Dietary diacylglycerol suppresses high fat and high sucrose diet-induced body fat accumulation in C57BL/6J mice

Takatoshi Murase, Tomohito Mizuno, Toshiko Omachi, Kouji Onizawa, Yumiko Komine, Hidehiko Kondo, Tadashi Hase, and Ichiro Tokimitsu[1]

Biological Science Laboratories, Kao Corporation, 2606, Akabane, Ichikaimachi, Hagagun, Tochigi 321-3497, Japan

Abstract Diacylglycerol (DG) comprises up to approximately 10% of various edible oils. In the present study, we examined the effects of dietary DG consisting mainly of 1,3-species on body weight, body fat accumulation, and mRNA levels of various genes involved in energy homeostasis in obesity-prone C57BL/6J mice. Five-month feeding with the high triacylglycerol (TG) diet (30% TG + 13% sucrose) resulted in significant increases in body weight, visceral fat accumulation, and circulating insulin and leptin levels compared with mice fed the control diet (5% TG). Compared with mice fed the high TG diet, body weight gain and visceral fat weight were reduced by 70% and 79%, respectively, in those fed the high DG diet (30% DG + 13% sucrose). In addition, circulating leptin and insulin levels were reduced to the respective control levels. Compared with high TG feeding, high DG feeding suppressed the elevation of leptin mRNA expression in adipose tissue, and upregulated acyl-coenzyme (Co)A oxidase and acyl-CoA synthase mRNA expression in the liver. These results indicate that dietary DG is beneficial for suppression of high fat diet-induced body fat accumulation. Furthermore, it is suggested that structural differences in DG and TG, but not the composition of fatty acid, markedly affect nutritional behavior of lipids.—Murase, T., T. Mizuno, T. Omachi, K. Onizawa, Y. Komine, H. Kondo, T. Hase, and I. Tokimitsu. **Dietary diacylglycerol suppresses high fat and high sucrose diet-induced body fat accumulation in C57BL/6J mice.** ***J. Lipid Res.*** **2001.** 42: **372–378.**

Supplementary key words glycerol • obesity • visceral fat • insulin • leptin

Obesity is a metabolic disorder resulting from disequilibrium between energy uptake and expenditure, and is known to be a strong risk factor for non-insulin-dependent diabetes mellitus associated with insulin resistance (1–4). Increases in body fat mass are accompanied by elevation of circulating free fatty acids, insulin, tumor necrosis factor (TNF)α, and leptin, suggesting that these molecules play important roles in the development of insulin resistance due to obesity (5–10).

Obesity is caused by a combination of genetic and environmental factors. Among the environmental factors, high fat intake has been shown to contribute to the development of both obesity and diabetes in humans and rodents (11–13). Therefore, numerous studies on the bioavailability of various lipids have been conducted for the management of obesity (14–16). Fish oil rich in n-3 polyunsaturated fatty acids [eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA)] is considered a favorable dietary component for alleviation of obesity with lowering of insulin level (17).

We have been studying the nutritional characteristics and dietary effects of diacylglycerol (DG) (18–20). DG, which consists of 1,3-DG and 1,2(or 2,3)-DG, comprises up to approximately 10% of various dietary oils, and is widely consumed in our daily diet. Recent studies on dietary effects of DG have shown that serum triacylglycerol (TG) concentration in rats fed a DG diet mainly composed of 1,3-DG was significantly lower than that of rats fed a TG diet of similar fatty acid composition (18). In addition, intragastric infusion of an emulsion containing DG significantly retarded the lymphatic transport of TG as chylomicrons in rats (19). More recently, Nagao et al. (20) reported that dietary DG, in contrast to TG, decreased both body weight and visceral fat mass, as determined by semiquantitative analysis using computed tomography in healthy men. These effects of dietary DG are probably caused by the different metabolic fates due to the different structures of the lipids because fatty acid compositions of DG and TG used in these studies were adjusted to be approximately equivalent.

Abbreviations: ACO, acyl-CoA oxidase; ACS, acyl-CoA synthase; DG, diacylglycerol; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; PPAR, peroxisome proliferator-activated receptor; SREBP, sterol regulatory element binding protein; TG, triacylglycerol; UCP, uncoupling protein; WAT, white adipose tissue.

[1] To whom correspondence should be addressed.
e-mail: 157473@kastanet.kao.co.jp

The present study was designed to quantitatively examine the effects of dietary DG on the development of obesity and resulting hyperinsulinemia and hyperleptinemia in obesity- and diabetes-prone C57BL/6J mice. The C57BL/6J mice are reported as a good model of human obesity and type II diabetes in which the disease results from the interaction between environmental and genetic factors (21–23). These mice develop obesity, hyperinsulinemia, and hyperleptinemia when raised on a high fat and high sucrose diet, but remain lean if the fat content of the diet is limited. As the mechanisms of the effects of dietary DG on obesity and diabetes are unclear, we compared the effects of DG and TG on mRNA expression of genes involved in lipid metabolism and development of obesity in adipose tissue and the liver in these mice.

MATERIALS AND METHODS

Test oils

TG oil was prepared by mixing soybean and rapeseed oil. The DG-rich oil was prepared by esterifying glycerol with fatty acids from the above TG oil by the method of Brigitte et al. (20, 24). As shown in **Table 1**, the fatty acid composition of DG-rich oil was similar to that of TG oil. The DG-rich 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.

Animals and diets

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 7:00 AM to 7:00 PM). The mice were fed laboratory chow for 1 week to stabilize the metabolic conditions. Mice were randomly divided into three groups, and were allowed ad libitum access to water and one of three synthetic diets using Roden caffe (Oriental, Tokyo, Japan) as follows: a control (low TG) diet containing 5% (w/w) TG, 20% protein, and 66.5% starch; a high TG diet containing 30% (w/w) TG, 20% protein, 28.5% starch, and 13% sucrose; and a high DG diet containing 30% (w/w) DG instead of TG. The compositions of the diets are listed in **Table 2**. Animals were maintained on these diets for 5 months. The energy values for DG and TG were calculated by the method described by Livesey (25). The energy values for each diet were calculated from the macronutrient composition using values of 4.1, 4.1, 9.4, and 9.2 kcal/g for carbohydrate, protein, TG, and DG, respectively. 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.

TABLE 1. Fatty acid composition of test oil

	TG	DG
	%	
16:0	7.1	4.4
18:0	2.0	3.6
18:1	44.8	44.2
18:2	39:3	39.7
18:3	4.6	4.4
20.0	0.5	1.0
20:1	0.7	0.7
22:0	0.2	0.3
22:1	0.2	0.1

TABLE 2. Composition of the diets

Ingredients	Control	High TG	High DG
		%	
Triacylglycerol	5.0	30.0	—
Diacylglycerol	—	—	30.0
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
Starch	66.5	28.5	28.5
Energy (kcal/100 g)	401.7	534.2	528.2
Fat energy (% of total energy)	11.7	52.8	52.3

Body weight, food intake, and feed efficiency

Body weight was measured weekly throughout the study. Food intake was measured on a per-cage basis for 24 h once per week. The feed efficiency was calculated as follows: body weight gain per cage (g)/kcal of food consumed per cage per day (26).

Blood analysis

On the final day of experiments, blood was collected via the postcaval vein from anesthetized animals after 12 h of food deprivation. Serum TG, total cholesterol, nonesterified fatty acids (NEFA), glucose, glutamic oxaloacetic transaminase (GOT), and γ-glutamic pyruvic transaminase (γ-GPT) concentrations were determined using enzyme assay kits; L-type Wako TG-H, L-type Wako CHO-H, NEFA-HA test Wako, L-type Wako Glu2, L-type Wako GOT, and L-type Wako GPT (Wako, Osaka, Japan), respectively. Serum insulin and leptin levels were measured using a mouse insulin enzyme-linked immunosorbent assay (EIA) kit and leptin EIA kit (Morinaga, Yokohama, Japan) according to the manufacturer's instructions.

Fat pad weights

The animals were killed to determine the weights of four fat pads: retroperitoneal, epididymal, mesenteric, and perirenal. The fat pads were dissected from each animal according to defined anatomical landmarks.

Extraction of total fat from the feces

Feces were collected three times on a per-cage basis for 24 h on the final week of feeding. After freeze drying, total lipids of feces were extracted by the procedure of Folch, Leens, and Sloane-Stanley (27), and the amount of extracted lipid was determined gravimetrically.

Measurement of liver triacylglycerol and cholesterol

Total lipids in the liver were extracted by the procedure of Folch, Leens, and Sloane-Stanley (27). The extracts dissolved in $CHCl_3$ were diluted by 10% Triton X100/2-propanol, followed by measurement of TG and cholesterol contents using TG Test Wako and Cholesterol Test Wako kits (Wako, Osaka, Japan).

Reverse transcription polymerase chain reaction

Liver and epididymal white adipose tissue (WAT) was dissected from each animal and was frozen in liquid nitrogen for subsequent RNA isolation. Total RNA was isolated using Isogen (Wako, Osaka, Japan) according to the manufacturer's instructions. The extracted RNA was analyzed by reverse transcription polymerase chain reaction (RT-PCR) using RNA PCR kits

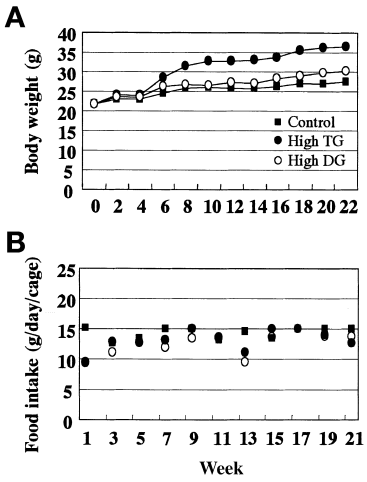

Fig. 1. Comparison of body weight (A) and food intake (B) in C57BL/6J mice on the three diets during 5 months. A: Each point represents the mean body weight of five animals. B: Each point represents food intake per cage for 24 h.

(Takara, Shiga, Japan) according to the supplier's instructions, except that RT was performed with oligo $d(T)_{18}$. PCR was carried out in a MJ Research (Watertown, MA) PTC-100 programmable thermal controller for 18 to 25 cycles according to the following thermal profile: 95°C for 1 min to denature the DNA, 62°C for 2 min to allow primer annealing, and 72°C for 1.5 min for extension. Following PCR, 4-μl aliquots were removed and electrophoresed on a 1.5% agarose gel, stained with ethidium bromide, and analyzed with a digital fluorodensitometer (FM-BIO100, Hitachi Software Engineering, Kanagawa, Japan). Amounts of PCR products were compared for the cycle in which the logarithmic amplification was attained before reaching saturation.

The primers used were Leptin F: 5′-ATGTGCTGGAGAC CCCTGTGTC-3′; Leptin R: 5′-TCAGCATTCAGGGCTAACATC CAA-3′; β-actin F: 5′-TTGTAACCAACTGGGACGATATGG-3′; β-actin R: 5′-GATCTTGATCTTCATGGTGCTAGG-3′; TNFα F: 5′-TTCTGTCTACTGAACTTCGGGGTGATCGGTCC-3′; TNFα R: 5′-GTATGAGATAGCAAATCGGCTGACGGTGTGGG-3′; plasminogen activator inhibitor-1 (PAI-1) F: 5′-CTATGCTGCAGATG ACCACAGC-3′; PAI-1 R: 5′-CTGAGCCATCATGGGCACAGAG-3′; uncoupling protein (UCP)-2 F: 5′-CATTCTGACCATGGTGCG TACTGA-5′; UCP-2 R: 5′-GTTCATGTATCTCGTCTTGACCAC 3′; peroxisome proliferator-activated receptor (PPAR)α F: 5′-GGATGTCACACAATGCAATTCGCT-3′; PPARα R: 5′-TCACA GAACGGCTTCCTCAGGTT-3′; acyl-coenzyme (Co)A oxidase (ACO) F: 5′-CCAACATGAGGACTATAACTTCCT-3′; ACO R: 5′-TACATACGTGCCGTCAGGCTTCAC-3′; acyl-coA synthase (ACS) F: 5′-TGAAGCCATCACGTACATAGTCAAC-3′; ACS R: 5′-TCGACTGTACTTTGTGGAAGATCAG-3′; sterol regulatory element binding protein (SREBP)-1 F: 5′-ACTTCATCAAGGCA GACTCACTG-3′; and SREBP-1 R: 5′-CCTGCTTGTGCTTCTG GTTGCTGT-3′.

Statistical analysis

All values are presented as means ± SD. Statistical analysis was performed by analysis of variance with Duncan's multiple range tests. Statistical significance was defined as $P < 0.05$.

RESULTS

Body weights during 5 months are shown in **Fig. 1A** and **Table 3**. Consistent with previous reports (21–23), feeding with the high TG diet for 5 months resulted in significant increases in body weight compared with mice fed the control diet. On the other hand, body weight gain was markedly reduced by feeding with the DG diet. In addition, consistent with body weight data, feed efficiency of high DG-fed mice was lower than that of high TG-fed mice (Table 3), suggesting the reduced accumulation of energy derived from dietary DG.

The average energy intake of high fat (TG and DG)- fed mice was higher than that of low fat (control)-fed mice. However, there was no significant difference between high TG- and high DG-fed mice (Table 3). On a per-cage basis, the total lipid amount in feces was increased in both groups of high fat-fed mice compared with controls (Table 3). The amount of fecal lipid in high DG-fed mice was

TABLE 3. Final body weight, feed efficiency, fasting serum cholesterol, triglyceride, glucose, NEFA, GOT, γ-GTP, energy intake, and fecal lipid

	Control	High TG	High DG
Final body weight (g)[a]	27.6 ± 1.7	36.4 ± 2.3[e]	30.2 ± 2.4[f]
Feed efficiency[b]	0.519	1.052	0.654
Total cholesterol (mg/dl)[c]	119.2 ± 15.1	132.8 ± 19.9	102.2 ± 14.4[f]
Triacylglycerol(mg/dl)[c]	67.5 ± 23.2	35.3 ± 2.9[e]	38.3 ± 4.0[d]
Glucose (mg/dl)[c]	228.7 ± 47.0	281.6 ± 62.5[d]	251.7 ± 31.0
NEFA (mEq/l)[c]	1.13 ± 0.25	0.86 ± 0.08[d]	0.92 ± 0.13
GOT (IU/l)[c]	45.2 ± 23.2	46.7 ± 13.1	46.2 ± 10.4
γ-GPT (IU/l)[c]	14.7 ± 17.5	18.5 ± 11.5	12.3 ± 4.6
Energy intake (kcal/cage/day)	57.2 ± 3.9	69.7 ± 8.1[e]	65.2 ± 8.4[e]
Fecal lipid (mg/g dried feces)	19.1 ± 2.9	64.3 ± 1.1[e]	60.5 ± 7.9[e]

Results are means ± SD (n = 5). NEFA, nonesterified fatty acids.
[a] Mice were killed after 5 months of feeding with each diet, and final body weight was determined.
[b] The feed efficiency was calculated as follows: [body weight gain per cage (g)]/[kcal of food consumed per cage per day].
[c] On the final day of experiments, blood was collected after 12 h of food deprivation.
[d] $P < 0.05$ as compared with controls.
[e] $P < 0.01$ as compared with controls.
[f] $P < 0.01$ as compared with High TG group.

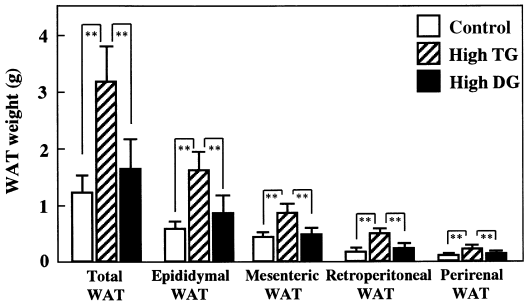

Fig. 2. Fat pad weight in C57BL/6J mice after 5 months on the three diets. Values are means ± SD of five animals. ** $P < 0.01$.

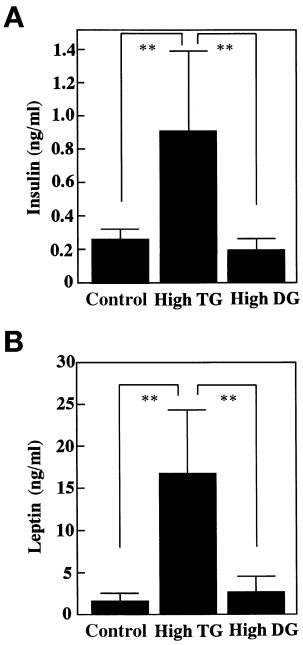

Fig. 3. Fasting serum insulin (A) and leptin (B) levels in C57BL/6J mice after 5 months on the three diets. Values are means ± SD of five animals. ** $P < 0.01$.

nearly equivalent to that in high TG-fed mice, indicating that absorption rate of dietary DG was not significantly different from TG. Therefore, it is unlikely that the difference in energy intake altered the body fat accumulation in mice fed TG and DG.

To examine the effects of diet on regional fat accumulation, we analyzed the distribution of four individual fat pads. After 5 months of feeding with the high TG diet, the weights of the fat pads were significantly increased. Compared with control mice, high TG feeding increased epididymal, mesenteric, retroperitoneal, and perirenal fat pad weights by 2.8-, 1.9-, 3.3-, and 3.0-fold, respectively (**Fig. 2**). In contrast, high DG diet significantly suppressed fat accumulation in all four areas. These fat pad weights were reduced by 73%, 92%, 76%, and 89%, respectively, compared with mice fed the high TG diet. These findings parallel the results of body weight gain shown in Table 3.

To further examine the local accumulation of fat, we determined the liver TG and cholesterol contents. As shown in **Table 4**, diet showed significant effects on the liver. The weight of the liver was increased by 37% by the high TG diet, and the increase was suppressed by the DG diet. There was no significant difference in liver TG concentration between control and high TG-fed mice. However, TG concentration of high DG-fed mice was 26% lower than that of high TG-fed mice. Total TG content per liver was increased by 41% by the high TG diet, whereas it was unchanged in high DG diet-fed mice. On the other hand, there were no significant differences in cholesterol concentrations between the groups. These results indicate that DG diet might be useful for prevention of TG accumulation in the liver.

Serum total cholesterol levels of mice fed the high DG diet were significantly lower than those of mice fed the high TG diet (Table 3). Serum TG concentrations were lower in high TG- and DG-fed mice compared with controls, whereas no significant differences were observed between high TG and DG groups. Fasting serum glucose levels were higher in high TG-fed mice, and were slightly decreased in the high DG group. There were no noticeable differences in GOT or γ-GPT between these groups.

Diet showed significant effects on serum insulin and leptin levels. As shown in **Fig. 3A**, the insulin level was 3.9-fold higher in high TG-fed mice compared with controls, indicating the development of hyperinsulinemia during 5 months of feeding. However, in contrast to high TG diet-fed mice, DG diet did not cause such hyperinsulinemia. Leptin levels in high TG-fed mice were elevated by 10-fold over those of controls (Fig. 3B). In contrast, the diet containing DG significantly suppressed the elevation of leptin levels.

TABLE 4. Liver weight, triglyceride, and cholesterol

	Control	High TG	High DG
Weight (g)	0.907 ± 0.057	1.246 ± 0.106[a]	1.104 ± 0.050[b]
Triacylglycerol(mg/g liver)	79.4 ± 11.7	81.6 ± 13.7	60.4 ± 5.5[a,b]
Cholesterol (mg/g liver)	3.81 ± 0.41	3.69 ± 0.48	3.46 ± 0.35
Total triacylglycerol(mg)	71.9 ± 11.4	101.6 ± 18.2[a]	66.7 ± 7.0[c]
Total cholesterol (mg)	3.46 ± 0.42	4.59 ± 0.61[a]	3.81 ± 0.33[b]

Results are means ± SD (n = 5).
[a] $P < 0.01$ as compared with controls.
[b] $P < 0.05$ as compared with High TG group.
[c] $P < 0.01$ as compared with High TG group.

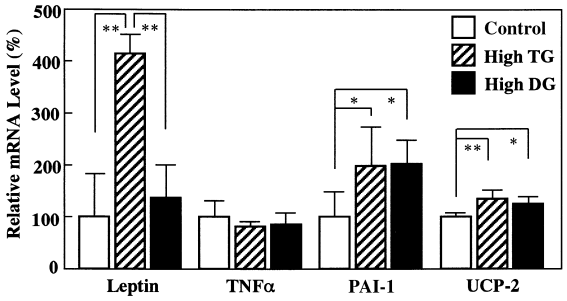

Fig. 4. mRNA levels in epididymal WAT of C57BL/6J mice. After 5 months of feeding with each diet, total RNA isolated from epididymal WAT was subjected to reverse transcription polymerase chain reaction and visualized by ethidium bromide staining as described in Materials and Methods. The amounts of mRNA were quantified with a digital fluorodensitometer (FM-BIO) and are expressed as percentages of those in controls. Values are means ± SD of five animals. TNFα, tumor necrosis factor α; PAI, plasminogen activator inhibitor. * $P < 0.05$; ** $P < 0.01$.

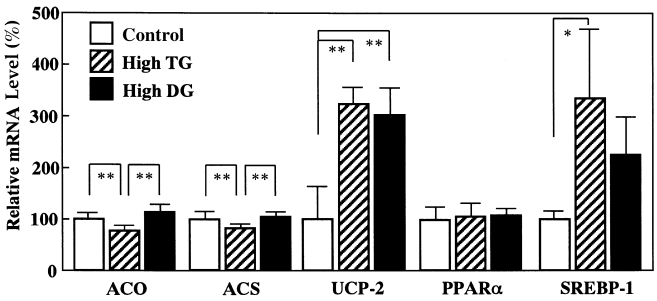

Fig. 5. mRNA levels in the liver of C57BL/6J mice. After 5 months of feeding with each diet, total RNA isolated from the liver was subjected to reverse transcription polymerase chain reaction and visualized by ethidium bromide as described in Materials and Methods. The amounts of mRNA were quantified with a digital fluorodensitometer (FM-BIO) and are expressed as percentages of those in controls. Values are means ± SD of five animals. * $P < 0.05$; ** $P < 0.01$.

To gain insight into the regulation of genes involved in obesity, lipid metabolism, and energy expenditure by dietary DG, we analyzed various mRNA levels in WAT and the liver by semiquantitative RT-PCR. In mice fed the high TG diet, the leptin mRNA level in epididymal adipose tissue was significantly higher than that in mice fed the control diet (**Fig. 4**). When normalized by the level of β-actin mRNA, the leptin mRNA level in high TG-fed mice was more than 4-fold higher than that in the control diet group. In contrast, elevation of the leptin mRNA level was significantly suppressed in mice fed the high DG diet compared with high TG-fed mice. Thus, DG diet suppressed elevation of both leptin mRNA expression in adipose tissues and the circulating levels. The levels of TNFα, which is a possible candidate mediating insulin resistance, were not changed by high fat diet. The levels of PAI-1 and UCP-2 mRNA in adipose tissue were elevated by both high TG and high DG diets. However, there were no differences between high TG and high DG diet groups. In accordance with the previous report (39) that DG feeding up-regulated ACO activity in the liver, ACO and ACS mRNA levels were significantly increased in mice fed the high DG diet compared with those mice fed the high TG diet (**Fig. 5**). Hepatic UCP-2 mRNA levels in high fat diet-fed mice were elevated by 3-fold over those of control mice, whereas there was no difference between mice fed high TG and high DG diets.

Many genes related to lipid metabolism such as ACO and UCP are known to be regulated by PPARα in the liver (28, 29). Therefore, we examined the levels of PPARα mRNA. However, we found no significant differences among the three groups. It has been reported that fish oil feeding decreases mature SREBP-1 by down-regulation of mRNA in the liver, which accounts for its beneficial effects (30, 31). Therefore, we also examined the levels of SREBP-1 mRNA in the liver. In mice fed high fat diets, SREBP-1 mRNA level was significantly higher than that in the mice fed the control diet. Although SREBP-1 mRNA level in high DG-fed mice was slightly lower than that in the high TG group, no significant differences were observed among high fat diet-fed mice.

DISCUSSION

In this study, we examined the effects of dietary DG in a diet-induced model of obesity and diabetes that is similar to human forms of these disorders (22, 23). The results of this study indicate that structural differences between DG and TG, but not the composition of fatty acid, markedly affect nutritional behavior of lipids including body fat accumulation, serum lipid profile, and development of hyperinsulinemia and hyperleptinemia.

Many studies have been conducted to determine the preventive or therapeutic effects of various dietary oils on obesity. Differences in fatty acid composition markedly affect the physiological actions of dietary oil (15, 32, 33). In a previous study to examine the effects of various dietary oils on obesity and diabetes in C57BL/6J mice, n-6 fatty acid-rich oils such as safflower and soybean oil produced similar levels of obesity and diabetes, whereas n-3 fatty acid-rich oils such as perilla and fish oils showed less induction of these diseases (34). Fish oils rich in EPA and DHA are known to lower serum and liver lipid contents, in part, by stimulation of fatty acid oxidation in the liver (35). Moreover, dietary fish oil has been found to reduce the activities of enzymes involved in fatty acid and TG synthesis and to stimulate the fatty acid oxidative pathway in the liver (36). These effects of fish oil are considered to be the result of the combined effects of PPARα activation and down-regulation of SREBP-1 (30, 31). Thus, certain dietary oils, possibly due to their constituent fatty acids, show beneficial effects on lipid metabolism and, thereby, on obesity and diabetes.

In contrast to previous reports, the present study indicates that dietary DG is beneficial for the suppression of body fat accumulation compared with TG, despite their similar fatty acid compositions. The precise mechanism

responsible for the apparent reduction of body fat accumulation by DG feeding is not clear. However, because energy intake and fecal lipid excretion were not significantly different between the high TG- and DG-fed mice, reduced body fat accumulation in the DG group was not related to reduced energy intake. Hara et al. (18, 19) showed that the extent of postprandial serum TG increase, especially chylomicron TG, after a single dose of DG emulsion was less than that observed after administration of TG emulsion. Impaired postprandial TG clearance was shown to be associated with visceral obesity (37, 38). Therefore, suppression of postprandial serum TG increases may, at least in part, explain the reduction of visceral fat after long-term feeding with DG. In addition, Murata, Ide, and Hara (39) reported that dietary DG compared with TG decreased the activities of enzymes involved in fatty acid synthesis, but increased those of enzymes involved in the fatty acid oxidation pathway in rat liver.

Consistent with the previous report that dietary DG increases ACO activity in the liver in rats (39), ACO and ACS mRNA levels were also increased in high DG-fed mice compared with high TG-fed mice, indicating that dietary DG up-regulates β-oxidation of fatty acid in the liver. These observations suggested that stimulation of fatty acid oxidation in the liver may be partially responsible for the beneficial effects of dietary DG. In addition, lower hepatic TG content and serum cholesterol levels in DG-fed mice may be related to up-regulated lipid metabolism in the liver.

UCP-2 is a mitochondrial membrane transporter that may regulate thermogenesis through dissipation of the proton gradient across the inner mitochondrial membrane. Although no consensus has yet been reached concerning the physiological role of UCP-2, some previous studies suggested its involvement in regulation of thermogenesis and body weight control (40–42). Therefore, it is possible that stimulation of fatty acid oxidation accompanied by UCP-2 up-regulation plays a role in the metabolic efficacy of dietary DG.

Alteration of lipid metabolism by DG feeding in the liver led us to speculate that this may have been due to changes in the levels of transcription factors that regulate fatty acid metabolism. However, we found no significant differences in PPARα or SREBP-1 mRNA levels in the liver between high fat diet-fed groups. The precise mechanism(s) by which dietary DG stimulates lipid metabolism in the liver remains to be elucidated.

A significant effect of dietary DG is suppression of fasting leptin and mRNA expression levels in adipose tissue (Fig. 3B, Fig. 4). The regulatory mechanisms of leptin production in the development of obesity associated with hyperleptinemia are unclear, but as the increase in leptin production occurs early in the process (21, 43), the suppressive effect of dietary DG on leptin gene expression may be an important factor responsible for the reduced body fat accumulation by dietary DG in C57BL/6J mice. As serum leptin concentration has been shown to be closely correlated with the amount of body fat, circulating leptin level may simply be a marker of the adipose tissue mass. However, some previous studies indicated that the level of leptin mRNA did not always correlate with adipose tissue mass (33, 44), and that leptin level was affected by the kind of diet independent of body weight gain (33, 45). Similarly, the present results provide a possibility that expression of leptin mRNA and circulating leptin levels are influenced by the structure of dietary acylglycerol. Consistent with previous reports (21, 45), the present results also indicate that up-regulation of leptin production is accompanied with enhanced insulin production. The precise mechanism(s) by which dietary DG suppresses leptin and insulin production relevant to body fat accumulation remains to be elucidated.

In summary, we showed that dietary DG compared with TG of similar fatty acid composition significantly suppressed body fat accumulation and elevation of circulating insulin and leptin levels in obesity- and diabetes-prone C57BL/6J mice. In addition, DG feeding affected expression of genes related to energy homeostasis including leptin in WAT, ACO, and ACS in the liver. These results indicated that DG may be beneficial for alleviation and prevention of high fat-induced body fat accumulation and possibly reduce the risk of associated diseases including diabetes and coronary heart disease. Moreover, understanding the nutritional characteristics of dietary DG and the molecular mechanisms may provide new insight for the management of obesity.

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