Anti-obesity effect of dietary diacylglycerol in C57BL/6J mice: dietary diacylglycerol stimulates intestinal lipid metabolism

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Abstract **We examined the long-term effects of dietary diacylglycerol (DG) and triacylglycerol (TG) with similar fatty acid compositions on the development of obesity in C57BL/6J mice. We also analyzed the expression of genes involved in lipid metabolism at an early stage of obesity development in these mice. Compared with mice fed the high-TG diet, mice fed the high-DG diet accumulated significantly less body fat during the 8-month study period. Within the first 10 days, dietary DG stimulated β-oxidation and lipid metabolism-related gene expression, including acyl-CoA oxidase, medium-chain acyl-CoA dehydrogenase, and uncoupling protein-2 in the small intestine but not in the liver, skeletal muscle, or brown adipose tissue, suggesting the predominant contribution of intestinal lipid metabolism to the effects of DG. Furthermore, analysis of digestion products of [^{14}C]DG and those of [^{14}C]TG revealed that the radioactivity levels detected in fatty acid, 1-monoacylglycerol, and 1,3-DG in intestinal mucosa were significantly higher after intrajejunal injection of DG rather than TG. Thus, dietary DG reduces body weight gain that accompanies the stimulation of intestinal lipid metabolism, and these effects may be related to the characteristic metabolism of DG in the small intestine.**—Murase, T., M. Aoki, T. Wakisaka, T. Hase, and I. Tokimitsu. **Anti-obesity effect of dietary diacylglycerol in C57BL/6J mice: dietary diacylglycerol stimulates intestinal lipid metabolism.** ***J. Lipid Res.*** **2002.** 43: **1312–1319.**

Supplementary key words triacylglycerol • small intestine • β-oxidation • obesity

In Westernized countries, obesity is an important health problem. It is becoming clear that obesity is a major risk factor for various diseases, including diabetes, hypertension, and atherosclerosis. As the high fat content of the typical Western diet is considered a major cause of obesity, many studies have been conducted on dietary modification to counter obesity (1–4).

We have been studying the nutritional characteristics and dietary effects of diacylglycerol (DG) (5–8). DG, which consists mainly of 1,3-DG, is a minor component of various edible oils and is widely consumed in our diet. Murata et al. reported that after a single dose of DG emulsion, the extent of increase in postprandial serum triacylglycerol (TG) levels, especially for chylomicron TG, was less than the increase observed after administration of TG emulsion (5). Nagao et al. reported that dietary DG, in contrast to TG, decreased both body weight and visceral fat mass as determined by computed tomography (CT) in healthy men (6). In addition, we have recently shown that dietary DG suppresses the accumulation of high-fat and high-sucrose diet-induced body fat in C57BL/6J mice (7). These results suggested that the structure of acylglycerol, but not the fatty acid composition, markedly affects the nutritional behavior of lipids. However, the mechanisms underlying the various effects of dietary DG have yet to be fully elucidated.

In this study, to gain insight into the dietary effects of DG, we examined the long-term effects of dietary DG on the development of obesity, and analyzed mRNA expression of genes involved in energy metabolism in various organs, including the liver, small intestine, brown adipose tissue, and skeletal muscle at an early stage of obesity development in C57BL/6J mice. Furthermore, we investigated the metabolic characteristics of DG in the small intestine by analyzing the digestion products in the lumen and TG synthesis-intermediates in the mucosa. We report here that dietary DG suppresses the accumulation of body fat accompanying intestinal gene expression, and that this effect may be related to the characteristic metabolism of DG in the small intestine.

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Abbreviations: ACO, acyl-CoA oxidase; DG, diacylglycerol; L-FABP, liver-fatty acid binding protein; MCAD, medium-chain acyl-CoA dehydrogenase; PPAR, peroxisome proliferator activated receptor; TG, triacylglycerol; UCP, uncoupling protein.

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MATERIALS AND METHODS

Oils

DG-rich oil was prepared from rapeseed oil as described previously (9). As shown in **Table 1**, the fatty acid composition of the DG oil was similar to that of TG oil (rapeseed oil). The DG oil contained approximately 90% DG and 10% TG. The DG was comprised of 1,3-DG and 1,2-DG at a ratio of 7:3 (w/w). [Carboxyl-^{14}C]triolein (99% pure, 102.0 mCi/mmol) was purchased from NEN Life Science Products, Inc. (Boston, MA). 1,3-[Carboxyl-^{14}C]diolein (99% pure, 13.3 mCi/mmol) was obtained from Daiichi Pure Chemicals Co., Ltd. (Tokyo, Japan). Triolein and 1,3-diolein were purchased from Sigma (St. Louis, MO).

Animals and diets

Experiment 1: Male C57BL/6J mice obtained from Japan Clea (Tokyo, Japan) at 7-weeks of age were maintained at 22 ± 1°C under a 12 h light-dark cycle (lights on from 7 AM to 7 PM). The mice were divided into three groups (n = 10 or 20, 5 mice/cage), and were allowed ad libitum access to water, and one of the three synthetic diets described in **Table 2** using Roden caffe (Oriental Yeast Co., Tokyo, Japan) to minimize dispersion of diets. The energy values for each diet were calculated from the macronutrient composition using values of 4 kcal/g, 4 kcal/g, and 9 kcal/g for carbohydrate, protein, and oil, respectively. The animals were maintained on their respective diets for 8 months. During the experiments, the animals were cared for in accordance with the principles for the use of animals for research and education, following the Statement of Principles adopted by the FASEB Board.

Experiment 2: Mice were divided into three groups (n = 6, 3 mice/cage) and were allowed ad libitum access to the respective synthetic diets for 10 days. On the final day of the experiment, the mice in all groups were sacrificed, and each organ was rapidly dissected for β-oxidation assay and Northern blotting analysis.

Food intake

Food intake in Experiment 1 was measured on a per-cage basis over the course of 24 h 1 day per week. In Experiment 2, food intake was measured on a per-cage basis every day throughout the study.

Blood analysis

On the final day of the experiments, blood was collected from anesthetized mice in the non-fasting condition via the post-caval vein. For mice under the fasting condition, blood samples were collected by cutting the tail after 12 h of fasting 10 days prior to the end of Experiment 1. Plasma TG, total cholesterol, non-esterified fatty acids (NEFA), and glucose concentrations were determined using the enzyme assay kits L-type Wako TG-H, L-type Wako CHO-H, NEFA-HA test Wako, and L-type Wako Glu2 (Wako, Osaka, Japan), respectively. Plasma insulin and leptin levels were measured using a mouse insulin EIA kit and leptin EIA kit (Morinaga, Yokohama, Japan), respectively, according to the manufacturer's instructions.

TABLE 1. Fatty acid compositions of test oils (%)

	TG Oil	DG Oil
16:0	4.2	4.2
18:0	1.9	2.0
18:1	60.3	62.0
18:2	20.2	20.1
18:3	9.3	6.4
20:0	0.6	0.7
20:1	1.3	1.3
22:0	0.3	0.4
22:1	0.2	0.2
Others	1.5	2.7

TABLE 2. Compositions of the diets (%)

Ingredients	Low-TG	High-TG	High-DG
Triacylglycerol	5.0	25.0	10.0
Diacylglycerol	—	—	15.0
Lard	—	5.0	5.0
Sucrose	—	13.0	13.0
Casein	20.0	20.0	20.0
Cellulose powder	4.0	4.0	4.0
Mineral mixture	3.5	3.5	3.5
Vitamin mixture	1.0	1.0	1.0
Potato starch	66.5	28.5	28.5
Energy (kcal/100g)	391	516	516

Fat pad weights

The fat pads were dissected from each animal, and the weights of epididymal white adipose tissue (WAT), perirenal WAT, and interscapular WAT, as well as that of interscapular brown adipose tissue (BAT), were determined.

β-Oxidation activity

β-oxidation activity was measured as reported previously (10), with minor modifications. Frozen mouse liver and intestinal mucosa were thawed and homogenized on ice with 5 vol of 250 mM sucrose containing 1 mM EDTA and 10 mM HEPES (pH 7.2), and centrifuged at 600 *g* for 5 min. The resultant supernatant was used for assay. The reaction mixture contained 50 mM Tris-HCl (pH 8.0), 40 mM NaCl, 2 mM KCl, 2 mM $MgCl_2$, 1 mM DTT, 5 mM ATP, 0.2 mM L-carnitine, 0.2 mM NAD^+, 0.06 mM flavin adenine dinucleotide (FAD), 0.12 mM CoA, 0.1 μCi [^{14}C]palmitic acid, and the extract containing 100 μg protein in a final volume of 200 μl. The reaction was started by adding the substrate and incubating the preparation at 37°C for 20 min. The reaction was terminated by adding 200 μl of 0.6 N perchloric acid, followed by centrifugation. The supernatant was extracted three times with 800 μl of *N*-hexane to remove residual radiolabeled palmitate. The radioactivity of the aqueous phase was measured. Protein concentrations were determined using a DC protein assay kit (BioRad, Hercules, CA).

RNA extraction and Northern blotting analysis

On the final day of Experiment 2, mice were sacrificed between 9 AM and 11 AM, and the small intestine, liver, skeletal muscle (gastrocnemius and soleus), and interscapular BAT were dissected from each animal and frozen in liquid nitrogen for subsequent RNA isolation. Total RNA was isolated using Isogen (Wako) according to the manufacturer's instructions. Purified RNA (20 μg) was electrophoresed on 1% agarose-formaldehyde gels, and blotted onto Hybond-N^+ membranes (Amersham Pharmacia Biotech, Buckinghamshire, UK). Blotted membranes were hybridized with a ^{32}P-labeled cDNA probe at 42°C overnight. Membranes were washed in 2× SSC-0.1% SDS at room temperature, and again in 0.1× SSC-0.1% SDS at 42°C, then autoradiographed and analyzed with a BAS2500 bioimage analyzer (Fuji Photo Film, Tokyo, Japan). The membranes were also hybridized with a ^{32}P-labeled 36B4 probe, and the mRNA levels were calculated relative to the 36B4 mRNA levels. Normalized values were expressed as percentages using the value of mice fed a low-TG diet as 100%. Each cDNA probe was prepared by reverse transcription and polymerase chain reaction (RT-PCR) by use of first-strand cDNA from mouse or rat tissue total RNA. The PCR-generated cDNA probes were as follows: acyl-CoA oxidase (ACO) (GenBank AF006688, nt 218–880), medium-chain acyl-CoA de-

hydrogenase (MCAD) (J02791, nt 671–1199), liver-fatty acid binding protein (L-FABP) (J00732, nt 1–440), fatty acid transporter (FAT) (L19658, nt 878–1483), 36B4 (X15267, nt 97–860), uncoupling protein (UCP)-1 (U63419, nt 380–1089), UCP-2 (AB012159, nt 296–1225), and UCP-3 (AB008216, nt 31–571). cDNA probes were radiolabeled with [α-^{32}P]dCTP by use of Ready-To-Go DNA labeling beads (Amersham Pharmacia Biotech).

Analysis of acylglycerol metabolites

TG emulsion was prepared by sonicating 10 mM Tris (pH 7.0), 150 mM NaCl, 10 mM taurocholate (Sigma), 10 mM of triolein, and 1×10^7 dpm/ml of [carboxyl-^{14}C]triolein. DG emulsion was prepared using 15 mM of 1,3-diolein and 1×10^7 dpm/ml of 1,3-[carboxyl-^{14}C]diolein instead of triolein, each of which contained 30 mM fatty acid.

The C57BL/6J mice that were fasted for 12 h were operated on through a subcostal incision under diethylether anesthesia. TG or DG emulsion (200 μl), containing 2×10^6 dpm [^{14}C]triolein or [^{14}C]diolein, respectively, was injected into the jejunum at 2 cm distal from the ligament of Treitz. Five minutes after injection, the upper part of the small intestine (3–18 cm from the pylorus) was excised, and immediately placed on ice. The small intestine was then washed with 2 ml of ice-cold 150 mM NaCl. The washing solution was used for analysis of the lipid digestion products. The small intestine was further washed once with ice-cold 20 ml of 150 mM NaCl, and once with 150 mM NaCl containing 0.2% Triton X-100, and then rinsed twice with 40 ml of 150 mM NaCl. The intestine was opened lengthwise, and the mucosa was scraped off using a glass microscope slide. The washing solution and the homogenate were frozen in liquid nitrogen and stored at −80°C. All manipulations were performed within 5 min after dissection.

The lipids were extracted by the procedure of Folch et al. (11), dried under a stream of nitrogen, and re-dissolved in chloroform-methanol (2:1, v/v). The extracted lipids were separated by thin-layer chromatography (TLC) using a silica gel 60 F254 TLC plate (Merck, Darmstadt, Germany) and chloroform-acetone (96:4, v/v) as the development solvent. The isomeric MGs were separated (i.e., the 1(3)-MG from the 2-MG) using boric acid-impregnated silica gel 60 high-performance TLC plates and chloroform-acetone (4:1, v/v). The TLC plates were exposed to a Fuji Imaging Plate (Fuji Photo Film Co., Tokyo, Japan), and the obtained fluorograms were analyzed with a BAS2500 bioimaging system (Fuji Photo Film).

Statistical analysis

All values are presented as mean ± SD. Statistical comparisons between groups were made using ANOVA, and each group was compared with the others by Fisher's protected least significant difference test (StatView: SAS Institute Inc., Cary, NC). Statistical significance was defined as $P < 0.05$.

TABLE 3. Body weight, energy intake, and fat weight

	Low-TG (n = 10)	High-TG (n = 20)	High-DG (n = 10)
Body weight (g)	30.8 ± 2.1[c]	41.9 ± 5.4	37.1 ± 4.1[a]
Energy intake (kcal/cage/day)	54.8 ± 8.9[c]	65.3 ± 3.8	62.2 ± 8.7
Epididymal WAT (g)	0.98 ± 0.23[c]	1.81 ± 0.47	1.38 ± 0.56[a]
Perirenal WAT (g)	0.11 ± 0.03[c]	0.31 ± 0.16	0.14 ± 0.09[b]
Interscapular WAT (g)	0.24 ± 0.08[b]	0.62 ± 0.40	0.38 ± 0.14
Interscapular BAT (g)	0.13 ± 0.02[b]	0.29 ± 0.11	0.16 ± 0.11[b]

Mice were sacrificed after 8 months of feeding with the respective diets, then body fat weight was determined. Food intake was measured on a per-cage basis over the course of 24 h one day per week. Values are the means ± SD.

[a] $P < 0.05$.
[b] $P < 0.01$.
[c] $P < 0.001$ versus the high-TG group.

TABLE 4. Plasma analysis

	Low-TG (n = 10)	High-TG (n = 20)	High-DG (n = 10)
Non-fasting condition			
Triacylglycerol (mg/dl)	42.95 ± 16.81	40.32 ± 18.11	29.16 ± 11.00
Total cholesterol (mg/dl)	122.52 ± 27.28	143.88 ± 27.11	132.25 ± 23.24
NEFA (mEq/l)	0.82 ± 0.17[c]	0.40 ± 0.12	0.44 ± 0.11
Glucose (mg/dl)	172.04 ± 55.43[b]	222.92 ± 34.60	229.54 ± 29.56
Insulin (ng/ml)	1.61 ± 0.87[a]	2.79 ± 1.41	2.17 ± 1.41
Leptin (ng/ml)	6.55 ± 3.80[a]	17.57 ± 11.82	17.02 ± 10.11
Fasting condition			
Glucose (mg/dl)	67.64 ± 12.65[c]	111.65 ± 25.18	83.28 ± 13.20[b]
Insulin (ng/ml)	0.60 ± 0.13[a]	1.45 ± 1.04	0.64 ± 0.57[a]
Leptin (ng/ml)	3.47 ± 1.23[c]	24.00 ± 18.67	7.40 ± 8.62[b]

On the final day of Experiment 1, blood was collected from anesthetized mice in the non-fasting condition via the post-caval vein. Blood from mice in the fasting condition was collected by cutting the tail after 12 h of fasting 10 days prior to the end of the experiment. Values are the means ± SD.

[a] $P < 0.05$.
[b] $P < 0.01$.
[c] $P < 0.001$ versus the high-TG group.

RESULTS

Consistent with our earlier report (7), dietary DG significantly reduced the body fat accumulation induced by a high-fat diet in C57BL/6J mice. Compared with the low-TG diet, feeding with the high-TG diet for 8 months produced significant increases in body weight and adipose tissue weight. On the other hand, the high-DG diet reduced body weight gain, epididymal WAT, perirenal WAT, and interscapular BAT weight by 43%, 52%, 85%, and 81%, respectively, as compared with the high-TG diet (**Table 3**).

Although there were no significant differences in plasma parameters between the high-TG-fed and high-

TABLE 5. Plasma analysis

	Low-TG	High-TG	High-DG
Body weight (g)	21.7 ± 1.2	23.1 ± 1.9	23.1 ± 1.2
Energy intake (kcal/cage/day)	32.1 ± 4.0	34.3 ± 4.8	34.9 ± 6.1
Plasma analysis			
Triacylglycerol (mg/dl)	52.23 ± 10.54	45.91 ± 8.30	48.41 ± 5.65
Total cholesterol (mg/dl)	101.77 ± 16.06	108.19 ± 19.16	111.00 ± 8.7
NEFA (mEq/l)	0.71 ± 0.25	0.58 ± 0.12	0.69 ± 0.12
Glucose (mg/dl)	218.37 ± 16.07[a]	245.70 ± 17.85	234.04 ± 18.16
Insulin (ng/ml)	2.58 ± 0.83	2.61 ± 0.90	2.51 ± 0.50
Leptin (ng/ml)	3.39 ± 1.45	5.88 ± 5.10	5.22 ± 1.37

Mice were fed the respective diets for 10 days (Experiment 2), then body weight was determined. Energy intake was measured on a per-cage basis throughout the study. Blood was collected under non-fasting conditions and analyzed as described in Materials and Methods. Values are the means ± SD of six animals.

[a] $P < 0.05$ versus the high-TG group.

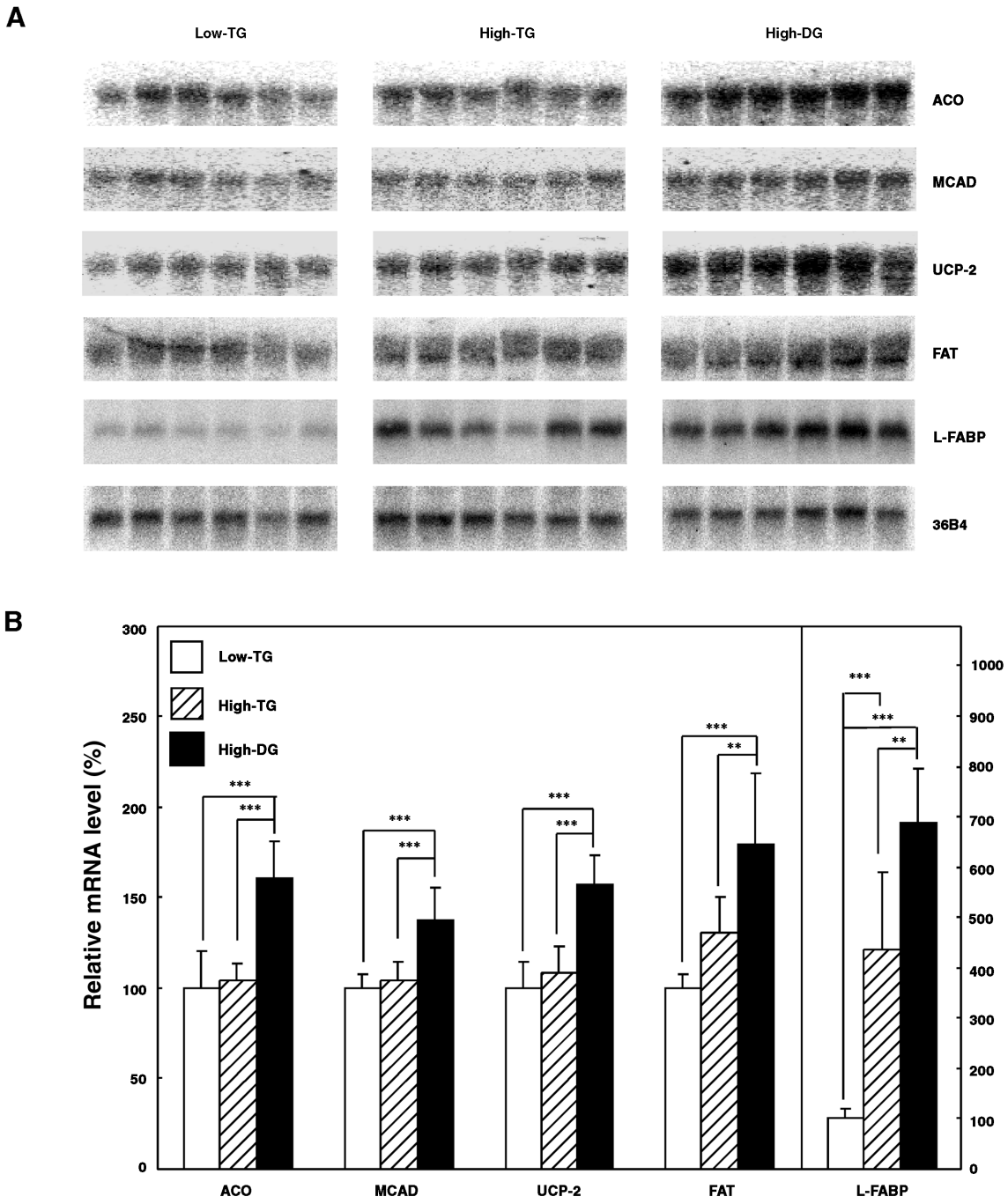

Fig. 1. Gene expression in the small intestine. After the mice were fed their respective diets for 10 days, total RNA isolated from the small intestine was subjected to Northern blotting as described in Materials and Methods. The amounts of mRNA were quantified with a BAS2500 bioimage analyzer and expressed as percentages of corresponding amounts in the low-TG group. Values are the means ± SD of six animals. $^{**}P < 0.01$, $^{***}P < 0.001$.

DG-fed mice under the non-fasting conditions after 8 months of feeding, glucose, insulin, and leptin concentrations under the fasting conditions in the DG-fed mice were significantly lower than those in the high-TG-fed mice (**Table 4**).

There was no significant difference in the average energy intake between the high-TG diet group and the high-DG diet group (Table 3). In addition, we confirmed previously that lipid absorption did not differ significantly between high-TG-fed and high-DG-fed mice (7, 8), suggesting that reduced accumulation of body fat in the DG group was not related solely to reduced energy intake.

To elucidate the mechanisms underlying the beneficial effects of dietary DG, we examined the effects of DG on the mRNA expression of genes involved in lipid metabolism in various tissues. However, it was difficult to deter-

mine whether the changes in lipid metabolism were a primary cause or a secondary consequence of the reduced body fat accumulation. Therefore, we investigated mRNA levels after 10 days of feeding (Experiment 2), at which time no significant differences were observed in body weight, food intake, or plasma parameters among the high-fat groups (**Table 5**). As the small intestine is the first and most susceptible of the organs exposed to dietary components, we examined the effects of DG on intestinal gene expression. In mice fed the high-DG diet, the acyl-CoA oxidase (ACO:peroxisomal β-oxidation enzyme) mRNA level in the small intestine was significantly higher than that in mice fed the low-TG or the high-TG diet (**Fig. 1**). When normalized by the level of 36B4 mRNA, the ACO mRNA levels in DG-fed mice were 60% and 53% higher than those in the low-TG and high-TG diet groups, respectively. Medium-chain acyl-CoA dehydrogenase (MCAD:mitochondrial β-oxidation enzyme) mRNA levels in the high-DG-fed mice were also higher than those of the low-TG-fed and high-TG-fed mice, by 37% and 32%, respectively. L-FABP and FAT are expressed in the small intestine, and are believed to participate in the uptake and metabolic processing of fatty acids (12). The L-FABP mRNA level in the small intestine was 587% higher in mice fed the high-DG diet than in mice fed the low-TG diet, and 58% higher than in the high-TG-fed mice. FAT mRNA in the high-DG-fed mice was also increased by 80% and 38% compared with the low-TG-fed and high-TG-fed mice, respectively. Uncoupling proteins (UCPs) have been proposed to influence metabolic efficacy (13, 14). Our previous study showed that UCP-2 is expressed abundantly in the small intestine and is upregulated by dietary fat

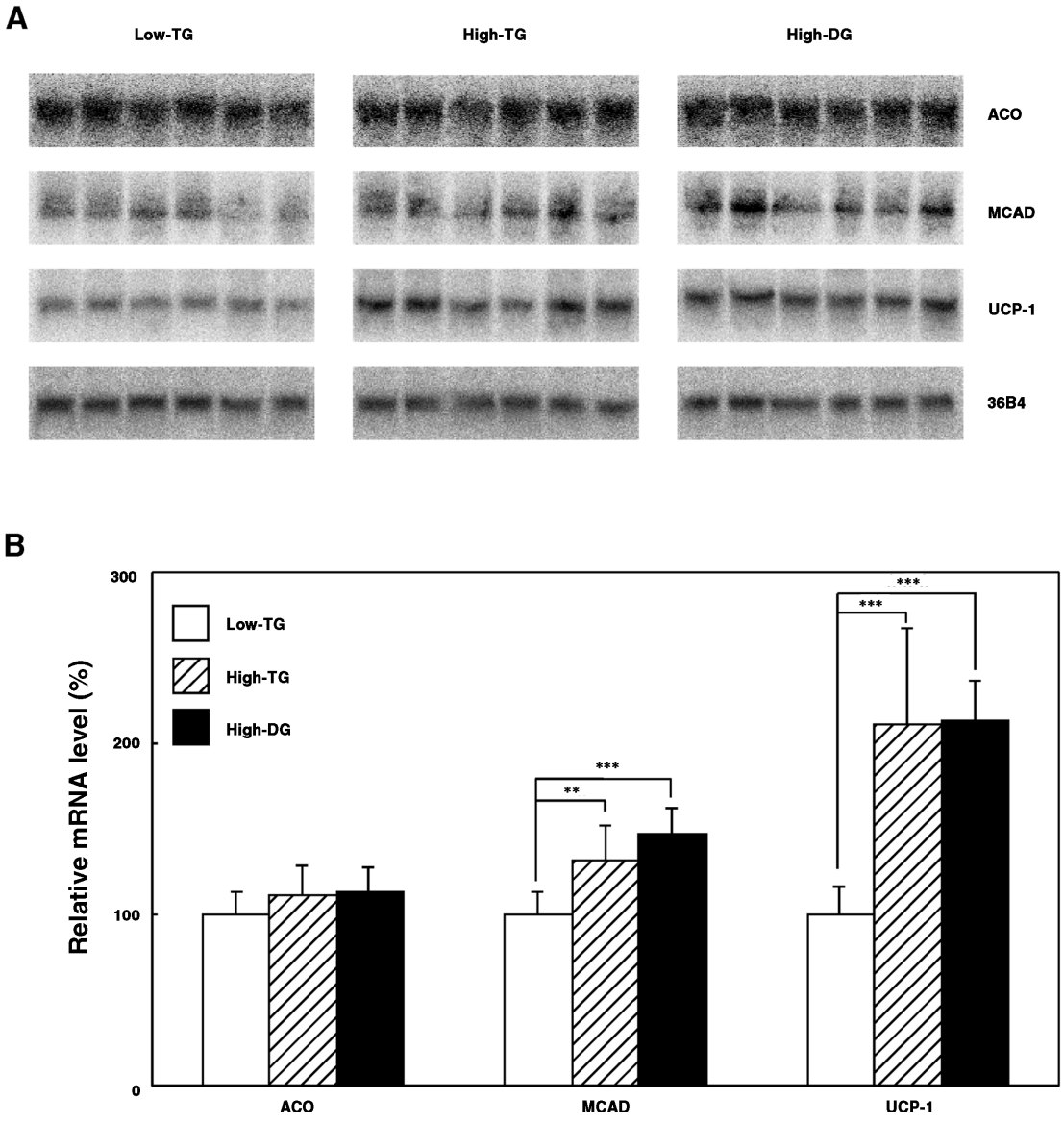

Fig. 2. Gene expression in the brown adipose tissue (BAT). Total RNA isolated from BAT was subjected to Northern blotting as described in Materials and Methods. The amounts of mRNA were quantified and expressed as percentages of corresponding amounts in the low-TG group. Values are the means ± SD of six animals. $^{**}P < 0.01$, $^{***}P < 0.001$.

(15). In the present study, the level of intestinal UCP-2 mRNA was elevated by the high-DG diet, while no marked effect was observed by the high-TG diet. The high-DG diet induced a greater increase in UCP-2 than either the low-TG or high-TG diet by 57% and 44%, respectively.

On the other hand, there were no marked differences in the normalized mRNA levels of ACO, MCAD, or L-FABP in the liver between the high-fat groups (data not shown). Furthermore, the mRNA levels of ACO, MCAD, and UCP-3 in the skeletal muscle were not influenced by the high-DG diet (data not shown). The levels of MCAD and UCP-1 mRNA in BAT were elevated by both the high-TG and high-DG diets; however, there was no difference between the extents of up-regulation (**Fig. 2**). No marked differences were observed in ACO mRNA levels among the three experimental groups in BAT. These results suggested that dietary DG up-regulates expression of the genes involved in lipid metabolism, especially in the small intestine.

To further confirm the effects of DG on intestinal lipid metabolism, we examined the β-oxidation activity after DG feeding. As shown in **Fig. 3**, the high-DG diet increased β-oxidation activity significantly by 139% and 102% as compared with the low-TG and high-TG diet groups, respectively, indicating that feeding with the DG diet stimulates lipid catabolism in the small intestine. On the other hand, no significant differences were observed in hepatic β-oxidation among the three experimental groups. Overall, these results suggested that stimulation of intestinal lipid metabolism might be one of the factors responsible for the beneficial effects of dietary DG.

To gain insight into the mechanism by which dietary DG up-regulates intestinal lipid metabolism, we investigated the metabolic characteristics of DG in the small intestine by analyzing the digestion products in the lumen and TG synthesis-intermediates in the mucosa 5 min after intrajejunal injection of DG. This time point was chosen for analyses based on a previous study showing time course of intraduodenally injected lipid metabolism (16). Digestion products' profiles of TG and DG in the intestinal lumen are shown in **Table 6**. In the DG injection group, 9.1- and 7.0-fold higher radioactivity levels were detected in 1-MG and fatty acid, respectively, compared with the TG injection group. In the intestinal mucosa, the radioactivity levels for 1-MG, 1,3-DG, and fatty acid were 7.5-, 12.6-, and 2.8-fold higher than those in the TG injection group, respectively (**Table 7**). These results suggested that DG is metabolized through characteristic pathways leading to alteration of the mucosal lipid profile, and this may affect the regulation of gene expression in the small intestine.

DISCUSSION

Most of the studies on the dietary effects of fat have been conducted from the standpoint of fatty acid composition. As a result, it has become accepted that the fatty acid composition of dietary fat consisting mainly of TG markedly affects the development of obesity, diabetes, and hyperlipidemia (1, 17, 18). On the other hand, little attention has been given to other factors, such as the number of fatty acids, or a fatty acid's binding position in the acylglycerol molecule. We have shown in both the present and our previous studies that the structure of acylglycerol is a factor affecting the nutritional behavior of lipids. Our results indicated that dietary DG, compared with TG with a similar fatty acid composition, significantly suppresses body weight gain accompanying the up-regulation of genes involved in lipid metabolism in the small intestine.

Obesity results from a disequilibrium between energy intake and expenditure. Previously, we confirmed that the energy value per weight and digestibility of DG is similar to that of TG (8), and that energy intake was not significantly different between high-TG-fed and high-DG-fed mice, suggesting that reduced body fat accumulation in the latter was not related solely to reduced energy intake; rather, some additional mechanism would be involved in the anti-obesity effects of DG. As shown in this study, in the early stage of DG feeding, marked changes were observed in β-oxidation and related gene expression in the small intestine. As the small intestine is the first organ exposed to dietary fat, it seems reasonable that it is the most susceptible to dietary DG. The DG diet was found to up-regulate the mRNA level involved in fatty acid transport (FAT and L-FABP), β-oxidation (ACO and MCAD), and thermogenesis (UCP-2) in the small intestine. These results suggested that 1,3-DG structure potently stimulates intestinal lipid metabolism. As UCPs have been proposed to influence energy expenditure and the development of obesity (13, 14, 19, 20), up-regulation of UCP-2 in the small intestine may lead to the stimulation of energy expenditure. In addition, Guerra et al. reported that defects in one of the enzymes associated with β-oxidation of fatty

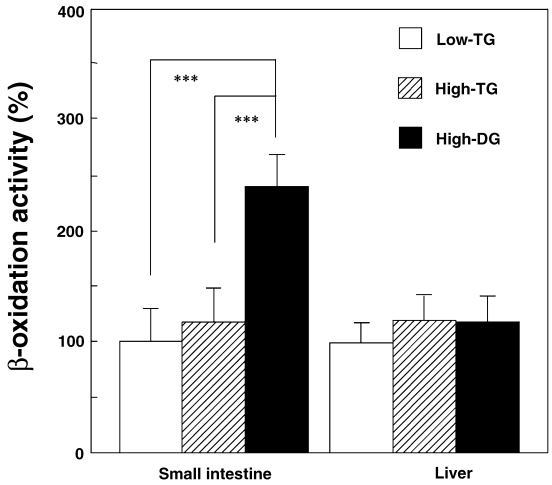

Fig. 3. β-oxidation activity in the small intestine and liver. The small intestines and livers of mice fed the respective diets for 10 days were homogenized, and palmitic acid oxidation activities were measured as described in Materials and Methods. Values are the means ± SD. ***$P < 0.001$.

TABLE 6. Analysis of digestion products of 1,3-[carboxyl-^{14}C]diolein or [carboxyl-^{14}C]triolein in the intestinal lumen

	[^{14}C]triolein Injection		[^{14}C]diolein Injection	
	PSL ($\times 10^3$)	Ratio	PSL ($\times 10^3$)	Ratio
		%		%
1(3)-Monoacylglycerol	5.6 ± 3.9	1.5 ± 0.5	51.2 ± 31.4[b]	14.2 ± 4.8[b]
2-Monoacylglycerol	69.4 ± 34.3	22.3 ± 13.5	21.6 ± 6.1[b]	7.2 ± 2.8[a]
1,2(2,3)-Diacylglycerol	45.7 ± 38.1	10.8 ± 3.6	10.0 ± 1.6[a]	3.0 ± 1.4[b]
1,3-Diacylglycerol	6.4 ± 8.9	1.2 ± 1.2	60.0 ± 61.4	15.1 ± 13.4[a]
Triacylglycerol	181.0 ± 164.0	41.9 ± 21.5	4.2 ± 3.7[a]	1.2 ± 0.7[b]
Fatty acid	70.5 ± 26.6	22.3 ± 12.5	186.0 ± 67.5[b]	59.1 ± 13.3[b]

Five minutes after injection of DG or TG emulsion containing ^{14}C -labeled diolein or triolein, the upper half of the small intestine was excised. Lipid profile of intestinal lumen was analyzed as described in Materials and Methods. Values are the means ± SD of six animals.

[a] $P < 0.05$.

[b] $P < 0.001$ versus the triolein injection group.

PSL, phosphostimulated luminescence.

acids resulted in defects in thermogenesis (21); therefore, stimulation of fatty acid β-oxidation is expected to lead to increased energy expenditure. Considering the fact that the small intestine is one of the largest and most active organs on β-oxidation (22, 23), it is likely that increased expression of intestinal UCP-2 and accompanying β-oxidation resulted in increased energy expenditure and contributed to the suppression of body fat accumulation. Further studies on intestinal energy metabolism are necessary to evaluate the precise contribution of the small intestine in diet-induced obesity.

Although the precise molecular mechanism by which dietary DG stimulates intestinal lipid metabolism and related gene expression remains to be elucidated, an increase in mucosal fatty acid may be involved in the process. The peroxisome proliferator-activated receptor (PPAR) is a nuclear transcription factor activated by fatty acids through direct interaction with the receptor (24, 25). PPAR is well known to activate transcription by binding to the promoter regions of target genes including ACO, MCAD, FABP, FAT, and UCP-2 (26, 27). Among the PPAR isoforms, PPARα is known to be expressed abundantly in the small intestine (28), and it is known to play an important role in the expression of the target genes (29, 30). Accordingly, PPAR is thought to be an excellent candidate for mediating the physiological and dietary control of genes encoding cellular fatty acid utilization enzymes. In this context, it is possible that the increase in fatty acid in the mucosa after DG feeding resulted in the induction of lipid-metabolizing enzymes through the PPAR-mediated pathway. Determination of the precise involvement of PPARs in the dietary DG-induced up-regulation of intestinal lipid metabolism must await further analyses using transgenic mice with gene knockout or overexpression.

The increase in fatty acid in the intestinal mucosa may be explained by the characteristic metabolic pathway of DG. The majority of ingested TG is hydrolyzed to 2-monoacylglycerol (MG) and fatty acids, then absorbed into intestinal mucosal cells and immediately resynthesized into TG molecules (31–33). On the other hand, 1,3-DG is presumed to be hydrolyzed to form 1(or 3)-MG and fatty acid, which is probably absorbed into the mucosa or further hydrolyzed into glycerol and fatty acid. Thus, the metabolic pathway of 1,3-DG is probably different from that of TG. As shown in Table 6, the amount of [^{14}C]fatty acid in the lumen of DG-injected mice was higher than that of TG-injected mice. Larger production of fatty acid in the lumen and absorption into the mucosa may lead to the higher content of fatty acid in the mucosa. Furthermore, compared with TG-injected mice, DG-injected mice had larger amounts of labeled 1,3-DG in the mucosa (Table 7). 1,3-DG has been shown to be little utilized as a substrate for TG synthesis by diacylglycerol acyltransferase (DGAT) (34), which mediates the acylation of DG. The increase in mucosal 1,3-DG reflects the low substrate speci-

TABLE 7. Analysis of ^{14}C-labeled lipids in the intestinal mucosa

	[^{14}C]triolein Injection		[^{14}C]diolein Injection	
	PSL ($\times 10^3$)	Ratio	PSL ($\times 10^3$)	Ratio
		%		%
1(3)-Monoacylglycerol	0.3 ± 0.2	0.1 ± 0.1	2.6 ± 1.8[a]	0.9 ± 0.4[c]
2-Monoacylglycerol	2.6 ± 1.9	1.0 ± 0.5	2.0 ± 1.5	0.6 ± 0.3
1,2(2,3)-Diacylglycerol	6.3 ± 3.2	2.6 ± 0.5	8.6 ± 4.5	2.6 ± 0.7
1,3-Diacylglycerol	0.8 ± 0.6	0.3 ± 0.2	9.8 ± 4.1[c]	3.3 ± 1.0[c]
Triacylglycerol	214.5 ± 71.1	92.2 ± 1.9	284.7 ± 150.1	84.9 ± 4.2[b]
Fatty acid	9.1 ± 5.0	3.7 ± 1.1	25.1 ± 14.2[a]	7.8 ± 2.5[b]

Five minutes after injection of DG or TG emulsion containing ^{14}C-labeled diolein or triolein, the upper half of the small intestine was excised. The lipid profile of intestinal mucosa was analyzed as described in Materials and Methods. Values are the means ± SD of six animals.

[a] $P < 0.05$.

[b] $P < 0.01$.

[c] $P < 0.001$ versus the triolein injection group.

ficity of DGAT, which may also contribute to the increase in mucosal fatty acid after DG injection.

In summary, we showed that the structure of acylglycerol (i.e., the structural difference between TG and DG) affects body fat accumulation, expression of genes involved in lipid metabolism and thermogenesis, and their metabolic fate in the small intestine in C57BL/6J mice. Understanding the nutritional characteristics of dietary DG and its molecular mechanisms, especially in the small intestine, may provide fresh insight for the management of obesity as well as for lipid nutrition.

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