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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