95°C to activate the hot start *Taq* polymerase (AmpliTaQ Gold DNA polymerase; Applied Biosystems), and then for 40 cycles, with each cycle consisting of a step at 95°C for 15 s, followed by a step at 60°C for 1 min. Calibrations and samples were run simultaneously. Initial

real-time amplifications were examined using agarose gel electrophoresis followed by ethidium bromide staining to verify that the primer pairs amplify a single product of the predicted size.

**BODIPY-labeled fatty acid uptake.** BODIPY-labeled fatty acid uptake was utilized for determining transmembrane permeation of long-chain fatty acids into enterocytes (26). The assay was performed as previously described in adipocyte and jejunal cells. Briefly, 125  $\mu$ l of the isolated mucosal cells suspended in Krebs-Ringer-HEPES ( $2 \times 10^6$  cells/ml) was preincubated in serum-free medium for 1 h at 37°C. The cells were washed with Dulbecco's complete (containing calcium and magnesium) PBS and incubated with 5–20  $\mu$ M BODIPY 3823 and PBS containing 20  $\mu$ M BSA (fatty acid free; 0.4–1:1 molar ratio) for 120 s at 37°C. Cells were then washed extensively at 4°C with PBS containing 0.1% BSA to remove surface-associated BODIPY. Cells were suspended in complete DMEM buffer with HEPES to pH 7.5 for cytometric analysis. Cell emission of BODIPY fluorescence was measured by flow cytometry with fluorescence-activated cell sorting (FACStar Plus 6-parameter cytometer/sorter with a dual argon

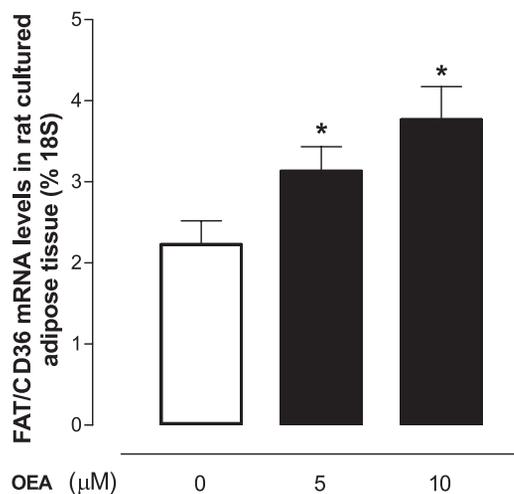


Fig. 4. Effect of different doses of OEA on FAT/CD36 mRNA expression in adipose tissue in vitro. OEA dose-dependently increased FAT/CD36 mRNA expression. All values are means  $\pm$  SE from FAT/CD36 densitometric analysis ( $n = 5$ ). \* $P < 0.05$  vs. control.

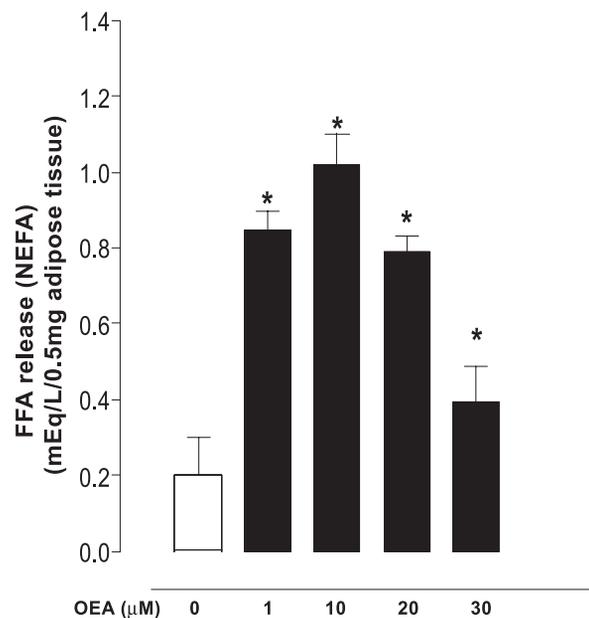
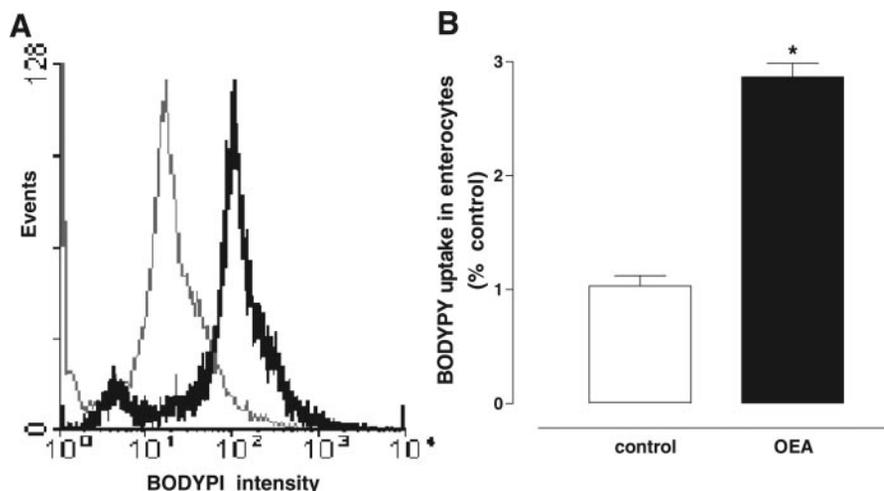


Fig. 5. Effect of OEA on free fatty acid (FFA) release at adipose tissue. The isolated adipocytes were treated with a different dose of OEA, and OEA increased free fatty acid release. OEA at 10  $\mu$ M induced the highest free fatty acid release. All values are means  $\pm$  SE ( $n = 5$ ). \* $P < 0.05$  vs. control.

Fig. 6. Uptake of BODIPY-labeled fatty acid at enterocytes. *A*: cell number is displayed on the ordinate and fluorescence (on a log scale) on the abscissa (BODIPY concentration, 5  $\mu$ M). *B*: histogram of BODIPY uptake in enterocytes treated with OEA. \* $P < 0.05$  vs. control. Experiments were repeated 3 times.



ion laser; Becton-Dickinson, San Jose, CA), and results were analyzed using CellQuest software (Becton-Dickinson).

**Statistical analysis.** Animal and tissue weights are expressed as means  $\pm$  SE. Results of FAT/CD36 mRNA levels are expressed as mean percentages ( $\pm$ SE) of ribosomal 18S mRNA levels determined as a control of each sample. ANOVA was used to test for significant differences between groups.  $P$  values  $< 0.05$  are considered significant.

## RESULTS

**Effects of OEA administration on rat food intake and body weight.** To examine the effect of OEA on food intake and body weight, we administered OEA (5 mg/kg) to rats intraperitoneally for 7 days, while the control group was treated with vehicle. The food intake and body weight were monitored daily. Our results are shown in Fig. 1. OEA significantly decreased rat food intake and body weight. Average food consumed was decreased by 28% and body weight was reduced by 25% in the OEA-treated group compared with the control group. This finding is consistent with previous reports (13). To determine whether OEA alters serum lipid profiles, we measured serum lipid profiles in OEA-treated rats. Our results indicate that OEA resulted in the decrease of total serum cholesterol and triglycerol levels but had no effect on HDL level (Fig. 2A). OEA had no effect on total nonesterified fatty acid (NEFA) content (Fig. 2B).

**Effect of OEA administration on adipocyte CD36 mRNA expression and on adipocyte lipolysis.** It has been reported that OEA treatment affects adipocyte function in mice (13). To test whether chronic administration of OEA might regulate adipocyte FAT/CD36 expression in rats, we intraperitoneally injected OEA into rats for 7 days and examined the FAT/CD36 mRNA expression in adipocytes. Our results indicate that intraperitoneal OEA treatment significantly increased FAT/CD36 mRNA levels in adipocytes compared with those in control rats (Fig. 3). To test whether OEA has a direct effect on adipocyte FAT/CD36 mRNA expression and free fatty acid release, we treated rat isolated adipocytes with different concentrations of OEA for 2 h in vitro and measured FAT/CD36 mRNA expression and free fatty acid release. Our results indicate that OEA dose-dependently increased FAT/CD36 mRNA expression (Fig. 4) and induced fatty acid release.

Interestingly, high-dose OEA appeared to inhibit adipocyte lipolysis (Fig. 5).

**Effect of OEA treatment on fatty acid uptake and FAT/CD36 mRNA expression at intestinal mucosa.** To determine whether chronic administration of OEA might regulate fatty acid uptake and FAT/CD36 mRNA expression in enterocytes, we intraperitoneally injected OEA for 7 days and examined fatty acid uptake and FAT/CD36 mRNA expression in intestinal cells. We examined fatty acid uptake in isolated jejunal enterocytes utilizing BODIPY-labeled fatty acid. We found that OEA treatment significantly increased fatty acid uptake and FAT/CD36 mRNA expression in enterocytes  $\sim 75\%$  compared with that in the control group (Figs. 6 and 7). To examine whether OEA has a direct effect on enterocyte fatty acid uptake and FAT/CD36 mRNA expression, we also incubated isolated enterocyte with OEA for 2 h and then examined fatty acid

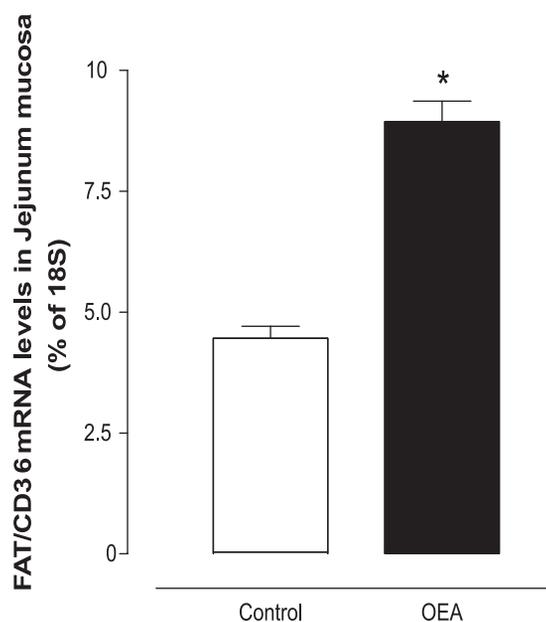


Fig. 7. Effect of OEA on FAT/CD36 mRNA expression in small intestine. FAT/CD36 mRNA levels in jejunal mucosa were increased after 7-day OEA treatment. All values are means  $\pm$  SE from FAT/CD36 densitometric analysis ( $n = 5$ ). \* $P < 0.05$  vs. control.

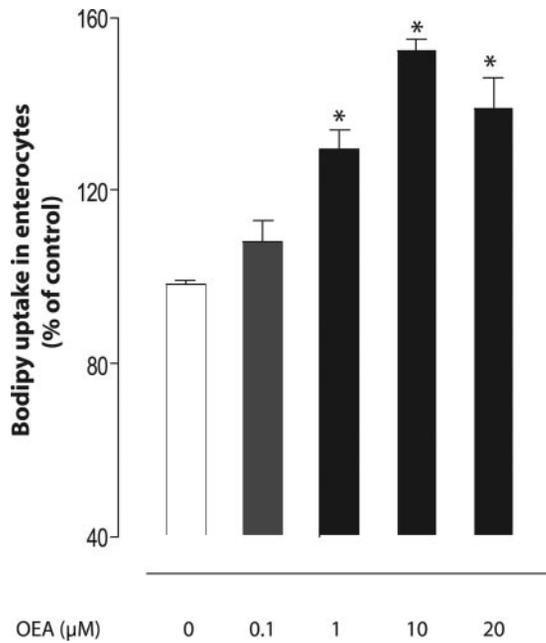


Fig. 8. Effect of OEA on fatty acid uptake at enterocytes in vitro. BODIPY uptake was dose-dependently increased after a different dose of OEA treatment. All values are means  $\pm$  SE ( $n = 5$ ). \* $P < 0.05$  vs. control.

uptake and FAT/CD36 mRNA expression in vitro. Our results indicate that OEA acutely dose-dependently increased fatty acid uptake and FAT/CD36 mRNA expression in isolated enterocytes (Figs. 8 and 9).

To determine the role of OEA on PPAR expression, we also examined PPAR- $\alpha$  and - $\gamma$  mRNA expression at the isolated enterocyte. Our results indicate that OEA significantly increased in PPAR- $\alpha$  mRNA expression but had no effect on PPAR- $\gamma$  mRNA expression (Fig. 10).

## DISCUSSION

In the present study, we have shown that OEA treatment reduces rat food intake and body weight as expected. Our

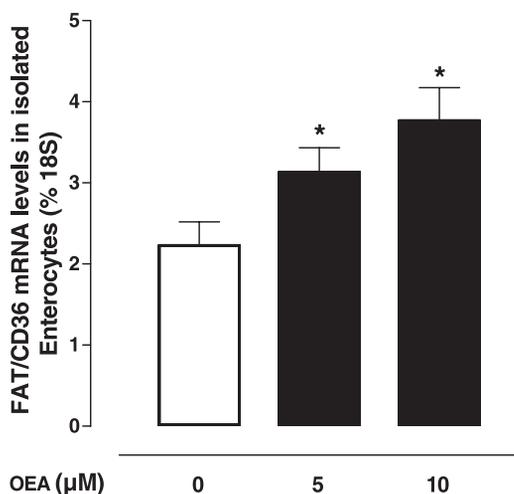


Fig. 9. Effect of OEA on FAT/CD36 mRNA expression in enterocytes in vitro. The isolated enterocytes were treated with a different dose of OEA. FAT/CD36 mRNA levels in enterocytes were increased in a dose-dependent manner. All values are means  $\pm$  SE from FAT/CD36 densitometric analysis ( $n = 5$ ). \* $P < 0.05$  vs. control.

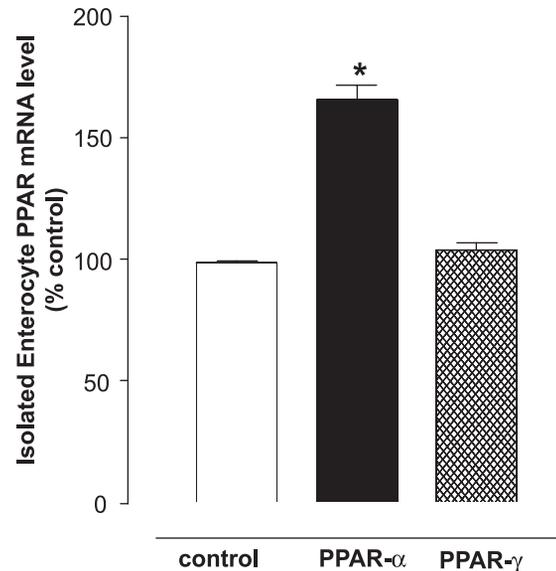


Fig. 10. Effect of OEA on peroxisome proliferator-activated receptor (PPAR) mRNA levels in isolated enterocytes. OEA treatment significantly induced PPAR- $\alpha$  mRNA but had no effect on PPAR- $\gamma$  mRNA expression. All values are means  $\pm$  SE ( $n = 5$ ). \* $P < 0.05$  vs. control.

results indicate that OEA increases FAT/CD36 mRNA and protein expression at both adipocytes and enterocytes, suggesting that an increase in adipocyte lipid oxidation mediated by OEA may be the reason for reduced body weight, whereas increases in fatty acid uptake in enterocytes may enhance energy utilization in the small intestine. FAT/CD36 may be involved in OEA-mediated regulation of lipid metabolism at both adipocytes and intestinal cells.

LCFAs are fuels that can be used to generate ATP efficiently, primarily through  $\beta$ -oxidation in mitochondria and peroxisomes. In addition, fatty acids are precursors for the biosynthesis of complex membrane lipids and for lipid-signaling molecules and serve as ligands for transcription factors that control cellular metabolic gene expression. Adipocytes are the primary site for lipid storage and mobilization, and as such, one of their major roles is the uptake and release of LCFA. The permeation of LCFAs across the adipocyte plasma membrane relies on a high-affinity, low-capacity carrier-facilitated transport system (2). FAT/CD36, an 88-kDa integral membrane protein, has been proposed to an important candidate for free fatty acid uptake in adipocytes (1). The important role of FAT/CD36 for LCFA uptake in adipocytes has been extensively studied. CD36 mRNA is induced during preadipocyte differentiation (5), and when expressed in fibroblasts lacking the protein, saturable LCFA transport is induced (21). Regulation of FAT/CD36 expression in vivo also is consistent with a role in LCFA transport with increased expression in the muscle of diabetic animals (16), and a recent report demonstrates that CD36 deficiency underlies insulin resistance, defective fatty acid metabolism, and hypertriglyceridemia in a hypertensive rat model of insulin resistance (3). OEA-reduced food intake and increased adipocyte lipolysis has been reported in mice, and our results confirm that OEA increases adipocyte lipolysis in rats.

The essential step in intestinal absorption of long-chain fatty acids is the permeation of the jejunal microvillus plasma

membrane. It has been reported that rat enterocyte and cultured Caco-2 cell LCFA uptake is saturable (27, 28), suggesting that a protein facilitates plasma membrane permeation. It also has been reported that FAT/CD36 mRNA is expressed predominantly in the rat proximal small intestine and that mRNA expression is increased by a LCFA in a high-fat diet (23). However, the effect of OEA on movement of LCFAs across cellular plasma membranes of enterocytes is unclear. Several proteins have been proposed as candidates for plasma membrane LCFA transporter proteins. CD36 expression distribution favors tissues with high metabolic capacity for LCFA in intestine, whereas it is absent from tissues like brain that do not utilize LCFA (1). After hydrolysis of dietary lipids, LCFAs are readily absorbed in the mammalian small intestine. To examine the role of OEA on fatty acid uptake at the small intestine, we first examined the effect of OEA on fatty acid uptake in enterocytes. Our results demonstrate that chronic OEA treatment significantly increases fatty acid uptake in the small intestine, and further experiments indicated that OEA directly enhances fatty acid uptake at enterocytes. The fact that OEA reduces food intake and body weight while it increases enterocyte fatty acid uptake suggests that OEA-mediated enterocyte fatty acid uptake may be due to decreased food intake and body weight. The increases of the fatty acid uptake by OEA would enhance the utilization of nutrients in the small intestine. These results suggest that OEA may contribute to the peripheral regulation of feeding and that FAT/CD36 plays an important role in OEA-mediated fatty acid uptake. Our findings support evidence that FAT/CD36 may play an important role in OEA-mediated reduction of food intake and body weight control.

PPAR- $\alpha$  is abundantly expressed in intestine and is known to play a pivotal role in the regulation of lipid homeostasis (6, 8). It has been reported that OEA content in the rat small intestine rises in the fasting state, and OEA activates PPAR- $\alpha$  in adipocytes (13, 24), suggesting that postprandial levels of endogenous OEA can fully activate PPAR- $\alpha$  receptor. To determine whether an increase of FAT/CD36 expression is mediated by OEA at enterocytes through activation of PPAR- $\alpha$ , we examined the effect of OEA on PPAR- $\alpha$  expression at enterocytes. Our results indicate that OEA selectively activates PPAR- $\alpha$ , whereas it does not engage PPAR- $\gamma$  activation, supporting that OEA stimulates adipocyte lipolysis and enterocyte fatty acid uptake through the activation of the nuclear receptor PPAR- $\alpha$ . Recently, an orphan receptor GPR119 has been identified to be the receptor of OEA (22). This receptor is expressed predominantly in the human and rodent pancreas and gastrointestinal tract and also in rodent brain, suggesting that the OEA effect in our study may be mediated, at least in part, via the GPR119 receptor.

In conclusion, we have identified that OEA has a direct effect on the increase of FAT/CD36 mRNA at adipocytes and enterocytes. The findings that OEA treatment increases adipocyte lipolysis and fatty acid uptake in the intestinal cell support the hypothesis that FAT/CD36 may play a role in lipid metabolism, especially the intestinal absorption of LCFA. Therapeutic strategies designed to alter or manipulate intestinal FAT/CD36 expression or function may have important implications in treating a variety of pathological conditions such as morbid obesity, short bowel syndrome, and other lipid malabsorption conditions.

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