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Oleoylethanolamide, an endogenous PPAR- α agonist, lowers body weight and hyperlipidemia in obese rats

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Abstract

The fatty-acid ethanolamide, oleoylethanolamide (OEA), is a naturally occurring lipid that regulates feeding and body weight [Rodríguez de Fonseca, F., Navarro, M., Gómez, R., Escuredo, L., Nava, F., Fu, J., Murillo-Rodríguez, E., Giuffrida, A., LoVerme, J., Gaetani, S., Kathuria, S., Gall, C., Piomelli, D., 2001. An anorexic lipid mediator regulated by feeding. *Nature* 414, 209–212], and serves as an endogenous agonist of peroxisome proliferator-activated receptor- α (PPAR- α) [Fu, J., Gaetani, S., Oveisi, F., Lo Verme, J., Serrano, A., Rodriguez De Fonseca, F., Rosengarth, A., Luecke, H., Di Giacomo, B., Tarzia, G., Piomelli, D., 2003. Oleoylethanolamide regulates feeding and body weight through activation of the nuclear receptor PPAR- α . *Nature* 425, 90–93], a ligand-activated transcription factor that regulates several aspects of lipid metabolism [Desvergne, B., Wahli, W., 1999. Peroxisome proliferator-activated receptors: nuclear control of metabolism. *Endocr. Rev.* 20, 649–688]. OEA reduces food intake in wild-type mice, but not in mice deficient in PPAR- α (PPAR- $\alpha^{-/-}$), an effect that is also observed with the PPAR- α agonists Wy-14643 and GW7647 [Brown, P.J., Chapman, J.M., Oplinger, J.A., Stuart, L.W., Willson, T.M. and Wu, Z., 2000. Chemical compounds as selective activators of PPAR- α . *PCT Int. Appl.*, 32; Willson, T. M., Brown, P. J., Sternbach, D. D., Henke, B. R., 2000. The PPARs: from orphan receptors to drug discovery. *J. Med. Chem.* 43, 527–550]. By contrast, specific agonists of PPAR- δ/β (GW501516) or PPAR- γ (ciglitazone) have no such effect. In obese Zucker rats, which lack functional leptin receptors, OEA reduces food intake and lowers body-weight gain along with plasma lipid levels. Similar effects are seen in diet-induced obese rats and mice. In the present study, we report that subchronic OEA treatment (5 mg kg⁻¹, intraperitoneally, i.p., once daily for two weeks) in Zucker rats initiates transcription of PPAR- α and other PPAR- α target genes, including fatty-acid translocase (FAT/CD36), liver fatty-acid binding protein (L-FABP), and uncoupling protein-2 (UCP-2). Moreover, OEA decreases neutral lipid content in hepatocytes, as assessed by Oil red O staining, as well as serum cholesterol and triglyceride levels. The results suggest that OEA regulates lipid metabolism and that this effect may contribute to its anti-obesity properties.

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Keywords: Oleoylethanolamine (OEA); Peroxisome proliferator-activated receptor- α (PPAR- α); Obesity; Zucker rat; Lipid metabolism

1. Introduction

The alarming increase in the prevalence of obesity highlights the need to identify the molecular and cellular mechanisms involved in control of feeding and energy balance. One such mechanism, which has recently been identified, may involve the natural fatty-acid ethanolamide, OEA. OEA is structurally similar to

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the endogenous cannabinoid anandamide (arachido-*noylethanolamide*), but does not bind to or activate cannabinoid receptors. Rather, pharmacological and molecular biological experiments have demonstrated that OEA induces satiety (Gaetani et al., 2003; Rodríguez de Fonseca et al., 2001) and reduces body-weight gain in mice and rats (Rodríguez de Fonseca et al., 2001) through activation of the nuclear receptor PPAR- α (Fu et al., 2003). 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 (Berger and Moller, 2002; Bocher et al., 2002). In the present article, we review current evidence indicating that the effects of OEA on feeding behaviour and body weight are mediated by PPAR- α , and report new findings suggesting that OEA may inhibit body-weight gain in obese animals by enhancing lipid utilization.

2. Materials and methods

2.1. Animals

We purchased male C57BL/6, PPAR- $\alpha^{-/-}$ (129S4/SvJae-*Ppara*^{tm1Gonz}) and wild-type mice (129S1/SvImJ) from Jackson Laboratory (Bar Harbor, ME). Animals were maintained on a 12/12-h light/dark cycle with ad libitum water and chow (RMH 2500, ProLab).

2.1.1. Obese animals

We fed wild-type, PPAR- $\alpha^{-/-}$ mice and Wistar rats with a high-fat diet (60 kcal % fat; D12492; Research Diets, New Brunswick, NJ) for 7 weeks; body-mass indices were $0.36 \pm 0.01 \text{ g cm}^{-2}$ for wild type ($n = 13$) and $0.41 \pm 0.01 \text{ g cm}^{-2}$ for PPAR- $\alpha^{-/-}$ mice ($n = 15$) (Gregoire et al., 2002). Zucker rats (5–6 weeks) were supplied by Charles River (Wilmington, MA).

2.2. Tissue preparation

We euthanized mice by decapitation. We collected the liver tissue, stored it in RNALater™ (Ambion, Austin, TX) for RNA isolation or froze it in liquid N₂ for histology.

2.3. RNA isolation and cDNA synthesis

We extracted total RNA using TRIzol™ (Invitrogen, Carlsbad, CA), and quantified it with Ribogreen™ (Molecular Probes, Eugene, OR). cDNA was synthesized with 0.2 μg of total RNA and oligo(dT)_{12–18} primer by using Superscript II RNase H-reverse transcriptase (Invitrogen) following the manufacturer's instructions.

2.4. Real-time quantitative PCR

Real-time quantitative (RTQ) PCR was performed by ABI PRISM 7700 sequence detection system (Applied Biosystems, Foster City, CA). We designed primer/probe sets using the Primer Express™ software base on gene sequences available from the GenBank™ database. Primers and fluorogenic probes were synthesized at TIB (Adelphia, NJ). The primer/probe sequences for rat genes were: PPAR- α , Forward (F): TGGTGGACCTCCGGCA, Reverse (R): TCTTCTTGATGACCTGCACGA, Probe (P): CTGGTCACGGAGCATGCGCAGT; FAT/CD36, F: CGGCGATGAGAAAGCAGAA, R: CAACCAGGCCAGGAGC, P: TGTTTCAGAAACCAAGTGACCGGGAAAATAA; L-FABP, F: GGCTTCGCCACCAGGAA; R: CCCTTCTACGCTGATGATCAAGT; P: TGGCCGGTATGGCCAAGCCC; UCP-2, F: CCAGAGCACTGTCAAGCC, R: TTCCAGAGGCCCGGA, P: ACAAGACCA TTGCACGAGAGGAAGGG; ACs, F: AGGAGGAAATGTCGCATGA, R: CATGAATCGGTGTGTCTGGG, P: TGTCCGGCGGTGCCCCACT; GAPDH, F: AAGTATGATGACATCAAGAAGGTGGT, R: AGCCAGGATGCCCTTTAGT, P: AAGCAGGCCGCCGAGGGC. RNA levels were normalized by using glyceraldehyde 3-phosphate dehydrogenase (GAPDH) as an internal standard as described (Schmittgen et al., 2000).

2.5. Histological analyses

We cut 10- μm -thick liver sections with a cryostat and fixed them for 1 h in 4% paraformaldehyde. To detect neutral lipids, we immersed the sections in propylene glycol for 5 min, and stained them with Oil red O (Sigma, St Louis, MO) for 15 min. After rinsing them with 85% propylene glycol and water, we counterstained the sections with hematoxylin OS (Vector labs, Burlingame, CA), and mounted them.

2.6. Biochemical analyses

Serum lipids were measured at the University of California, Irvine pathology laboratory.

2.7. Feeding experiments

2.7.1. Acute experiments

We administered drugs or vehicle (70% DMSO in saline, 4 ml kg⁻¹; i.p.) at 5:00–5:30 PM to free-feeding animals habituated to the experimental setting. Food intake was monitored with an automated system (SciPro, N. Tonawanda, NY) (Gaetani et al., 2003).

2.7.2. Subchronic experiments

We divided the obese wild-type and PPAR- $\alpha^{-/-}$ mice into 2 groups ($n = 6-8$ /group) and treated them for 4 weeks with vehicle (saline/polyethylene glycol/Tween 80, 90/5/5, 1 ml kg $^{-1}$, once daily, i.p.) or OEA (5 mg kg $^{-1}$, once daily, i.p.). We treated diet-induced obese rats ($n = 15$) and genetically obese Zucker rats ($n = 15$) for 2 weeks with vehicle (saline/polyethylene glycol/Tween 80, 90/5/5, 1 ml kg $^{-1}$, once daily, i.p.) or OEA (5 mg kg $^{-1}$, once daily, i.p.). We measured food intake and body weight daily.

2.8. Statistical analyses

Results were expressed as the mean \pm s.e.m. of n separate experiments. The significance of differences between the groups was evaluated by one-way analysis of variance (ANOVA) followed by a Dunnett's test for multiple comparisons or Student's t test. Analyses were done with GraphPad Prism software (GraphPad 2.0).

3. Results

3.1. The effect of OEA on feeding behaviour is mediated by PPAR- α

In normal rats, systemic administration of OEA dose-dependently reduced food intake (Fig. 1A). This effect was significant for doses of 3 mg kg $^{-1}$ or greater. Under the same conditions, anandamide and oleic acid had no effect, while palmitoylethanolamide (PEA) was significantly less potent than OEA (Fig. 1A). These results indicate that OEA reduces feeding in a structurally selective manner.

Since OEA binds PPAR- α with high affinity (median effective concentration, EC $_{50} = 120$ nM) (Fu et al.,

2003), we hypothesized that the effect of OEA on feeding may be mediated by activation of this nuclear receptor. To test this idea, we examined several selective PPAR agonists on food intake. First, we used the synthetic PPAR- α agonists Wy-14643 (Brown et al., 2000) (40 mg kg $^{-1}$, i.p.) and GW7647 (Lee et al., 1995) (10 mg kg $^{-1}$, i.p.). Both compounds inhibited food intake in C57BL/6 mice (Fig. 1B). Next, to determine the receptor selectivity of this effect, we tested potent agonists of PPAR- β/δ (GW501516) (Oliver et al., 2001) (5 mg kg $^{-1}$, i.p.) and PPAR- γ (ciglitazone) (Chang et al., 1983) (15 mg kg $^{-1}$, i.p.), which resulted ineffective (Fig. 1C). These findings suggest that OEA regulates feeding through selective activation of PPAR- α . This possibility was confirmed by showing that the effects of OEA are absent in PPAR- $\alpha^{-/-}$ mice (Fu et al., 2003).

3.2. OEA inhibits body-weight gain in obese animals

To study the impact of prolonged OEA treatment on body weight, we induced obesity in wild-type and PPAR- $\alpha^{-/-}$ mice by exposure to a high-fat diet and treated them with OEA for 4 weeks (5 mg kg $^{-1}$, once daily, i.p.). The drug reduced food intake (Fig. 2A), inhibited body-weight gain (Fig. 2B) and lowered plasma cholesterol levels (Fig. 2C) in wild-type mice ($n = 6-7$ per group), whereas it had no such effect in PPAR- $\alpha^{-/-}$ mutant mice (Fig. 2A–C, $n = 7-8$ per group). Thus, PPAR- α expression is necessary for the weight-reducing and lipid-lowering actions of OEA (Fu et al., 2003).

To further explore the anti-obesity properties of OEA, we used two additional animal models of obesity. In diet-induced obese Wistar rats, a 2-week regimen with OEA (5 mg kg $^{-1}$, once daily, i.p.) produced a modest, but significant inhibition of feeding (Fig. 3A, $n = 7-8$) and markedly reduced body-weight gain (Fig. 3B,

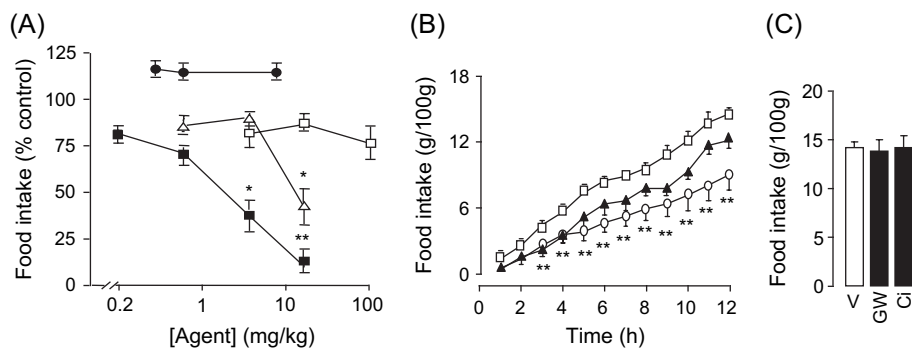


Fig. 1. OEA suppresses food intake in rats. (A) Dose-dependent effects of i.p. injection of OEA (closed squares), palmitoylethanolamide (open triangles), oleic acid (open squares) and anandamide (closed circles). Food intake was measured 60 min after injection of drugs or vehicle ($n = 8-12$). Food intake in vehicle-injected animals (70% DMSO in saline, 4 ml kg $^{-1}$, i.p.) was 7.1 ± 0.5 g/animal. Modified from Rodríguez de Fonseca et al. (2001). (B) Time-course of the effects of vehicle (open squares) or the PPAR- α agonists Wy-14643 (closed triangles 40 mg kg $^{-1}$ i.p.) and GW7647 (open circles 20 mg kg $^{-1}$ i.p.) on food intake in C57BL/6 mice (vehicle, $n = 10$; drugs, $n = 4-7$). (C) Lack of effect of the PPAR- β/δ agonist GW501516 (GW, 5 mg kg $^{-1}$ i.p.) and PPAR- γ agonist ciglitazone (Ci, 15 mg kg $^{-1}$ i.p.) on food intake in C57BL/6 mice ($n = 4-6$). Modified from Fu et al. (2003). * $P < 0.05$ and ** $P < 0.01$, one-way ANOVA followed by Dunnett's test.

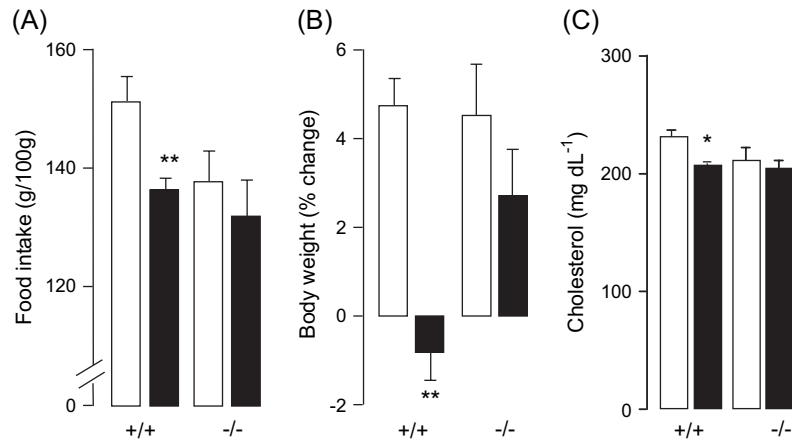


Fig. 2. Subchronic treatment with OEA reduces body-weight gain and serum cholesterol levels in diet-induced obese mice. Effects of OEA (closed bars, 5 mg kg⁻¹, once daily for 4 weeks, i.p.) or vehicle (open bars) on food intake (A), body-weight gain (B) and plasma cholesterol levels (C) in high-fat diet-induced obese (DIO) wild-type mice (+/+) ($n = 6-7$ per group) or high-fat DIO PPAR- α mice (-/-) ($n = 7-8$ per group). Modified from Fu et al. (2003). * $P < 0.05$ and ** $P < 0.01$, Student's t test.

$n = 7-8$) (Guzmán et al., 2004). Interestingly, the OEA-induced inhibition of food intake cannot account by itself for the weight-reducing effects of the compound, because pair-fed animals gained as much weight as did

vehicle-treated controls (Fig. 3B, $n = 7-8$). Similar effects were observed in genetically obese Zucker (*fa/fa*) rats (Fig. 3C, D, $n = 7-8$), which are homozygous for a mutated inactive form of the leptin receptor,

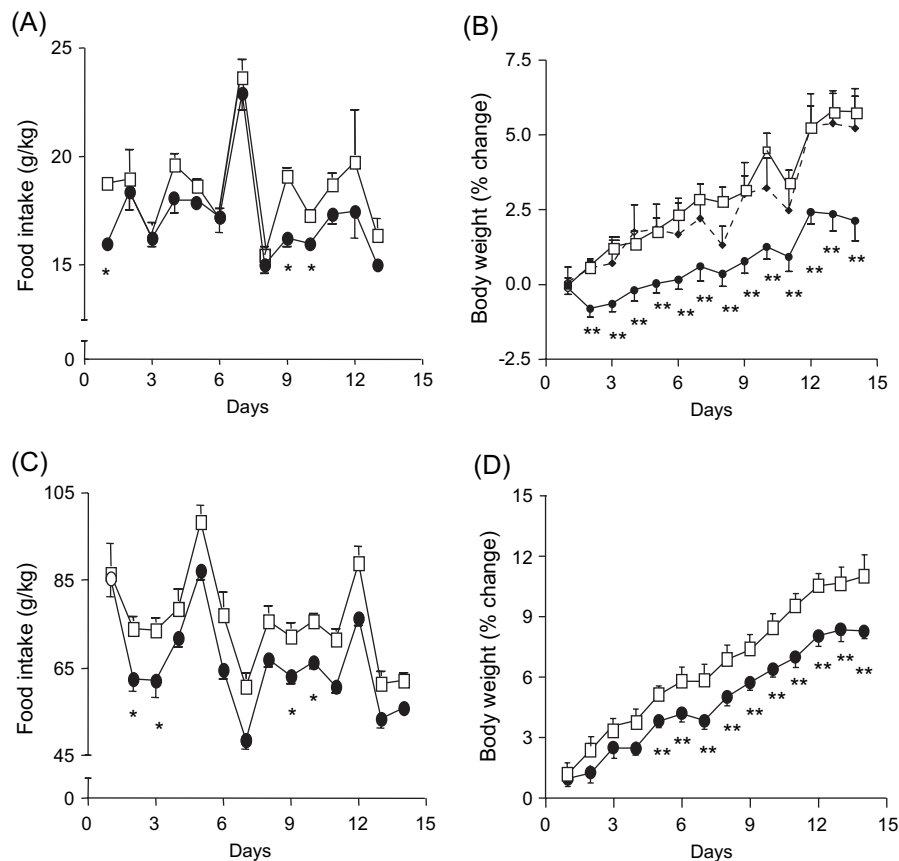


Fig. 3. Subchronic treatment with OEA reduces body-weight gain in obese rats. Effect of vehicle (open squares, $n = 7-8$), OEA (closed circles, 5 mg kg⁻¹, once daily for 2 weeks, $n = 7-8$) and pair-feeding (closed diamonds, $n = 7-8$) on food intake (A) and body-weight gain (B) in high-fat DIO rats. Modified from Guzmán et al. (2004). Effects of vehicle (open squares, $n = 7-8$), OEA (closed circles, 5 mg kg⁻¹, once daily for 2 weeks, $n = 7-8$) on food intake (C) and body-weight gain (D) in genetically obese Zucker rats. Modified from Fu et al. (2003). * $P < 0.05$ and ** $P < 0.01$, one-way ANOVA followed by Dunnett's test or Student's t test.

suggesting that OEA and leptin signalling occur through independent pathways (Fu et al., 2003).

3.3. OEA reduces lipid levels in Zucker rats

Lipid accumulation within the hepatocyte is a histological hallmark of the obese Zucker rats (Bray, 1977). To examine whether OEA corrects this defect, we measured neutral lipid content in liver tissue by using Oil red O staining (Koopman et al., 2001). After 2 weeks of OEA treatment (5 mg kg^{-1} , once daily, i.p.), lipid droplets in liver tissue were markedly reduced (Fig. 4). In addition, the treatment caused a significant decrease in serum cholesterol and triglyceride levels, but no change in glucose levels (Table 1).

3.4. OEA regulates the expression of genes involved in lipid metabolism

To begin to investigate the mechanisms responsible for the decreased lipid accumulation in OEA-treated Zucker rats, we tested whether subchronic OEA administration resulted in persistent changes in the expression of genes involved in lipid metabolism. We measured mRNA levels of PPAR- α and several of its downstream genes: fatty-acid translocase (FAT/CD36), liver fatty-acid binding protein (L-FABP), uncoupling protein-2 (UCP-2), and

Table 1
Effects of OEA on serum lipids and glucose in obese Zucker rats

	Vehicle	OEA
Cholesterol	99.88 ± 8.41	$66.14 \pm 7.06^*$
Triglycerides	565.29 ± 55.50	$394.17 \pm 49.40^*$
Glucose	229.29 ± 27.90	221.25 ± 23.80

OEA (5 mg kg^{-1} , i.p.) or vehicle was administered once daily for 2 weeks. Serum cholesterol, triglycerides and glucose were measured as described under Section 2 and were expressed in mg dl^{-1} . Results were the mean \pm s.e.m. ($n = 7-8$). $*P < 0.05$ vs vehicle by Student's t test.

acyl-CoA synthase (ACs). Two weeks treatment of OEA (5 mg kg^{-1} , i.p.) significantly increased expression of PPAR- α (Fig. 5A, 1.5-fold, $P < 0.01$, $n = 6$), FAT/CD36 (Fig. 5B, 1.7-fold, $P < 0.001$, $n = 6$), L-FABP (Fig. 5C, 2-fold, $P < 0.01$, $n = 6$), and UCP-2 (Fig. 5D, 2.2-fold, $P < 0.001$, $n = 6$). By contrast, OEA did not affect expression of ACs (Fig. 5E, $n = 6$).

4. Discussion

The findings reviewed in the present article indicate that OEA, a naturally occurring fatty-acid ethanolamide, induces satiety and inhibits body-weight gain in rats and mice through activation of the nuclear receptor PPAR- α (Fu et al., 2003; Gaetani et al., 2003; Rodríguez

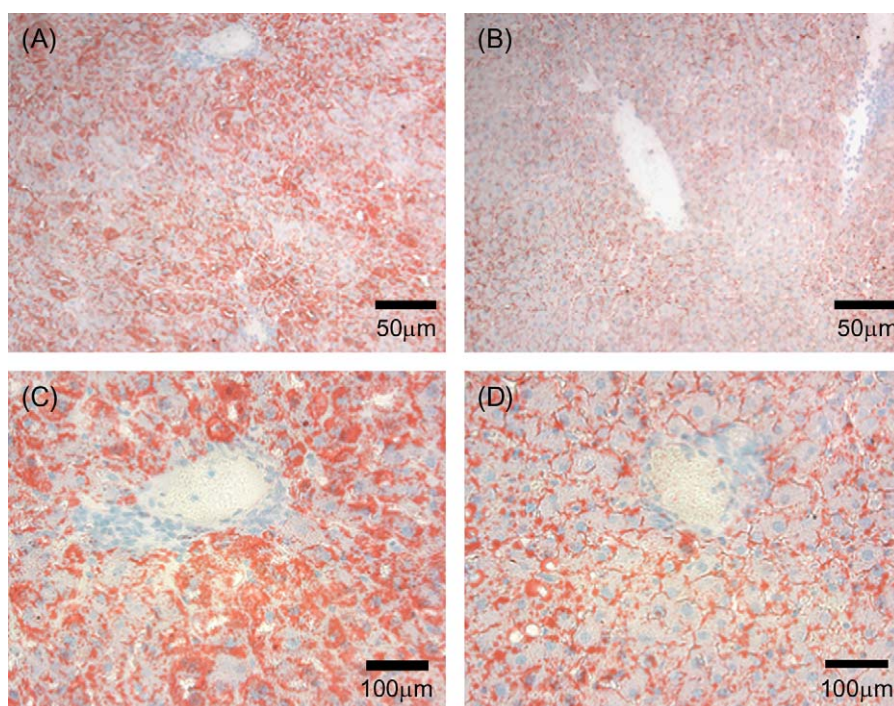


Fig. 4. Subchronic treatment with OEA reduces lipid accumulation in the liver of Zucker rats. The photomicrographs show histologic appearance of liver sections after treatment with vehicle (A, C) or OEA (B, D) (5 mg kg^{-1} , once daily for 2 weeks, i.p.). Red droplets indicate neutral lipid staining using Oil red O. Slides were counterstained with hematoxylin. (A, B) $10\times$ magnification, scale bars: $50 \mu\text{m}$; (C, D) $20\times$ magnification, scale bars: $100 \mu\text{m}$.

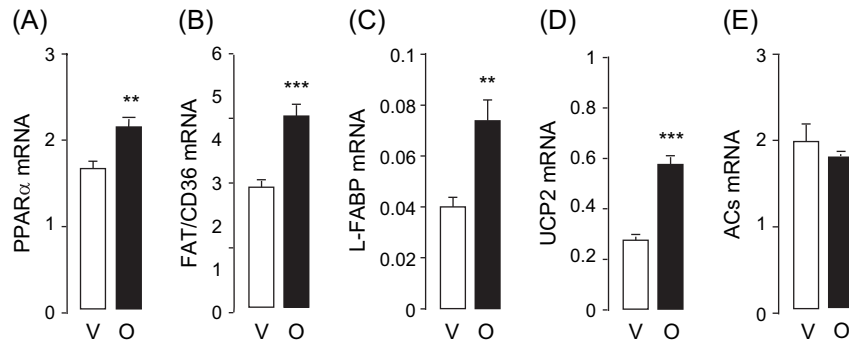


Fig. 5. Subchronic treatment with OEA regulates gene expression in the liver of Zucker rats. Effects of vehicle (V, $n = 6$) and OEA (O, 5 mg kg^{-1} , once daily for 2 weeks, i.p., $n = 6$) on mRNA levels of PPAR- α (A), FAT/CD36 (B), L-FABP (C), UCP-2 (D), and ACs (E). mRNA levels are expressed in arbitrary units. ** $P < 0.01$ and *** $P < 0.001$, Student's t test.

de Fonseca et al., 2001). The results also suggest that OEA lowers lipid levels in liver and blood, raising the possibility that this compound may exert its anti-obesity effects, at least in part, by enhancing lipid oxidation. Though still speculative, this idea is supported by three lines of evidence. First, OEA stimulates fatty-acid oxidation in isolated muscle, heart and liver cells of lean rats and mice (Guzmán et al., 2004). Second, pair-feeding experiments in rats show that hypophagia alone cannot account for the reduction in body-weight gain produced by OEA (Guzmán et al., 2004). Finally, OEA promotes the expression of genes encoding for proteins that are thought to be involved in lipid catabolism and energy balance in lean rats (Guzmán et al., 2004) and Zucker rats (present study).

Here, we found that a 2-week regimen with a relatively low dose of OEA (5 mg kg^{-1} , once daily, i.p.) significantly increased the expression of three PPAR- α target genes in the liver of obese Zucker rats: FAT/CD36, L-FABP and UCP-2. FAT/CD36 and L-FABP are involved in the uptake and transfer of free fatty acids in cells (Meunier-Durmort et al., 1996), respectively, and their expression is regulated by OEA in intestine and liver of lean rats and mice (Fu et al., 2003; Guzmán et al., 2004). UCP-2 is a mitochondrial protein that uncouples respiration from ATP synthesis in liver and muscle tissues, allowing energy to dissipate as heat. This thermogenic effect is thought to promote weight loss and may thus contribute to the anti-obesity effects exerted by OEA in Zucker rats. Collectively, these findings add new information on the biological effects of OEA, which may be useful to elucidate its mechanism of action and to evaluate its therapeutic potential as a scaffold for anti-obesity drugs.

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