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Research report

Oral administration of omega-7 palmitoleic acid induces satiety and the release of appetite-related hormones in male rats

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ABSTRACT

We have analyzed the effect of palmitoleic acid on short-term food intake in male rats. Administration of omega-7 palmitoleic acid by oral gavage significantly decreased food intake compared to palmitic acid, omega-9 oleic acid, or a vehicle control. Palmitoleic acid exhibited a dose-dependent effect in this context and did not cause general malaise. A triglyceride form of palmitoleate also decreased food intake, whereas olive oil, which is rich in oleic acid, did not. Palmitoleic acid accumulated within the small intestine in a dose-dependent fashion and elevated levels of the satiety hormone cholecystokinin (CCK). Both protein and mRNA levels of CCK were affected in this context. The suppression of food intake by palmitoleic acid was attenuated by intravenous injection of devazepide, a selective peripheral CCK receptor antagonist. Palmitoleic acid did not alter the expression of peroxisome proliferator-activated receptor alpha (PPARα) target genes, and a PPARα antagonist did not affect palmitoleic acid-induced satiety. This suggests that the PPARα pathway might not be involved in suppressing food intake in response to palmitoleic acid. We have shown that orally administered palmitoleic acid induced satiety, enhanced the release of satiety hormones in rats.

Introduction

Obesity is a growing problem around the world, representing a metabolic disorder that is associated with many severe, lifestyle-related diseases including cardiovascular disease, diabetes, hypertension, hyperlipidemia, and hyperuricemia (Friedman & Andrus, 2012; Van Gaal, Mertens, & De Block, 2006). A stable body weight is maintained by balancing energy intake and energy expenditure. As such, excessive energy intake is an established risk factor for developing obesity (Burkhalter & Hillman, 2011). Hunger and satiety are physiologically important in this context because they regulate energy intake. It is entirely conceivable, therefore, that appetite suppression represents an effective means of reducing energy intake.

The means by which different nutrients suppress appetite have been intensely studied. In general, fat is less satiating than protein, carbohydrates, or fiber, which may lead to the passive overconsumption of fatty foods (Blundell & Macdiarmid, 1997; Halton & Hu, 2004; van Dam & Seidell, 2007). In rodents, however, the ability of a high-fat diet to induce hyperphagia is associated with the diet's energy and carbohydrate content, not its fat content alone (Ramirez & Friedman, 1990). In fact, lipids suppress later food intake when present in the small intestine of both humans and animals (Castiglione, Read, & French, 1998; Van Wymelbeke, Himaya, Louis-Sylvestre, & Fantino, 1998; Woltman & Reidelberger, 1995). On the other hand, not all fats are equal in their effect on appetite and associated biological processes, and evidence both from human and animal studies suggest that unsaturated fatty acids are more readily oxidized than saturated fats and may be more satiating (Jones & Schoeller, 1988; Piers, Walker, Stoney, Soares, & O'Dea, 2002).

Monounsaturated fatty acids (MUFA) are found mainly in vegetable oils, nuts, and seeds. Studies have demonstrated that MUFA suppressed appetite and short-term food intake in overweight subjects (Flint, Helt, Raben, Toubro, & Astrup, 2003) and animals (Vögler et al., 2008). It has been demonstrated that intestinal infusion of MUFA oleate (C18:1 n-9) increases plasma levels of gut satiety hormones such as cholecystokinin (CCK) (French et al., 2000) and peptide YY (PYY) (Feinle-Bisset, Patterson, Ghatei, Bloom, & Horowitz, 2005). Furthermore, when oleate is infused into the duodenum, it acts as a substrate for the production of oleoylethanolamide (Schwartz et al., 2008), which regulates food intake by activating the nuclear receptor peroxisome proliferator-activated receptor alpha (PPARα) (Fu et al., 2003). This result is not seen with the saturated fatty acid palmitate. Taken together, these results are interesting and suggest that MUFA may be able to reduce appetite by the induction of satiety hormones as well as oleoylethanolamide. One the other hand, the MUFA used in these studies was almost always oleic acid. As such, it is unclear whether MUFAs of

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shorter chain length (<C18) can similarly suppress appetite. It also unclear whether orally administered fatty acids affect satiety, because most studies have used intestinal administration. In the current study, therefore, we aimed to examine the suppressive effects on appetite of orally administered shorter-chain MUFA palmitoleic acid (C16:1) compared to oleic acid (C18:1).

Omega-7 palmitoleic acid is a natural component of several plant products, including oils from macadamia nuts (Maguire, O'Sullivan, Galvin, O'Connor, & O'Brien, 2004) and sea buckthorn (Yang & Kallio, 2001). Palmitoleic acid is also found in animal products such as fish oils (Ozogul, Ozogul, Cicek, Polat, & Kuley, 2008). Experiments in cell culture (Morgan & Dhayal, 2010; Morgan, Dhayal, Diakogiannaki, & Welters, 2008), animal models (Cao et al., 2008; Matthan, Dillard, Lecker, Ip, & Lichtenstein, 2009; Yang, Miyahara, & Hatanaka, 2011), and humans (Garg, Blake, & Wills, 2003; Griel et al., 2008) have shown that palmitoleic acid (or a diet rich in palmitoleic acid) may favorably influence glucose and lipid metabolism. Furthermore, palmitoleic acid increases the release of CCK from STC-1 cells (Tanaka et al., 2008). Whether dietary palmitoleic acid can affect satiety, however, remains unclear. Here we have administered palmitoleic acid *via* p.o. gavage and measured the effect on satiety and levels of appetite-related hormones. We have also investigated whether palmitoleic acid acts through the PPARα pathway to influence appetite.

Methods

Materials

Reagent-grade chemicals were used for all experiments. Free fatty acid (FFA) forms of palmitoleic acid (C16:1 n-7), palmitic acid (C16:0), and oleic acid (C18:1 n-9) were purchased from Sigma Aldrich (St. Louis, MO, USA). GW6471 (a PPARα antagonist) and devazepide (a CCK antagonist) were also purchased from Sigma. Palmitoleate triglyceride (TG) was obtained from Toyo Chemical Industrial Co., Ltd. (Tokyo, Japan). Olive oil was purchased from Wako Pure Chemical Industries, Ltd. (Tokyo, Japan). The fatty acid composition of palmitoleate TG and olive oil (Table 1) was determined by first methylating samples using 14% boron trifluoride-methanol (BF3/methanol, Sigma) for 30 min at 80 °C. Fatty acid methyl esters were quantified *via* gas chromatography using an Agilent 6890 N Network Gas Chromatograph System (Agilent Technologies Japan, Ltd., Tokyo, Japan). Specific methyl esters were identified by comparing retention times to those of standard fatty acid methyl esters (Nu-Chek Prep, Inc., Elysian, MN, USA). All experimental oils were stored at −20 °C until use. Polyglycerol ester was obtained from Mitsubishi-Kagaku Foods Corporation (Tokyo, Japan). Final oil concentrations were obtained by dispersing the oil in 1.5% (w/w) of polyglycerol ester aqueous solution (the vehicle) *via* sonification.

Animals

Nine-week-old male Sprague Dawley rats (SLC, Shizuoka, Japan) were housed individually in stainless steel wire-mesh cages. Animals were exposed to a 12-h light/dark cycle, and a constant temperature of 24 ± 1 °C. To stabilize metabolic conditions at the beginning of the experiment, rats were given free access to distilled water and laboratory chow (Labo MR Stock, Nosan Corporation, Japan) for 1 week. After this stabilization period, rats were randomly divided into experimental groups (n = 8-10). 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 Japan SLC Inc.

Table 1
Fatty-acid composition (%) of olive oil and a triglyceride form of palmitoleate.

Fatty acid	Palmitoleate	Olive oil
C14:0	3.45	0.03
C14:1	0.69	N.D.
C16:0	22.37	10.29
C16:1 n-7	65.20	0.72
C18:0	0.07	2.83
C18:1 n-9	0.83	76.70
C18:2 n-6	0.07	6.57
C18:3 n-3	0.01	0.60
C20:0	N.D.	0.42
C20:4 n-6	N.D.	N.D.
C20:5 n-3	N.D.	N.D.
C22:5 n-3	N.D.	0.05
C22:6 n-3	N.D.	0.03

N.D.: not detected.

Food-intake studies

Five food-intake experiments were performed. In Experiments 1–3, for 24 h preceding the food-intake test, rats were deprived of food but had free access to water. After test oils or vehicle controls were administered by oral gavage, a small feeding tray, which contained a specific amount of powdered chow, was placed in the cage (time 0). Food intake was then recorded at designed time points. Food intake was calculated by subtracting the weight of the uneaten portion of food from the weight of the initial portion. Rats were allowed free access to water throughout the test. The test oils and detailed procedures used in Experiments 1–3 are described as below: In Experiment 1, rats were gavaged with palmitoleic acid FFA (50, 150 or 500 mg/10 mL/kg), palmitic acid FFA (500 mg/10 mL/kg), or a vehicle control (10 mL/kg) and food consumption was measured after 1 h of food exposure. In Experiment 2, rats were gavaged with palmitoleic acid FFA (150 or 500 mg/10 mL/kg), oleic acid FFA (500 mg/10 mL/kg), or a vehicle control (10 mL/kg) to compare the appetite-suppressive effect between palmitoleic acid and oleic acid. These rats were allowed to feed for 1 h and food consumption was measured, after which they were anesthetized and sacrificed. The duodenum, ileum, and jejunum were removed, rinsed with phosphate-buffered saline, snap frozen in liquid nitrogen, and stored at −80 °C until further analysis. Blood was collected from these animals by abdominal vein puncture. Plasma was obtained from these blood samples *via* centrifugation at 2000× for 15 min. Plasma samples were stored at −80 °C until hormone measurements. In Experiment 3, to determine whether a TG form of palmitoleate suppressed food intake, rats were gavaged with palmitoleate TG (770 mg/10 mL/kg; corresponding to 500 mg/kg of palmitoleic acid), olive oil (770 mg/10 mL/kg; corresponding to 500 mg/10 mL//kg of oleic acid), or a vehicle control (10 mL/kg). Food intake was measured 1 h after the oral administration.

In Experiment 4, in order to examine the role of gastrointestinal hormone CCK in appetite-suppressing effect of palmitoleic acid, devazepide (500 μg/5 mL/kg) or its vehicle (5% dimethyl sulfoxide/5% Tween 80/90% saline; 5 mL/kg) was administered *via* intraperitoneal injection 30 min before rats were gavaged with palmitoleic acid (500 mg/10 mL/kg). A feeding tray containing powdered chow was place in the cage after palmitoleic acid administration, and food intake was recorded 2 h later.

Another (fifth) experiment was performed in order to determine whether PPARα affects food intake following the oral administration of palmitoleic acid FFA. GW6471 (3 mg/5 mL/kg) or a vehicle control (10% dimethyl sulfoxide in physiological saline; 5 mL/kg) was administered *via* intraperitoneal injection 30 min

before rats were gavaged with palmitoleic acid (500 mg/10 mL/kg). Food intake was measured 2 h after oral gavage.

Conditioned taste aversion

Rats were deprived of water for 23 h and then allowed to drink from two graduated bottles during a 1-h test period. This procedure was repeated for 10 d. On day 11, the water in each bottle was substituted with a 0.15% aqueous solution of saccharin. Following a 1-h drinking period, rats were gavage palmitoleic acid (150 or 500 mg/10 mL/kg), a vehicle control (10 mL/kg), or lithium chloride (LiCl) (0.15 mol/L, 127 mg/10 mL/kg), which served as a positive control. On days 12 and 13 of the experiment rats were given water in each bottle during the 1-h drinking session. This allowed the rats to recover from any residual effects of sickness (if any). On day 14 (the test day), rats were given one bottle of saccharin and one bottle of water. The amount consumed from each bottle was measured during the 1-h drinking period. The saccharin preference ratio was calculated as the amount of saccharin consumed divided by the total amount of consumed liquids.

Hormone measurements in plasma

In food-intake Experiment 2, plasma concentrations of the satiety-related hormones CCK, PYY, leptin, and ghrelin were measured using the CCK Enzyme Immunoassay Kit (Phoenix Pharmaceuticals, Inc., Belmont, CA, USA), the Mouse/Rat PYY EIA Kit (Yanaihara Institute, Inc., Shizuoka, Japan), the Rat Leptin Elisa Kit (Yanaihara Institute, Inc., Shizuoka, Japan), and the Rat Ghrelin Enzyme Immunoassay Kit (SPI-Bio Bertin Pharma, Paris, France), respectively.

RNA isolation, cDNA synthesis, and quantitative real-time PCR (qRT-PCR)

In food-intake Experiment 2, total RNA was extracted from duodenum, ileum, and jejunum tissues using TRIzol reagent (Qiagen, Tokyo, Japan). cDNA was synthesized using 1 μg of total RNA, 0.5 μg oligo dT-adaptor primers, and the PrimeScript II First Strand cDNA Synthesis Kit (TaKaRa Bio, Otsu, Japan). QRT-PCR was performed using an Applied Biosystems 7500 Real-Time PCR System (Life Technologies Co., Tokyo, Japan). Table 2 lists the forward and reverse PCR primers, which were used at a final concentration of 10 μM. SYBR Premix Ex Taq (TaKaRa Bio) was also used. The PCR cycling parameters were: 30 s at 95 °C; followed by 40 cycles of 5 s at 95 °C, and 34 s at 60 °C. A final melting curve was generated by 15 s at 95 °C, 1 min at 60 °C, and 15 s at 95 °C. Expression levels were normalized to the gene encoding 18S ribosomal RNA.

Determining the fatty acid composition of gastrointestinal tissue

In food-intake Experiment 2, the fatty acid composition of duodenal tissue in rats was determined as described previously (Lepage & Roy, 1986). Briefly, lipids were extracted in a 4:1 (v/v) methanol:hexane solution, then methylated using acetyl chloride for 1 h at 80 °C. Fatty acid methyl esters were separated using a capillary column, then quantified using gas chromatography and compared with purified standards.

Table 2
qRT-PCR primer sequences.

Gene	Forward primer (5′–3′)	Reverse primer (5′–3′)
Cck	CATCCAGCAGGTCCGCAAA	TCCATCCAGCCCATGTAGTCC
Pyy	GCGGTATGGGAAAAGAGAAGTCC	GCAAGTGAAGTCGGTGTAGTTAGCA
Ppara	TCACACAATGCAATCCGTTT	GGCCTTGACCTTGTTCATGT
Fatp1	GTGCGACAGATTGGCGAGTT	GCGTGAGGATACGGCTGTTG
Cd36	CGGCGATGAGAAAGCAGAA	CCAGGCCCAGGAGCTTTATT

Statistical analysis

Results are expressed as the mean ± standard error (SE). Differences between groups were statistically analyzed by one-way analysis of variance (ANOVA) followed by Tukey's test. Analyses were performed using GraphPad Prism software (GraphPad Software, San Diego, CA, USA), and differences were considered statistically significant at $p < 0.05$.

Results

Effect of palmitoleic acid on short-term food intake

Administration of palmitoleic acid by oral gavage suppressed food consumption in a dose-dependent fashion, although there was no difference in food consumption between the palmitic acid and control group (Fig. 1). Higher doses of palmitoleic acid had more dramatic effects, as 50 mg/kg of palmitoleic acid did not affect food intake at the 1-h time point, whereas 150 mg/kg ($p < 0.05$) and 500 mg/kg ($p < 0.001$) doses significantly decreased food intake at this same time point as compared to control. Furthermore, compared with 500 mg/kg of palmitic acid, palmitoleic acid decreased ($p < 0.01$) food consumption at the same concentration. Figure 2 shows that the effect of palmitoleic acid was more dramatic than that of oleic acid, which is an omega-9 MUFA. In fact, rats administered 500 mg/kg of oleic acid FFA exhibited food-intake levels that were indistinguishable from controls (at both 30-min and 1-h time points), and palmitoleic acid significantly decreased ($p < 0.05$) food intake compared to either control or oleic acid group (Fig. 2A). Finally, when a TG form of palmitoleate was given to rats, food intake was significantly lower at the 1-h time point ($p < 0.01$), whereas olive oil did not affect consumption (Fig. 2B).

Conditioned taste-aversion test

To determine whether palmitoleic acid reduced food intake by inducing a non-specific state of behavioral suppression, we conducted a conditioned taste-aversion test. When saccharin consumption was paired with the oral administration of either palmitoleic acid (150 or 500 mg/kg) or a vehicle control, comparable preferences for saccharin were measured (Fig. 3). In contrast,

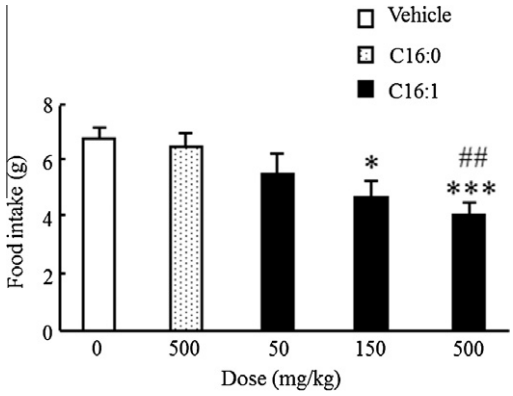

Fig. 1. Dose-dependent effect of palmitoleic acid on food intake. Rats were orally administered palmitoleic acid (C16:1), palmitic acid (C16:0), or a vehicle control. Food intake was measured after 1 h of food exposure. Values represent the mean ± SE ($n = 10$). $^{*}p < 0.05$, $^{***}p < 0.001$ compared to controls. $^{\#\#}p < 0.01$ compared to the palmitic acid group.

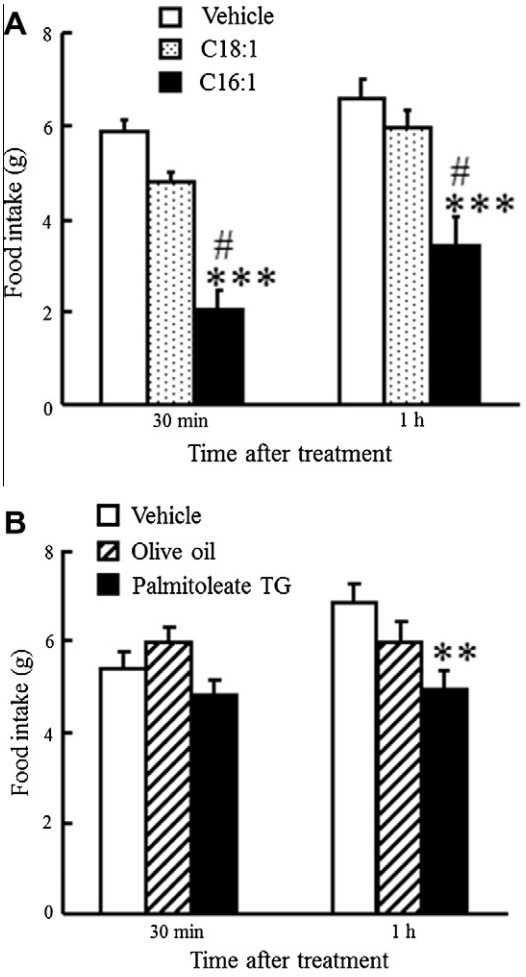

Fig. 2. Effects of palmitoleic acid and oleic acid on food intake. (A) Rats were orally administered palmitoleic acid FFA (C16:1; 500 mg/kg), oleic acid FFA (C18:1; 500 mg/kg), or a vehicle control. Food intake was measured at the time points indicated. (B) Rats were orally administered a TG form of palmitoleate (C16:1 concentration: 500 mg/kg), olive oil (C18:1 concentration: 500 mg/kg), or a vehicle control. Food intake was measured at the time points indicated. Values represent the mean ± SE ($n = 10$). $^{**}p < 0.01$, $^{***}p < 0.001$ compared to controls. $^{\#}p < 0.05$ compared to the oleic acid group.

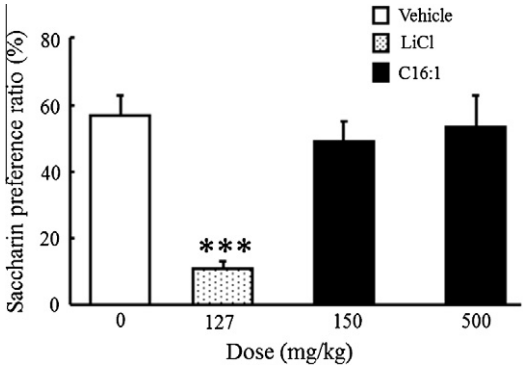

Fig. 3. Effects of LiCl (127 mg/kg), palmitoleic acid (C16:1), or a vehicle control on conditioned taste aversion. Values represent the mean ± SE ($n = 8$). $^{***}p < 0.001$ compared to controls.

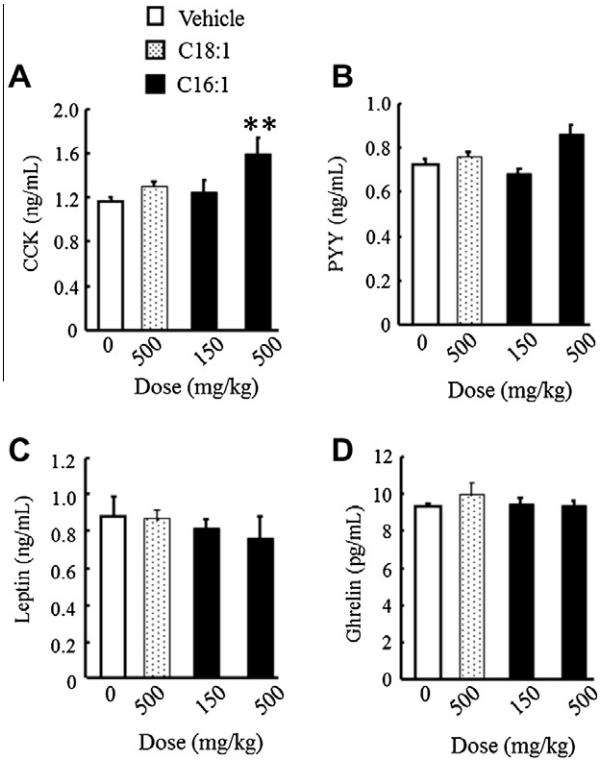

Fig. 4. Plasma levels of satiety-related hormones. Rats were orally administered palmitoleic acid (C16:1), oleic acid (18:1), or a vehicle control. Levels of (A) CCK, (B) PYY, (C) leptin, or (D) ghrelin were measured after 1 h of food exposure. Values represent the mean ± SE ($n = 10$). $^{**}p < 0.01$ compared to controls.

when saccharin was paired with LiCl, a significant decrease in saccharine preference was observed ($p < 0.001$; Fig. 3).

Effect of palmitoleic acid on levels of satiety-related hormones

To investigate the effect of palmitoleic acid on plasma satiety-related hormone levels, we measured plasma concentrations of CCK, PYY, leptin and ghrelin. One hour following the oral administration of palmitoleic acid (500 mg/kg), plasma levels of CCK were significantly elevated compared with controls ($p < 0.01$; Fig. 4A). This increase was not seen with 150 mg/kg of palmitoleic acid, indicating a dose-dependent effect. Administration of oleic acid (500 mg/kg) did not affect CCK levels (Fig. 4A). Furthermore, oral administration of either palmitoleic acid or oleic acid did not affect plasma concentrations of PYY, leptin, or ghrelin (Fig. 4B–D).

Effect of palmitoleic acid on the expression of satiety-related genes

To investigate the effect of palmitoleic acid on gene expressions of satiety-related hormone levels, we measured mRNA expression of *Cck* in duodenum and that of *Pyy* in ileum. One hour following the oral administration of palmitoleic acid (500 mg/kg), *Cck* mRNA levels were significantly elevated in duodenal tissue compared with vehicle controls ($p < 0.01$; Fig. 5A). *Pyy* mRNA levels in the ileum were similarly affected ($p < 0.05$; Fig. 5B). These changes were not seen with 150 mg/kg of palmitoleic acid, indicating a dose-dependent effect. In contrast, the administration of oleic acid (500 mg/kg) did not significantly affect *Cck* or *Pyy* mRNA levels compared with controls.

Effect of CCK receptor antagonist on food intake

When animals were injected (i.p.) with devazepide 30 min before the administration (p.o.) of palmitoleic acid (500 mg/kg), palmitoleate-induced short-term feeding suppression was attenuated (Fig. 6). In the devazepide-treated group, food intake was not lower after oral gavage of palmitoleic acid compared with that of

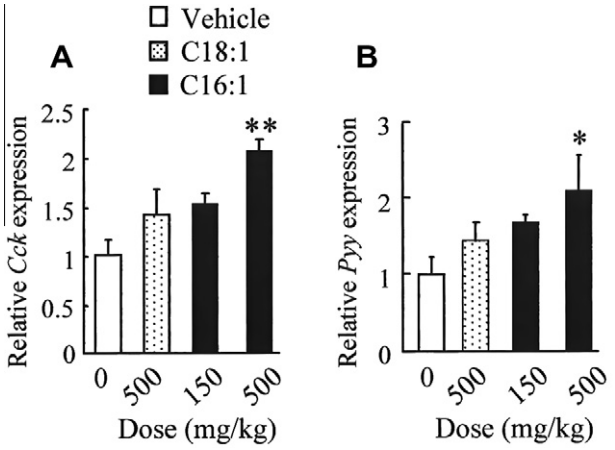

Fig. 5. Relative expression levels of satiety-related genes. Rats were orally administered palmitoleic acid (C16:1), oleic acid (18:1), or a vehicle control. mRNA levels of *Cck* in the duodenum (A) and *Pyy* in the ileum (B) were measured after 1 h of food exposure. Values represent the mean ± SE ($n = 10$). $^{*}p < 0.05$, $^{**}p < 0.01$ compared to controls.

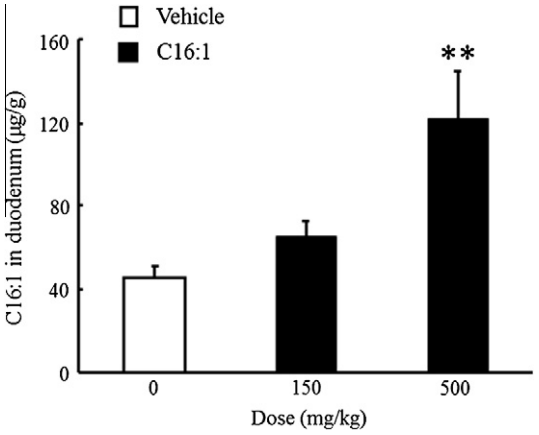

Fig. 7. Dose-dependent accumulation of palmitoleic acid (C16:1) in the duodenum. Rats were orally administered palmitoleic acid (C16:1), or a vehicle control. Levels of palmitoleic acid were determined after 1 h of food exposure. Values represent the mean ± SE ($n = 10$). $^{**}p < 0.01$ compared to controls.

the control, whereas intravenous pretreatment with the vehicle lowered food intake ($p < 0.05$). Food intake was not influenced by the oral gavage of vehicle regardless of devazepide pretreatment.

Accumulation of palmitoleic acid within the gastrointestinal tract

One hour following the oral administration of palmitoleic acid, levels of this fatty acid were measured in the duodenum. At a 500-mg/kg dose, levels of palmitoleic acid were significantly elevated within duodenal tissues compared with vehicle controls ($p < 0.01$; Fig. 7). This elevation was not seen when 150 mg/kg of palmitoleic acid was administered.

Involvement of PPARα in the food-intake response to palmitoleic acid

To determine whether the activation of PPARα contributes to the appetite-suppressing properties of palmitoleic acid, we assessed the effect of palmitoleic acid on the expression of PPARα target genes within the jejunum. Palmitoleic acid administration (500 mg/kg) did not significantly affect mRNA levels of *Ppara*; *solute carrier family 27 (fatty acid transporter), member 1* (*Slc27A1*) (also known as *fatty acid transport protein 1* (*Fatp1*)); or *CD36 molecule (thrombospondin receptor)* (*Cd36*) (also known as *fatty acid translocase* (*Fat*)) compared with controls (Fig. 8). Furthermore, when animals were injected (i.p.) with GW6471 (a PPARα inhibitor) 30 min before the administration (p.o.) of palmitoleic acid (500 mg/kg), a decrease ($p < 0.05$) in food intake was still observed. Injection of GW6471 on its own did not affect food intake compared with controls (Fig. 9).

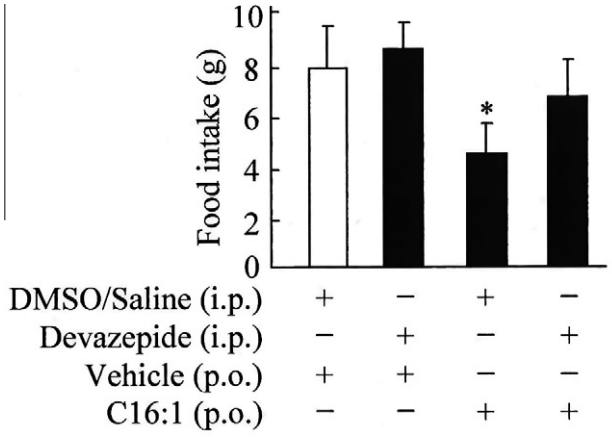

Fig. 6. Effect of CCK receptor antagonist on food intake. A bolus injection of devazepide (500 μg/kg) or a vehicle control was given to rats. Thirty minutes later, they were orally administered palmitoleic acid (C16:1; 500 mg/kg) or a vehicle control. Food intake was measured after 2 h of food exposure. Values represent the mean ± SE ($n = 10$). $^{*}p < 0.05$ compared to controls.

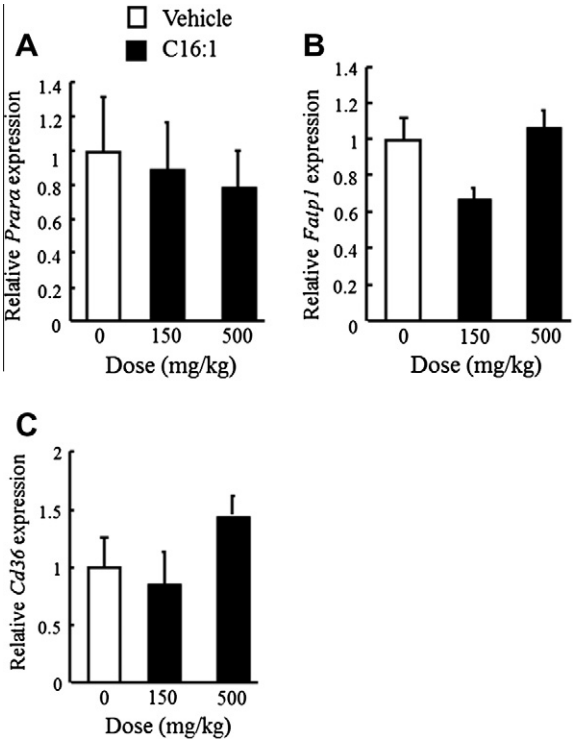

Fig. 8. Relative expression levels of PPARα target genes. Rats were orally administered palmitoleic acid (C16:1), or a vehicle control. After 1 h of food exposure, mRNA levels for (A) *Ppara*, (B) *Fatp1*, and (C) *Cd36* were measured in the jejunum. Values represent the mean ± SE ($n = 10$).

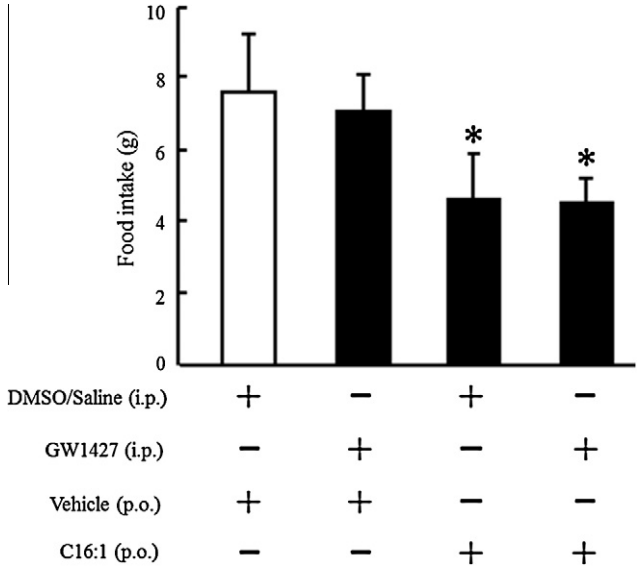

Fig. 9. Effect of PPARα antagonist on food intake. A bolus injection of GW6471 (3 mg/kg) or a vehicle control was given to rats. Thirty minutes later, they were orally administered palmitoleic acid (C16:1; 500 mg/kg) or a vehicle control. Food intake was measured after 2 h of food exposure. Values represent the mean ± SE ($n = 10$). $^{*}p < 0.05$ compared to controls.

Discussion

Oral administration of palmitoleic acid reduced food intake in a dose-dependent manner in food-deprived rats. In a conditioned taste-aversion test, palmitoleic acid (at both low (150 mg/kg) and high (500 mg/kg) doses) had little effect. This suggests that the fatty acid did not evoke aversive behavior per se. This was in contrast to LiCl, which did elicit taste aversion.

To investigate the mechanisms underlying the appetite-suppressive effect of palmitoleic acid, we measured levels of satiety-related hormones in response to palmitoleic acid administration. The CCK and PYY hormones are released within the proximal and distal small intestine, respectively, and regulate food intake. Physiologically, CCK and PYY stimulate pancreatic secretion, elicit gallbladder contraction, regulate gastric emptying, and induce satiety (Beglinger & Degen, 2004; Renshaw & Batterham, 2005). CCK also mediates the initial postprandial release of PYY (Lin, Chey, & Zhao, 2000; McFadden, Rudnicki, Kuvshinoff, & Fischer, 1992). In mammals, PYY release can be stimulated by a fatty meal, the perfusion of fat into the distal small intestine, or by exogenous CCK. These effects can be abolished *via* pretreatment with an antagonist of the CCK receptor. Infusion of exogenous CCK (Brennan et al., 2008; Gutzwiller et al., 2000) or PYY (Degen et al., 2005) reduces food intake and enhances satiety in humans. Furthermore, enterally administered lipids have been shown to modulate gut-hormone release and appetite (Pilichiewicz et al., 2006; Seimon et al., 2009). In the current study, orally administered palmitoleic acid elevated CCK levels in plasma and increased *Cck* and *Pyy* mRNA expression in the small intestine. Furthermore, the effect of palmitoleic acid on food intake suppression was attenuated by intravenous injection of the selective CCK receptor antagonist, devazepide. It is thus suggested that endogenous CCK may possibly mediate the reduction of food intake by orally administered palmitoleic acid and CCK receptors are at least partly involved in the reduction. Collectively, these data suggest that the ability of palmitoleic acid to suppress food intake can be partly attributed to steadily rising levels of appetite hormones in the gut. The fact that palmitoleic acid accumulated in the intestine in a dose-dependent fashion further supports the idea that appetite suppression resulted from exogenous palmitoleic acid. Worthy of note, 150 mg/kg of palmitoleic acid suppressed food intake yet had no effect on plasma levels of CCK. In addition, the gradation of the effect of increasing super-threshold doses (150 and 500 mg/kg) was not significant. It is thus suggested that multiple mechanisms may be involved in the suppressive effect of palmitoleic acid on satiety.

In addition to palmitoleic acid FFA, palmitoleate TG also decreased food intake. Olive oil, which is rich in oleic acid, did not affect food consumption. Compared with palmitoleic acid FFA, however, the TG form took longer to elicit satiety. Generation of long-chain free fatty acids from ingested fat (*via* lipase-mediated hydrolysis) represents a critical step toward the induction of satiety hormones within the gut. CCK secretion largely depends on the generation of long-chain fatty acids, as undigested triglycerides do not stimulate CCK release (Beglinger & Degen, 2004; Degen et al., 2007). It is possible, therefore, that the palmitoleate TG took longer to induce satiety because of this fat-hydrolysis step. Pasman et al. also demonstrated that the increase in plasma CCK levels was faster after ingestion of Korean pine nut oil FFA than TG in post-menopausal overweight women (Pasman et al., 2008). Nevertheless, effects of other components within the palmitoleate TG cannot be excluded. Additional studies are needed, therefore, to directly compare the TG and FFA forms of palmitoleic acid.

Compared with palmitoleic acid, oral administration of oleic acid (another long-chain MUFA) elicited very little appetite suppression. This was true both for its free fatty acid or triglyceride forms. Why oleic acid did not affect appetite in this context remains unknown, although the formation or transport of chylomicrons may have played a role. Long-chain fatty acids are absorbed into the lymphatic circulation system as chylomicrons (Kohan, Yoder, & Tso, 2010). Chylomicron transport has been linked to fat-induced appetite suppression (Glatzle et al., 2002; Whited, Lu, Tso, Kent Lloyd, & Raybould, 2005), as inhibition of chylomicron transport abolishes fat-induced satiety in rodents (Raybould, Meyer, Tabrizi, Liddle, & Tso, 1998). Furthermore, chylomicrons (or their products) release endogenous CCK into the intestinal mucosa (Liu, Doi, & Tso, 2003). Thus, it may be that dietary palmitoleic acid (but not oleic acid) may affect chylomicron formation or transport, leading to CCK release, the initiation of a vagovagal reflex, and the inhibition of gastric motor function. Further studies are needed to test this hypothesis.

Oleoylethanolamide is a fat-induced bioactive lipid amide that affects vagus nerve activity through PPARα. *Via* this mechanism, it induces satiety and decreases meal frequency (Fu et al., 2003; Schwartz et al., 2008). It is conceivable, therefore, that palmitoleic acid stimulates oleoylethanolamide to suppresses appetite. In this study, however, oral administration of palmitoleic acid did not alter the expression of *Ppara* or its target genes (e.g., *Fatp1* and *Cd36*). In addition, a PPARα antagonist did not abolish the satiety effect of palmitoleic acid. This suggests that PPARα may not be involved in palmitoleic acid-induced satiety. Nevertheless, in order to show that the PPARα antagonist is active under the conditions of the test, using positive control such as PPARα agonist is required in further study.

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