Dietary 1-monoolein decreases postprandial GIP release by reducing jejunal transport of glucose and fatty acid in rodents

Akira Shimotoyodome,1 Noriko Osaki,1 Koji Onizawa,1 Tomohito Mizuno,2 Chika Suzukamo,1 Fumiaki Okahara,1 Daisuke Fukuoka,1 and Tadashi Hase1

1Biological Science Laboratories, Kao Corporation, Tochigi, Japan; and 2Health Care Food Research Laboratories, Kao Corporation, Tokyo, Japan

Submitted 1 November 2011; accepted in final form 28 May 2012

Shimotoyodome A, Osaki N, Onizawa K, Mizuno T, Suzukamo C, Okahara F, Fukuoka D, Hase T. Dietary 1-monoolein decreases postprandial GIP release by reducing jejunal transport of glucose and fatty acid. Am J Physiol Gastrointest Liver Physiol 303: G298–G310, 2012. First published May 30, 2012; doi:10.1152/ajpgi.00457.2011.—Postprandial secretion of insulin and glucose-dependent insulino-tropic polypeptide (GIP) is differentially regulated by not only dietary carbohydrate but also fat. Recent studies have shown that the ingestion of diacylglycerol (DAG) results in lower postprandial insulin and GIP release than that of triacylglycerol (TAG), suggesting a possible mechanism for the antiobesity effect of DAG. The structural and metabolic characteristics of DAG are believed to be responsible for its beneficial effects. This study was designed to clarify the effect of 1-monoacylglycerol [oleic acid-rich (1-MO)], the characteristic metabolite of DAG, on postprandial insulin and GIP secretion, and the underlying mechanism. Dietary 1-MO dose dependently stimulated whole body fat utilization, and reduced high-fat diet-induced body weight gain and visceral fat accumulation in mice, both of which are consistent with the physiological effect of dietary DAG. Although glucose-stimulated insulin and GIP release was augmented by the addition of fat, coingestion of 1-MO reduced the postprandial hormone release in a dose-dependent manner. Either glucose or fatty acid transport into the everted intestinal sacs and enteroendocrine HuTu-80 cells was also reduced by the addition of 1-MO. Reduction of either glucose or fatty acid transport or the nutrient-stimulated GIP release by 1-MO was nullified when the intestine was pretreated with sodium-glucose cotransporter-1 (SGLT-1) or fatty acid translocase (FAT)/CD36 inhibitor. We conclude that dietary 1-MO attenuates postprandial GIP and insulin secretion by reducing the intestinal transport of the GIP secretagogues, which may be mediated via SGLT-1 and FAT/CD36. Reduced secretion of these anabolic hormones by 1-MO may be related to the antiobesity effect of DAG.

FAT/CD36; indirect calorimetry; insulin; intestinal transport; SGLT-1

THE WIDESPREAD PREVALENCE of obesity is now a worldwide health problem. Excess adiposity, especially excess abdominal fat accumulation, increases the risk of morbidity from a number of diseases, including diabetes, hypertension, and cardiovascular diseases and is also associated with a greater risk for certain cancers (25). Therefore, improvement of lifestyle, particularly dietary content, is often recommended for the primary prevention and treatment of these diseases.

Diacylglycerol (DAG), which consists of 1,3-DAG and 1,2(2,3)-DAG is contained in natural edible oils at a level of 2–10% (8) and is consumed in the daily human diet. Prior studies in animals and humans have shown that dietary DAG oil composed mainly of 1,3-DAG leads to the suppression of body fat accumulation, body weight loss, improved glucose tolerance, and lower postprandial lipemia compared with that of triacylglycerol (TAG), which has a similar fatty acid composition (32, 39). Recent studies have shown that the ingestion of a 1,3-DAG-rich diet results in a lower postprandial insulin response than that of TAG oil in humans (33, 40). Since a high-level postprandial insulin response has been shown to be associated with increased body fat (38) and the development of insulin resistance (34) these findings suggest a possible mechanism for the beneficial effects of 1,3-DAG.

Postprandial insulin secretion is regulated by not only dietary carbohydrate but also gut-derived incretins, glucose-dependent insulino-tropic polypeptide (GIP), and glucagon-like peptide-1 (GLP-1) (9). More recently, we have shown that 1,3-DAG-rich oil stimulates less GIP secretion compared with TAG-rich oil of a similar fatty acid composition (35), supporting the lower postprandial insulin response after 1,3-DAG-rich oil ingestion in combination with carbohydrate. There is strong evidence for its anabolic characteristics and role in the regulation of adipogenesis of GIP (14). Since increased blood GIP level has been shown in obese patients (7) and decreased GIP signaling attenuates obesity (1, 19), lower postprandial GIP response also seems to contribute to the antiobesity effect of 1,3-DAG.

The structural and metabolic characteristics of 1,3-DAG compared with those of TAG are believed to be responsible for its beneficial effects. It has been shown that 1,3-DAG is digested into 1-monoacylglycerol (1-MO) and free fatty acids (FFA) in the small intestine (17). However, the precise role of 1-MO in postprandial GIP release, the characteristic metabolite of 1,3-DAG, has not been clearly elucidated.

Therefore, this study was designed to provide evidence that 1-MO (oleic acid-rich) reduced postprandial GIP response and attenuated high-fat (HF) diet-induced obesity in mice. Furthermore, this study was carried out to clarify the underlying mechanism of reduction in GIP secretion after 1-MO ingestion.

Recent studies have shown that glucose-stimulated GIP secretion was blocked by phloridizin, an inhibitor of sodium-glucose cotransporter-1 (SGLT-1), in vitro (29) and in vivo (20), indicating that intestinal glucose transport via SGLT-1 triggers GIP secretion. Since very little is known about the apparent mechanism of fat-stimulated GIP secretion, we hypothesized that fat-induced GIP secretion may be triggered by intestinal fatty acid transport. Previous studies indicated that fat-stimulated GIP secretion was associated with chylomicron formation (26, 35). Either chylomicron formation (24) or fatty acid uptake (23) was shown to be mediated by fatty acid translocase (FAT)/CD36 in the proximal intestine. Accord-
ingly, we investigated how 1-MO affected intestinal transport of GIP secretagogues (glucose and fatty acid) via SGLT-1 and FAT/CD36 to elucidate the mechanism of reduction of GIP secretion by 1-MO.

MATERIALS AND METHODS

Materials. All reagents for the experiments were from Sigma-Aldrich Japan (Tokyo, Japan), unless otherwise stated. Commercially available 1-MO of food additive grade was from Kao Corporation (cat. no. O-95R; Tokyo, Japan). The ester distributions and fatty acid compositions of 1-MO were determined by gas chromatography. The fatty acid composition of 1-MO was oleic/stearic/linoleic acid/others = 71.33/4.39/13.48/10.80 (%).

Animals. Male C57BL/6J mice (7–8 wk old; CLEA Japan, Tokyo, Japan) were housed at four or five per cage in a temperature- and relative humidity-controlled (23 ± 2.0°C, 55 ± 10%) room with a 12:12-h light-dark cycle with lights on at 0700. Mice were fed standard chow (cat. no. CE-2; CLEA Japan) consisting of 3.47 kcal/g, with 4.6% fat, 51.4% carbohydrate, and 24.9% protein. Food and water were provided ad libitum. All animal experiments were conducted in the Experimental Animal Facility of Kao Tochigi Institute. The Animal Care Committee of Kao Tochigi Institute approved the present study.

HF diet-induced obesity. Eight mice were assigned to each of the indicated groups (Table 1). The average body weight was adjusted among each group. Each group had free access to the semipurified powder diet (Table 1). The composition of the respective diets was based on our previous study on the antiobesity effect of DAG (21). A dome-type cover on the feeding dish (model Roden CAFE; Oriental Yeast) was used to avoid the scattering of the powdered diet in the cage. The energy values for each diet were calculated from the macronutrient composition using values of 4, 4, and 9 kcal/g for carbohydrate, protein, and oil, respectively.

Mice were maintained for 9 wk. Individual body weights were monitored weekly. Food intake was measured on a per-cage basis throughout the study every 2 or 3 days. Food intake was determined by subtracting the remaining food weight from the initial food weight on the previous feeding day. The energy intake was calculated from the food intake and macronutrient composition of each diet. Mice were anesthetized by the inhalation of sevoflurane (Maruishi Pharmaceutical, Tokyo, Japan) and maintained on ice. After anesthetization, capillary blood samples were collected from the orbital sinus under anesthesia using a heparinized capillary tube. They were kept on ice until plasma preparation. After centrifugation, plasma was stored at −80°C until analysis.

For the first GIP analysis, we collected whole blood samples via the abdominal vein for single blood time points (0, 10 min) to estimate the peak GIP levels circulating in the whole body (35). Overnight-fasted mice were divided into six groups. One group was designated the 0 min group, and blood samples were taken from the abdominal vein. One group was administered glucose alone, and the remaining four were administered glucose plus TO with or without 1-MO. Blood samples were collected into capillary blood collection tubes containing dipeptidyl peptidase IV inhibitor (Millipore, Tokyo, Japan) and maintained on ice. After centrifugation, plasma was stored at −80°C until total GIP analysis.

Primary crypt culture. Primary duodenal crypt culture was prepared following the method described by Parker et al. (29) Freshly removed mouse duodenum was digested with collagenase XI (0.4 mg/ml) for 40 min at 37°C. Digested tissue was centrifuged and resuspended in DMEM (25 mmol/l glucose) supplemented with 10% FCS, 2 mmol/l L-glutamine, penicillin, and streptomycin. The tissue suspension was incubated in Matrigel (BD Biosciences, Bedford, MA)-coated 24-well plates for 24 h at 37°C under 5% CO2. Viability of the crypt culture was verified using LDH assay kit (cat. no. TOX7, In Vitro Toxicology Assay Kit, lactate dehydrogenase-based; Sigma).

In vitro GIP secretion study. GIP secretion study from primary crypt culture was also performed as described previously (29). Briefly, the primary crypt culture was washed twice and incubated in the lipid micelle [sodium taurocholate (2 mmol/l), oleic acid (0.4 mmol/l), and either 1-MO or 2-MO (0.2 mmol/l each)] suspended in bath solution (29) containing 0.1% fatty acid-free BSA for 2 h at 37°C. The composition of the lipid micelle was in accordance with that of a previous study (3), but slightly modified because of cell toxicity of the micelle components. The medium was collected and centrifuged to remove any contaminating cells. The plated cells were disrupted with lysin buffer (29) to extract intracellular GIP. Total GIP was determined in the supernatant fraction and cell lysis sample using a total GIP ELISA kit. GIP secretion in each well is expressed as a fraction of the crypt culture was verified using LDH assay kit (cat. no. TOX7, In Vitro Toxicology Assay Kit, lactate dehydrogenase-based; Sigma).

Table 1. Composition of the semipurified experimental diet

<table>
<thead>
<tr>
<th>Group</th>
<th>LF</th>
<th>HF</th>
<th>HF +3% 1-MO</th>
<th>HF +6% 1-MO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Potato starch, %</td>
<td>66.5</td>
<td>28.5</td>
<td>25.5</td>
<td>22.5</td>
</tr>
<tr>
<td>1-MO, %</td>
<td>0</td>
<td>3</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Sucrose, %</td>
<td>13</td>
<td>13</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>Triglycerides, %*</td>
<td>5</td>
<td>25</td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td>Lard, %</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Casein, %</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Cellulose, %</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Mineral mixture, %</td>
<td>3.5</td>
<td>3.5</td>
<td>3.5</td>
<td>3.5</td>
</tr>
<tr>
<td>Vitamin mixture, %</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Energy, kcal/g</td>
<td>4.0</td>
<td>5.2</td>
<td>5.4</td>
<td>5.5</td>
</tr>
</tbody>
</table>

LF, low-fat diet; HF, high-fat diet; MO, monoacylglycerol. *Fraction of fatty acid composition of triglycerides was oleic/stearic/linoleic acid/others = 35.24/20.00/48.15/14.61 (%).
of the total GIP measured in that well and is normalized to the basal
secretion measured in parallel on the same day.

**Western blot analysis.** The jejunum was removed, and mucosa was
scraped off from mice. Fifteen milligrams of protein were separated
by SDS-PAGE and transferred to a polyvinylidene difluoride mem-
brane (Immun-Blot polyvinylidene difluoride membrane; Bio-Rad,
Hercules, CA). Immunoblotting was performed using goat anti-CD36
(mouse) IgG (cat. no. AF2519, 1:2000; R&D Systems, Minneapolis,
MN). Antibody labeling was detected by chemiluminescence (Pierce
Western Blotting Substrate Plus; Thermo Scientific, Rockford, IL)
and visualized using a ChemiDoc XRS plus imager (Bio-Rad).

**Immunohistochemistry.** Mouse duodenum was fixed in 4% para-
formaldehyde. Frozen sections were blocked with 10% donkey serum
in PBS and incubated with a mixture of rabbit anti-GIP (porcine)
serum (cat. no. T-4340, 1:400; Peninsula Laboratories) and goat
anti-CD36 (mouse) IgG (cat. no. AF2519, 1:200; R&D Systems,
Minneapolis, MN) for 2 h at room temperature. Bound GIP and
FAT/CD36 antibodies were detected using an Alexa Fluor 488-
conjugated donkey anti-rabbit IgG and Alexa Fluor 555-conjugated
donkey anti-goat IgG secondary antibody (1:500; Invitrogen, Carls-
bad, California). Tissue samples stained with the normal rabbit IgG
(10 µg/ml; Dako, Glostrup, Denmark) served as a negative control.
Prolong Gold Antifade Reagent with 4,6-diamidino-2-phenylindole
(Invitrogen) was used for staining nuclei. All fluorescence images
were captured using an all-in-one fluorescence microscope (model
BIOREVO BZ-9000; Keyence, Osaka, Japan) with a ×100/NA 1.4
objective, at an optical slice thickness of 4 µm.

**Glucose and fatty acid transport.** Rat jejunal everted sacs were
prepared as described previously (37). The functional integrity of the
everted sac preparation was assessed on the basis of its ability to
accumulate radiolabeled D-glucose and oleate in the tissue and transfer
it to the serosal solution in a time-dependent manner. Glucose (5%), TO

---

**Fig. 1.** Average (A) and final body weights (B), white adipose tissue (WAT) weights (C), dietary intake (D), body weight gain (E), and WAT weights per calorie intake (F) in C57BL/6J mice fed on 5 or 30% triglycerides (TG)- or 1-monoacylglycerol (1-MO)-supple-
mented diets for 9 wk. Data are expressed as means ± SE; n = 8 in each group. Mice were
fed a low-fat diet (○), high-fat (HF) diet (●), or HF diet supplemented with 3 (▲) or 6% (▼)
1-MO. B–F: data are expressed as means ± SE; n = 8 in each group. LF, low-fat diet. Statistical
analysis was conducted using 1-way ANOVA followed by Dunnett post hoc test. *P < 0.05
(for the difference from the HF diet group). N.S., not significant.
Glucose and fatty acid uptake study with HuTu-80 cells. The HuTu-80/SGLT-1 cells were grown to 90% confluence. The cells were then split and seeded into 12-well plates (5 × 10⁵ cells/well). The cells were cultured in glucose-free and FBS-free DMEM medium for 1 h, before nutrient uptake.

To examine the effect of phloridzin on glucose uptake, the cells were washed twice with PBS containing 1.8 mM CaCl₂ and 1 mM MgCl₂, followed by incubation in 0.5 ml of PBS buffer containing 14C-labelled glucose (α-MG) (0.5 μCi/ml, cat. no. NET289, PerkinElmer) and 100 μM oleate for 30 min at 37°C.

In the case of 1-MO inhibition study, the cells were incubated in 0.5 ml of PBS buffer containing or without 0.5 mM SSO for 30 min at 37°C followed by incubation in [3H]oleate (0.5 μCi/ml, cat. no. NET289, PerkinElmer) and 100 μM oleate for 30 min at 37°C.

To determine the effect of 1-MO on nutrient uptake, the cells were incubated in 0.5 ml of PBS buffer containing or without 0.5 mM SSO for 30 min at 37°C followed by incubation in [3H]oleate (0.5 μCi/ml, 100 μM oleate and 1% TO with or without 0.2% 1-MO for 10 or 30 min at 37°C.

The cells were washed three times with PBS buffer and solubilized with 0.5 ml of 0.1 N NaOH, which was added to 10 ml of Scintillation Cocktail (Hionic-Fluor, PerkinElmer). The radioactivity of 3H and 14C in the tissue samples was measured using an automatic liquid-scintillation spectrometer (Tri-Carb 2550 TR/LL; Packard, Rungis, France).

**Effect of phloridzin on glucose-induced GIP secretion and glucose transport.** Overnight-fasted Sprague-Dawley rats (8 wk old; Japan SLC, Shizuoka, Japan) were gastrically administered 5% glucose and fatty acid transport.

**Effect of sulfo-N-succinimidyl oleate on fat-induced GIP secretion and fatty acid transport.** Sulfo-N-succinimidyl oleate (SSO), an inhibitor of FAT/CD36 (6), 30), was synthesized as previously reported (12). Overnight-fasted rats were anesthetized by the intraperitoneal injection of 7% urethane and 1.5% chloral hydrate. Under anesthesia, the duodenum was cannulated using a polyethylene tube and infused with Krebs buffer containing 0.2% BSA with or without 200 μM SSO at 37°C for 25 min followed by rinsing twice with 4 ml of Krebs buffer containing 0.2% BSA with for 20 s. The sacs were treated in glucose plus TO emulsion at 37°C for 10 min, followed by rinsing with 0.1% N-acetyl cysteine and 0.1% taurocholate.

**Effect of sulfo-N-succinimidyl oleate on fat-induced GIP secretion and fatty acid transport.** Sulfo-N-succinimidyl oleate (SSO), an inhibitor of FAT/CD36 (6, 30), was synthesized as previously reported (12). Overnight-fasted rats were anesthetized by the intraperitoneal injection of 7% urethane and 1.5% chloral hydrate. Under anesthesia, the duodenum was cannulated using a polyethylene tube and infused with Krebs buffer containing 0.2% BSA with or without 200 μM SSO at 37°C for 25 min followed by rinsing twice with 4 ml of Krebs buffer containing 0.2% BSA with for 20 s. The sacs were treated in glucose plus TO emulsion at 37°C for 10 min, followed by rinsing with 0.1% N-acetyl cysteine and 0.1% taurocholate.

**Effect of sulfo-N-succinimidyl oleate on fat-induced GIP secretion and fatty acid transport.** Sulfo-N-succinimidyl oleate (SSO), an inhibitor of FAT/CD36 (6, 30), was synthesized as previously reported (12). Overnight-fasted rats were anesthetized by the intraperitoneal injection of 7% urethane and 1.5% chloral hydrate. Under anesthesia, the duodenum was cannulated using a polyethylene tube and infused with Krebs buffer containing 0.2% BSA with or without 200 μM SSO at 37°C for 25 min followed by rinsing twice with 4 ml of Krebs buffer containing 0.2% BSA with for 20 s. The sacs were treated in glucose plus TO emulsion at 37°C for 10 min, followed by rinsing with 0.1% N-acetyl cysteine and 0.1% taurocholate.

**Effect of sulfo-N-succinimidyl oleate on fat-induced GIP secretion and fatty acid transport.** Sulfo-N-succinimidyl oleate (SSO), an inhibitor of FAT/CD36 (6, 30), was synthesized as previously reported (12). Overnight-fasted rats were anesthetized by the intraperitoneal injection of 7% urethane and 1.5% chloral hydrate. Under anesthesia, the duodenum was cannulated using a polyethylene tube and infused with Krebs buffer containing 0.2% BSA with or without 200 μM SSO at 37°C for 25 min followed by rinsing twice with 4 ml of Krebs buffer containing 0.2% BSA with for 20 s. The sacs were treated in glucose plus TO emulsion at 37°C for 10 min, followed by rinsing with 0.1% N-acetyl cysteine and 0.1% taurocholate.

**Effect of sulfo-N-succinimidyl oleate on fat-induced GIP secretion and fatty acid transport.** Sulfo-N-succinimidyl oleate (SSO), an inhibitor of FAT/CD36 (6, 30), was synthesized as previously reported (12). Overnight-fasted rats were anesthetized by the intraperitoneal injection of 7% urethane and 1.5% chloral hydrate. Under anesthesia, the duodenum was cannulated using a polyethylene tube and infused with Krebs buffer containing 0.2% BSA with or without 200 μM SSO at 37°C for 25 min followed by rinsing twice with 4 ml of Krebs buffer containing 0.2% BSA with for 20 s. The sacs were treated in glucose plus TO emulsion at 37°C for 10 min, followed by rinsing with 0.1% N-acetyl cysteine and 0.1% taurocholate.

**Effect of sulfo-N-succinimidyl oleate on fat-induced GIP secretion and fatty acid transport.** Sulfo-N-succinimidyl oleate (SSO), an inhibitor of FAT/CD36 (6, 30), was synthesized as previously reported (12). Overnight-fasted rats were anesthetized by the intraperitoneal injection of 7% urethane and 1.5% chloral hydrate. Under anesthesia, the duodenum was cannulated using a polyethylene tube and infused with Krebs buffer containing 0.2% BSA with or without 200 μM SSO at 37°C for 25 min followed by rinsing twice with 4 ml of Krebs buffer containing 0.2% BSA with for 20 s. The sacs were treated in glucose plus TO emulsion at 37°C for 10 min, followed by rinsing with 0.1% N-acetyl cysteine and 0.1% taurocholate.

**Effect of sulfo-N-succinimidyl oleate on fat-induced GIP secretion and fatty acid transport.** Sulfo-N-succinimidyl oleate (SSO), an inhibitor of FAT/CD36 (6, 30), was synthesized as previously reported (12). Overnight-fasted rats were anesthetized by the intraperitoneal injection of 7% urethane and 1.5% chloral hydrate. Under anesthesia, the duodenum was cannulated using a polyethylene tube and infused with Krebs buffer containing 0.2% BSA with or without 200 μM SSO at 37°C for 25 min followed by rinsing twice with 4 ml of Krebs buffer containing 0.2% BSA with for 20 s. The sacs were treated in glucose plus TO emulsion at 37°C for 10 min, followed by rinsing with 0.1% N-acetyl cysteine and 0.1% taurocholate.

**Effect of sulfo-N-succinimidyl oleate on fat-induced GIP secretion and fatty acid transport.** Sulfo-N-succinimidyl oleate (SSO), an inhibitor of FAT/CD36 (6, 30), was synthesized as previously reported (12). Overnight-fasted rats were anesthetized by the intraperitoneal injection of 7% urethane and 1.5% chloral hydrate. Under anesthesia, the duodenum was cannulated using a polyethylene tube and infused with Krebs buffer containing 0.2% BSA with or without 200 μM SSO at 37°C for 25 min followed by rinsing twice with 4 ml of Krebs buffer containing 0.2% BSA with for 20 s. The sacs were treated in glucose plus TO emulsion at 37°C for 10 min, followed by rinsing with 0.1% N-acetyl cysteine and 0.1% taurocholate.

**Effect of sulfo-N-succinimidyl oleate on fat-induced GIP secretion and fatty acid transport.** Sulfo-N-succinimidyl oleate (SSO), an inhibitor of FAT/CD36 (6, 30), was synthesized as previously reported (12). Overnight-fasted rats were anesthetized by the intraperitoneal injection of 7% urethane and 1.5% chloral hydrate. Under anesthesia, the duodenum was cannulated using a polyethylene tube and infused with Krebs buffer containing 0.2% BSA with or without 200 μM SSO at 37°C for 25 min followed by rinsing twice with 4 ml of Krebs buffer containing 0.2% BSA with for 20 s. The sacs were treated in glucose plus TO emulsion at 37°C for 10 min, followed by rinsing with 0.1% N-acetyl cysteine and 0.1% taurocholate.
RESULTS

HF diet-induced obesity. The body and visceral WAT weights of mice fed a HF diet were significantly higher than those of mice fed an low-fat diet. The mice fed the HF diet containing 3 or 6% 1-MO weighed less than those fed the HF diet (Fig. 1, A and B). The body and total WAT weights were significantly lower in the 6% 1-MO diet-fed mice than in those on the HF diet (Fig. 1, B and C). Since dietary intake tended to be lower ($P = 0.17$) in the 6% 1-MO diet-fed than in the HF diet-fed groups (Fig. 1D), we calculated the food efficiency by dividing the body weight gain and total WAT weight by the food intake. Dietary supplementation with 6% 1-MO reduced the food efficiency for either the body or the visceral WAT weight in a dose-dependent manner (Fig. 1, E and F). Body and visceral WAT weights per food intake were significantly lower in the 6% 1-MO-fed than in the HF diet groups.

Fig. 3. Plasma concentrations of glucose (A), insulin (C), and TG (E) and areas under the curve (AUCs) immediately before and 10, 30, 60, and 120 min after the administration of glucose alone [2 mg/g body wt (BW), ○], glucose plus glyceryl trioleate (TO; 2 mg/g body wt each, ■), or 1-MO [0.08 (gray diamonds), 0.2 (gray triangles), or 0.4 mg/g body wt (gray squares)] together with glucose plus TO (2 mg/g body wt each) through gastric gavage in overnight-fasted anesthetized male C57BL/6J mice. AUCs are calculated from 0–120 min for glucose (B), and from 0–30 min for insulin (D). Data are expressed as means ± SE; $n = 8$ in each group. Increase in the plasma concentration of glucose-dependent insulinotropic polypeptide (GIP) at 10 min after the administration of glucose alone (2 mg/g body wt) or glucose + TO (2 mg/g body wt each) with 1-MO (0–0.4 mg/g body wt) through gastric gavage in overnight-fasted anesthetized male C57BL/6J mice (F). Whole blood samples were collected via the abdominal vein for single blood time points (0, 10 min) to estimate total GIP circulating in the whole body. Data are calculated by subtracting the average initial concentration from that after 10 min, and expressed as means ± SE; $n = 12$ in each group. Effect of monoolein on GIP secretion from mouse duodenum crypt culture (G). The crypt culture was incubated for 2 h in the lipid micelle [sodium taurocholate (2 mmol/l), oleic acid (0.4 mmol/l), and either 1-MO or 2-MO (0.2 mmol/l each)] suspended in control buffer. Data are normalized to the basal secretion after control buffer, and expressed as means ± SE; $n = 6$ in each group. Statistical analysis was conducted using 1-way ANOVA followed by Dunnett post hoc test [for A–F, *$P < 0.05$ (for the difference from the high-fat diet group)] or Bonferroni/Dunn multiple comparison [for G; $a$, $b$, $c$, $d$ Means not sharing a given letter differ significantly ($P < 0.0167$)].
Energy expenditure and fat utilization. In the HF diet-fed condition, RER was significantly lower in the 1-MO-fed groups (0.844 ± 0.005 and 0.829 ± 0.006 for 3 and 6%, respectively) than in the control group (0.856 ± 0.006), while energy expenditure was similar between the groups. Thus, fat utilization was significantly higher in the 6% 1-MO, and tended to be higher (P = 0.084) in the 3% 1-MO groups, than in the control group (Fig. 2B), although total energy expenditure did not differ between the groups (Fig. 2A). Dietary intake of the mice housed in metabolic chambers was not different between the groups (P > 0.05). Body weight gains of the mice housed in metabolic chambers were 1.04 ± 0.27, 0.93 ± 0.24, and 0.58 ± 0.24 g in the control, 3%, and 6% 1-MO groups, respectively, and they were not significantly different (P > 0.05).

Postprandial blood responses. Glucose administration increased plasma glucose levels at 10 min, which thereafter declined (Fig. 3A). Insulin levels peaked at 10 min after glucose administration and thereafter declined, reaching the baseline after 30 min (Fig. 3C). When TO was administered together with glucose, the insulin level was significantly increased at 10 min, followed by a marked decline of blood glucose at 30 min (Fig. 3, A and C). The glucose response was significantly decreased after glucose plus TO compared with that after glucose (~30% decrease in AUCGlucose120 min, Fig. 3B).

The coadministration of 1-MO with glucose plus TO decreased peak insulin levels and increased blood glucose at 30 min in a dose-dependent manner (Fig. 3, C and A, respectively) but did not affect peak blood glucose levels. Blood glucose at 30 min was negatively (R = −0.636, P < 0.0001) and Kc (the glucose elimination rate between 10 and 30 min) was positively (R = 0.495, P < 0.01) correlated with the peak insulin level at 10 min. The postprandial insulin response (AUC) for 30 min was decreased dose dependently upon 1-MO administration (Fig. 3D), which was associated with an increased glucose response for 120 min (Fig. 3B). Peak insulin and triglycerides...
levels after glucose plus TO administration were significantly decreased by the addition of 0.4% 1-MO to the levels after the administration of glucose alone (Fig. 3, C and E).

To study the underlying mechanism of the inhibitory effect of 1-MO on the fat-promoted blood insulin response that occurred at 10 min after gavage, we examined the postprandial response of GIP. In our previous study, the blood GIP level was shown to peak at 10 min after gastric gavage (35).

Glucose administration increased circulating blood GIP levels at 10 min after gastric gavage (Fig. 3F). When TO was administered together with glucose, the GIP level was significantly increased. However, the addition of 1-MO significantly decreased the circulating GIP levels after glucose plus TO administration in a dose-dependent manner (Fig. 3F). The fat-promoted GIP response for 10 min was significantly inhibited by 0.4% 1-MO administration (P < 0.01) (Fig. 3F).

**Effect of 1-MO on GIP secretion in vitro.** To study how 1-MO affects the nutrient-induced GIP release from intestine, we examined the effect of lipid micelle containing either 1-MO or 2-MO on GIP secretion from duodenum crypt culture.

Whereas each lipid micelle stimulated GIP secretion from crypt culture into medium, significantly less GIP was secreted (P < 0.001) for lipid micelle containing 1-MO compared with that containing 2-MO (Fig. 3G). The GIP release for 1-MO lipid micelle was only half of that for 2-MO lipid micelle (Fig. 3G).

**Effect of 1-MO on postprandial blood responses in HF diet-fed obese mice.** Several studies have shown that postprandial GIP secretion was increased after chronic HF feeding (10) and in obese subjects (7). To examine the effect of 1-MO on GIP hypersecretion, mice were maintained on a semipurified HF diet for 23 wk to induce obesity, and we conducted glucose and fat loading tests. Fasting and postprandial blood glucose, insulin, and GIP levels after either glucose or glucose plus TO administration were higher in the HF diet-fed obese mice (44.5 ± 0.7 g in body wt, Fig. 4, E and F) than in the standard chow (CE-2)-fed normal mice (18.5 ± 0.1 g in body wt, Fig. 5, A–C). When 1-MO was administered with glucose plus TO, peak insulin and GIP levels decreased. Postprandial insulin (AUC<sub>Insulin</sub>30 min) and GIP (AUC<sub>GIP</sub>60 min) responses were significantly lower in the 1-MO-administered mice than in the control (glucose plus TO-administered) mice (Fig. 4, E and F). The blood glucose response did not differ between the 1-MO-administered and control groups (Fig. 4F). Fasting blood triglycerides levels and postprandial blood TG responses were lower in the obese mice than in the normal mice. The blood TG response did not differ between the 1-MO-administered and control groups (data not shown).

**Effect of 1-MO on intestinal glucose and fatty acid transport.** Transport of radiolabeled glucose, maltose, and oleate from the emulsion into the jejunal sacs for 10 min was significantly lower when the emulsion was supplemented with 1-MO (Fig. 5, A–C). Mannitol transport into the jejunal sacs from the emulsion was not changed by the addition of 1-MO (Fig. 5D).

**Effects of inhibitors of glucose and fatty acid transport on blood GIP response.** The coadministration of phloridzin with glucose significantly decreased the peak levels and postprandial increases of blood glucose (Fig. 6, A and B), insulin (Fig. 6, C and D), and GIP (Fig. 6, E and F) compared with those with glucose alone in rats.

In addition, the peak GIP levels during duodenal TO infusion were significantly reduced when TO was infused with SSO (Fig. 6G). The GIP response during the infusion period (60 min) was significantly lower in SSO-treated than in control groups.
Localization of FAT/CD36 in GIP-producing K cells. Goat anti-CD36 (mouse) IgG (cat. no. AF2519; R&D Systems) reacted with a single band of FAT/CD36 in Western blot analysis of mouse duodenal protein (Fig. 7A). Immunohistochemistry using the antibody showed the apical localization of FAT/CD36 in epithelial cells of the villus. GIP-positive staining was observed in the cytoplasmic area of isolated epithelial cells. Continual FAT/CD36-positive staining of the apical area and the GIP-positive cytoplasmic area were collocated in the isolated, spindle-shaped epithelial cells (Fig. 7B–F).

Effect of glucose or fatty acid transport inhibition on 1-MO activity. Figure 8, A and D shows the effects of 1-MO on glucose (Fig. 8A) and oleate (Fig. 8D) transport with SGLT-1 and FAT/CD36 inhibitors, respectively. Phloridzin reduced glucose transport (Fig. 8A), but did not affect oleate transport (Fig. 8B), from the emulsion into the everted sacs. Glucose transport into the...
tissue was significantly reduced by 1-MO when the emulsion did not contain phloridzin. However, glucose transport was not reduced further by adding 1-MO when the emulsion was supplemented with phloridzin (Fig. 8A). 1-MO reduced intestinal oleate transport from the emulsion either with (P/H0.0051) or without (P/H0.029) phloridzin (Fig. 8B).

Figure 8, C and D shows the effect of 1-MO on glucose (Fig. 8C) and oleate (Fig. 8D) transport with or without pretreatment of SSO. SSO reduced oleate transport (P/H0.023) (Fig. 8D), but did not affect glucose transport (Fig. 8C), from the emulsion into the tissue. Oleate transport was not reduced further by adding 1-MO when the intestine was pretreated with SSO (Fig. 8D). Glucose transport tended to be reduced by 1-MO whether the sacs were pretreated with SSO (P/H0.082) or not (P/H0.0274) (Fig. 8C).

**Effect of 1-MO on glucose or fatty acid uptake into human enteroendocrine cells.** Preliminary analysis by semiquantitative RT-PCR showed that HuTu-80, human duodenal endocrine cell line, expressed *Gip* and *Cd36*, but not *Sglt1* mRNA. Therefore, the cells were stably transfected with human SGLT-1 (HuTu-80/SGLT-1) to examine the effect of 1-MO on cellular uptake of glucose and fatty acid. HuTu-80/SGLT-1 cells had 10-fold greater glucose uptake activity than original HuTu-80 cells. Cellular uptake of radiolabeled α-MG and oleate was increased in a time-dependent manner, and significantly blocked by phloridzin and SSO (data not shown). Addition of 1-MO significantly reduced radiolabeled α-MG and oleate transported into the cells for 30 min to ~60% compared with those of control (Fig. 9, A and B).

**DISCUSSION**

This study had four major findings. First, this study demonstrated that dietary 1-MO stimulated fat utilization and attenuated HF diet-induced obesity and postprandial lipemia in mice, either of which is consistent with the physiological effect of dietary 1,3-DAG. Second, and more importantly, was that 1-MO attenuated postprandial GIP and insulin responses either in normal or in HF diet-fed obese mice. Third, and the most
importantly, was the novel finding that FAT/CD36 was located on the apical membrane of GIP-producing K cells in the mouse duodenum, and fat-stimulated GIP secretion was reduced by SSO, suggesting that fatty acid transport via FAT/CD36 plays a significant role in fat-stimulated GIP secretion from K cells. Finally, this study provides evidence that the luminal 1-MO decreased the intestinal transport of GIP secretagogues via SGLT-1 and FAT/CD36, but not passive transport of mannitol.

The increased fat utilization is thought to drain fatty acids from the body, reduce VLDL formation, and exert antiobesity effects (4). Several studies demonstrated that the upregulation of fat catabolism is associated with reduced fat accumulation in rodent models of HF diet-induced or genetic insulin resistance (2, 11, 22). The results in the present study suggest that the antiobesity effect of 1,3-DAG is attributable to 1-MO, its characteristic metabolite in the intestinal lumen. Coingestion of 1-MO also lowered the postprandial blood triglycerides response, suggesting that the decreased postprandial triglyceridemia after 1,3-DAG ingestion (32) may also be explained by the specific metabolism of 1,3-DAG in the small intestine. However, the effect of 1-MO of other fatty acids (e.g., linoleic acid) still remains to be studied.

Although not vitiating the significance of the findings, dietary supplementation with 1-MO reduced the cumulative energy intake of mice by ~9%. Although we cannot rule out the possibility that 1-MO reduced diet-induced obesity by a tendency of lowering the energy intake, body weight gain could not be explained entirely by reduced energy intake since feed efficiency (body weight gain per diet intake) was significantly lowered by 1-MO ingestion. We preliminarily confirmed that dietary 1-MO did not affect meal ingestion in the short feeding period, suggesting that 1-MO does not induce taste aversion in mice. Whereas calorie restriction has been shown to decrease the metabolic rate in rodents (2, 18), 1-MO did not affect the dietary intake and total energy expenditure, and rather increased fat utilization in our indirect calorimetry. The tendency of reduced cumulative energy intake in 1-MO-fed mice in the longer feeding period may be attributable to a lower energy requirement caused by a reduction of the body weight compared with that of the HF diet-fed mice. As another possibility, the long-term ingestion of 1-MO may directly regulate appetite and satiety, for example, by affecting appetite-regulating gut hormones, such as ghrelin, CCK, PYY, or GLP-1. Further studies are needed to clarify the effect of chronic 1,3-DAG or 1-MO ingestion on gut-derived satiety hormones and appetite/satiety control in the brain.

Although coingestion with 1-MO did not affect postprandial peak blood glucose levels, it decreased peak insulin levels and lowered the subsequent decrease in blood glucose. Blood insulin level after 1-MO ingestion does not fall below, and thereby blood glucose does not exceed, the level after glucose ingestion. Thus, higher blood glucose caused by reduced insulin response after 1-MO ingestion does not seem to be undesirable from a therapeutic perspective.

Decreased levels of the anabolic hormones seem to be beneficial to prevent the progression of obesity. Despite the lack of a change in the activity of GIP or active GIP secretion, this study suggests the intriguing possibility that luminal 1-MO reduces the secretion of GIP and thereby lowers postprandial insulin secretion. While we cannot exclude the possibility that 1-MO ingestion alters the renal or hepatic extraction of GIP or insulin, respectively, the present results are consistent with our previous findings (35), and suggest that lower GIP and insulin levels after 1,3-DAG ingestion than those after TAG may be explained by 1-MO.

From the result of indirect calorimetry, the magnitude of increased fat utilization by 6% 1-MO ingestion was estimated to be ~3 mg/g body wt/day. This increase in fat utilization explains half of the decrease of visceral fat weight after 1-MO ingestion for 9 wk. Furthermore, the inhibition of GIP signaling has been shown to increase fat oxidation and prevent the onset of obesity induced by a HF diet (19). Our previous study has shown that increased levels of circulating GIP or insulin decreased the hepatic gene expression of ACO, the rate-
limiting enzyme involved in peroxisomal fatty acid oxidation and fat oxidation capacity, and whole body fat utilization (37). Recently, Althage et al. (1) reported that the targeted ablation of GIP-producing cells in transgenic mice enhanced energy expenditure and reduced HF diet-induced obesity. The results in this study are consistent with the above findings, and suggest that lower blood GIP and insulin levels after 1-MO ingestion enhanced fat oxidation, and thereby prevented diet-induced obesity.

Gastric emptying was shown to contribute to the postprandial glucose concentrations in healthy subjects and patients with type 2 diabetes (16). However, it is unlikely that delayed gastric emptying is responsible for the acute changes in GIP secretion after the ingestion of 1-MO because the coinestion of 1-MO did not delay the peak blood glucose and triglycerides levels.

The results in this study suggested that fat-induced GIP secretion was triggered by fatty acid transport via SGLT-1 and FAT/CD36 located in GIP-producing K cells. Parker et al. (29) have shown the expression of Sglt1 mRNA and the apical localization of SGLT-1 protein in K cells and inhibition of glucose-induced GIP secretion by phloridzin. The inhibitory effect of phloridzin on GIP secretion in this study was consistent with their results, suggesting that glucose-induced GIP secretion is triggered by glucose transport via SGLT-1 into K cells.

Recent reports suggest that G protein-coupled fatty acid receptors, such as GPR40, 119, and 120, may play a role in the regulation of enteroendocrine hormone release (5, 13, 28). Hansen et al. (11) showed that 2-monoacylglycerol formed during fat digestion can activate GPR119 and cause GLP-1 release from the human intestine. Hirasawa et al. (13) have reported that long-chain fatty acids evoked ligand-induced internalization of GPR120 and stimulated GLP-1 secretion triggered by intracellular Ca^{2+} increase. Parker et al. (29) have shown that murine K cells expressed these fatty acid receptors including GPR119 and GPR120. Taking these findings together, long-chain fatty acids seem to stimulate GIP release by a similar mechanism to that of GLP-1 release. However, the molecular mechanism behind the relationship between GIP secretion and the intracellular metabolism of fat in K cells remains to be elucidated.

The reduction of glucose or fatty acid transport by 1-MO was nullified when it was blocked by phloridzin (Fig. 8A) or SSO (Fig. 8D), suggesting that 1-MO competes with each specific inhibitor for the inhibition of SGLT-1 or FAT/CD36 activity. The results of cellular uptake of the GIP secretagogues, which was reduced by phloridzin, SSO, or 1-MO, were consistent with those of nutrient-induced GIP secretion in vivo. Taken together, these results suggest the intriguing possibility that decreased GIP secretion due to 1-MO is attributable to reduced or retarded active transport of the GIP secretagogues via the transcellular pathway mediated by SGLT-1 or FAT/CD36.

The possibility that 1-MO reduced fat absorption as the mechanism behind its antiobesity effect cannot be excluded since we did not examine overall lipid absorption from the intestine. However, it seems to be unlikely that 1-MO ingestion decreases whole-intestine lipid absorption, since 1,3-DAG-rich oil did not increase lipid fecal excretion in rodents (27) even though 1,3-DAG reduced fatty acid transport from the jejunal sacs (Shimotoyodome A et al., unpublished data). In addition, lipid administration to Cd36-deficient mice did not alter the fecal lipid output (24) since FAT/CD36 mainly contributes to fatty acid uptake by the proximal intestine (23). Taking these results into account, dietary 1-MO does not seem to inhibit whole-gut lipid absorption enough to increase fecal lipid output.

Although intestinal uptake of glucose and fatty acid via SGLT-1 and FAT/CD36 is basically common in rodents and humans, there may be a limitation in this study due to the difference of species since this study relied on different species for postprandial GIP response, intestinal and cellular uptake of nutrients. The molecular and cellular mechanism of reduced GIP secretion by 1-MO remains to be definitively characterized.

In conclusion, this study provides evidence that 1-MO ingestion lowers nutrient-stimulated GIP secretion by reducing glucose and fatty acid transport via SGLT-1 and FAT/CD36. The present study suggests that 1-MO serves as an exogenous physiological regulator of hormone secretion with an influence on energy homeostasis. This should be important for the further development of GIP-based therapy for the treatment of obesity. Therefore, more detailed studies of GIP kinetics should be undertaken in relation to energy homeostasis in larger animals and humans.

ACKNOWLEDGMENTS

The authors thank Nanami Sugino for excellent technical support. The authors also thank Kojiro Hashizume for support in the chemical synthesis of sulfo-N-succinimidyl oleate.
REFERENCES


