Chapter 9

Antiobesity Effect of Long-Term Consumption of Dietary Diacylglycerol in Experimental Animal Models

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Introduction

Obesity is a major worldwide health problem that is caused by a combination of genetic and environmental factors. Previous studies indicated that obesity is a major risk factor for a variety of lifestyle-related diseases, including diabetes, hypertension, hyperlipidemia, and arteriosclerosis (1–3). Obesity is a metabolic disorder resulting from disequilibrium between energy uptake and expenditure. Although both diacylglycerol (DAG) oil and triacylglycerol (TAG) oil have similar energy values and absorption rates (4), studies (Chapter 4) showed that the ingestion of DAG oil activates lipid metabolism in the small intestine and liver, and enhances whole-body energy expenditures (5,6). The studies discussed in this chapter focus only on the effects on body fat accumulation as the result of the long-term consumption of DAG oil in experimental animal models. Other physiological effects are discussed in other chapters.

Antiobesity Effect of Diacylglycerol

A series of comparative studies of DAG and TAG to determine the long-term effect of DAG were conducted in animals. In the studies described below, DAG was prepared by esterifying glycerol with fatty acids from rapeseed oil and soybean oil by the method of Huge-Jensen *et al*. (7) and was comprised of the structural isomers 1,2-DAG and 1,3-DAG in a ratio of 3:7. TAG was prepared by mixing rapeseed oil, soybean oil, and safflower oil to give a fatty acid composition that was similar to that of the DAG.

Watanabe *et al*. (8) analyzed the body fat accumulation of male SD rats fed a diet containing either 10% (w/w) DAG oil or TAG oil. The percentage of body fat, measured by bioimpedance (EM-SCAN SA-2), was monitored periodically at 3 and 4 wk. No differences in food consumption were noted in the two groups, but in the DAG diet group, the percentage of body fat was significantly lower than that in the TAG diet group at both 3 and 4 wk (Fig. 9.1). Sugimoto *et al*. investigated the efficacy of DAG or TAG oil on body fat accumulation in male Wistar rats (9) and female Wistar fatty rats (10). There were no significant differences of body fat accumulation between the DAG diet group and the TAG diet group. Thus, the efficacy of DAG in Wistar rats is a matter of argument. In any case, the difference in body fat between the treatment groups was relatively small compared with the obese animal model described below.

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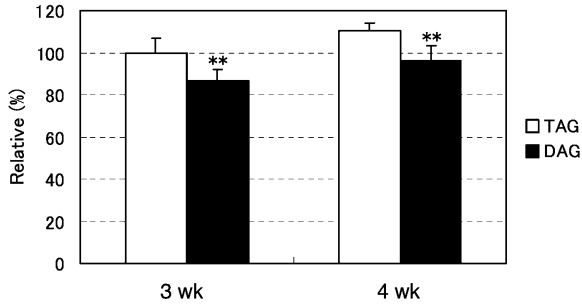

Fig. 9.1. Relative change in body fat ratio in rats. Values are the means ± SD, n = 5. **Different from triacylglycerol ingestion, $P < 0.01$. *Source*: Ref. 8.

Additional research demonstrated that dietary DAG suppresses the accumulation of high-fat and high-sucrose diet-induced body fat in mice (9). This study was designed to examine the effects of dietary DAG on the development of obesity and the resulting hyperinsulinemia and hyperleptinemia in C57BL/6J mice, known as a model of diet-induced obesity and diabetes mellitus. Male C57BL/6J mice (7 wk old) were randomly assigned to 1 of 3 study groups in which they were allowed free access to 1 of 3 synthetic diets: a control (low-TAG) containing 5% (w/w) TAG oil, 20% protein, and 66.5% starch; a high-TAG containing 30% (w/w) TAG oil, 20% protein, 28.5% starch and 13% sucrose; or a high-DAG containing 30% (w/w) DAG oil instead of TAG oil. The mice were maintained on these diets for 5 consecutive months and monitored for several variables including body weight, food intake, feed efficiency [body weight gain per cage (g)/kcal of food consumed per cage by day], fat pad weights, liver triacylglycerol levels, liver cholesterol level, and serum insulin and leptin levels. Feces were collected 3 times on a per-cage basis for 24 h during the final week of feeding.

Mice that consumed the high-TAG diet for 5 mo had a significantly greater weight gain compared with those that were fed the control diet. However, mice that were fed a high-DAG diet had a significant reduction in body weight compared with the high-TAG diet group (Fig. 9.2, Table 9.1). High fat–fed mice (TAG and DAG) consumed more energy on average than the low fat–fed mice (control); however, no difference was observed between the TAG- and DAG-fed groups. Consistent with this, mice fed high DAG had a significantly lower feed efficiency than mice fed high TAG. When fecal lipid quantity was examined, both groups of high fat–fed mice had increases in fecal lipids compared with the controls. The amounts of fecal lipid in the high DAG–fed and high TAG–fed mice were nearly equivalent, indicating that the DAG and TAG adsorption rates did not differ (Table 9.1).

After 5 mo of high-TAG diet consumption, the weights of the 4 individual fat pads (epididymal WAT, mesenteric WAT, retroperitoneal WAT, perirenal WAT) were significantly increased. In contrast, a high-DAG diet significantly suppressed fat accumulation in all 4 areas (Fig. 9.3). These findings parallel the results for body weight gain.

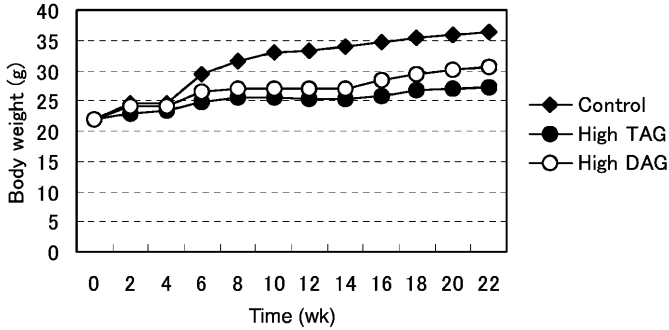

Fig. 9.2. Comparison of body weight in C57BL/6J mice fed the 3 diets for 5 mo. Each point represents the mean body weight of 5 mice. *Source*: Ref. 11.

TABLE 9.1
Final Body Weight, Feed Efficiency, Energy Intake, and Fecal Lipid[a]

	Control	High TAG	High DAG
Final body weight[b] (g)	27.6 ± 1.7	36.4 ± 2.3**	30.2 ± 2.4††
Feed efficiency[c]	0.519	1.052	0.654
Energy intake [kcal/(cage·d)]	57.2 ± 3.9	69.7 ± 8.1**	65.2 ± 8.4**
Fecal lipid (mg/g dried feces)[d]	19.1 ± 2.9	64.3 ± 1.1**	60.5 ± 7.9**

[a]Values are means ± SD, n = 5. *Source*: Ref. 11.
[b]Mice were killed after 5 mo of consuming each diet, and final body weight was determined.
[c]Feed efficiency was calculated as follows: [body weight gain per cage (g)]/[kcal of food consumed per cage per day].
[d]Feces were collected 3 times on a per-cage basis for 24 h during the final week of feeding.
**Different from controls, $P < 0.01$; ††different from the High-TAG group, $P < 0.01$.

The diet had a significant effect on the liver. Mice in the high-TAG diet group had an increased liver weight, whereas the increase in liver weight was suppressed by the high-DAG diet. Although there was no significant difference in liver triacylglycerol concentrations (mg/g liver) between the control and high TAG-fed mice, the high DAG-fed mice had a significantly lower triacylglycerol concentration than that of the high TAG-fed mice. The total liver triacylglycerol (mg) of TAG-fed mice was significantly higher than that of the controls. However, the total liver triacylglycerol of DAG-fed mice was significantly lower than that of the TAG-fed mice. Although no difference in cholesterol concentrations (mg/g liver) was observed between the groups, the liver total cholesterol (mg) of DAG-fed mice was significantly lower than that of TAG-fed mice (Table 9.2). These findings suggest that a DAG diet might be useful in preventing triacylglycerol and cholesterol accumulation in the liver.

The diet had significant effects on serum insulin and leptin levels. Elevated serum insulin levels in the high TAG–fed mice indicated the development of hyperinsulinemia during the 5 mo of feeding. This hyperinsulinemia was not observed in mice fed the DAG diet. Leptin levels in the high TAG-fed group were 10-fold higher than those

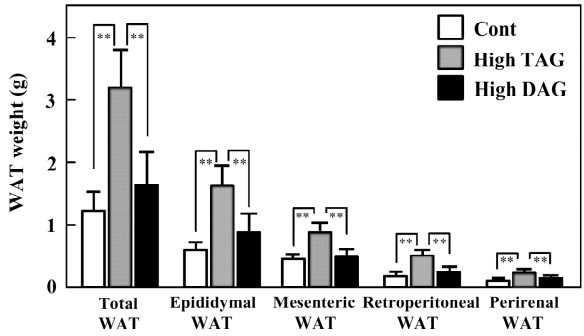

Fig. 9.3. White adipose tissue (WAT) weight in C57BL/6J mice after 5 mo of consuming the 3 diets. Values are means ± SD, n = 5. **Different from control and high-TAG, $P < 0.01$. *Source*: Ref. 11.

TABLE 9.2
Liver Weight, Triglyceride, and Cholesterol[a]

	Control	High TAG	High DAG
Weight (g)	0.907 ± 0.057	1.246 ± 0.106**	1.104 ± 0.050†
Triacylglycerol (mg/g liver)	79.4 ± 11.7	81.6 ± 13.7	60.4 ± 5.5**,†
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**	66.7 ± 7.0††
Total cholesterol (mg)	3.46 ± 0.42	4.59 ± 0.61**	3.81 ± 0.33†

[a]Values are means ± SD, n = 5. *Source*: Ref. 11.
**Different from controls, $P < 0.01$; †different from the High-TAG group, $P < 0.05$; ††different from High-TAG group, $P < 0.01$.

in the control group, whereas the high-DAG diet significantly suppressed the elevation of leptin levels (data not shown).

Furthermore, Murase *et al.* (5) examined the effects of dietary DAG and TAG with similar fatty acid compositions on the development of obesity in C57BL/6J mice during 8 mo of feeding. In this study, the antiobesity effect of a lower content of DAG in the feed was examined. Male C57BL/6J mice (7 wk old) were randomly assigned to 1 of 3 study groups in which they were allowed free access to 1 of 3 synthetic diets: a control (low TAG) containing 5% (w/w) TAG oil, 20% protein, and 66.5% starch; a high TAG containing 25% (w/w) TAG oil, 20% protein, 28.5% starch and 13% sucrose; or a high DAG containing 15% (w/w) DAG oil and 10% (w/w) TAG oil. The mice were fed these diets for 8 consecutive months. The results are as follows. Dietary DAG consistently reduced body fat accumulation induced by a high-fat diet. Compared with the low-TAG diet, mice that were fed the high-TAG diet for 8 mo had significant increases in body weight and adipose tissue weight. Conversely, in mice fed the high-DAG diet, body weight gain, epididymal WAT, perirenal WAT, and interscapular BAT weight were reduced by 43, 52, 85, and 81%, respectively, com-

pared with the high-TAG diet (Table 9.3). In addition, under conditions of food deprivation, DAG-fed mice had significantly lower glucose, insulin, and leptin concentrations than those of high TAG-fed (Table 9.3).

No differences in mean energy intake were observed between the high-DAG and high-TAG groups. It was demonstrated previously that lipid absorption does not differ between these groups (4,11), suggesting that the reduced energy intake in the DAG group does not account for the reduced accumulation of body fat.

These results stimulated our interest in the mechanisms of the antiobesity effects of DAG. The effects of DAG on the mRNA expression of genes involved in lipid metabolism in various tissues were also analyzed. The details of these studies are given in Chapter 4. In the above studies, DAG was prepared by esterifying glycerol with fatty acids from rapeseed oil and soybean oil, which contain mainly oleic acid and linoleic acid as fatty acid constituents. These results suggest that DAG, which contains mainly oleic acid and linoleic acid as fatty acids, is very effective in preventing and altering obesity compared with TAG with a similar fatty acid composition.

Antiobesity Effect of α-Linolenic Acid–Rich Diacylglycerol

The above results suggest that the structure of the acylglycerol, but not the fatty acid composition, markedly affects the nutritional behavior of this class of lipids. On the other hand, n-3 polyunsaturated fatty acids (n-3 PUFA), such as eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), and α-linolenic acid (ALA) were reported to have a number of physiologic functions, such as anti-arteriosclerosis, anti-hypertension, and immunity control (12–16). The n-3 PUFA affect lipid metabolism, resulting in a decreased lipid concentration in the blood and a reduction in body weight and body fat weight (17–20). In the three studies below, α-linolenic acid–rich DAG (ALA-DAG), which has a 1,3-DAG structure and fatty acids containing >60%

TABLE 9.3
Body Weight, Energy Intake, Fat Weight and Plasma Analysis[a]

	Control	High TAG	High DAG
n	10	20	10
Body weight[b] (g)	30.8 ± 2.1***	41.9 ± 5.4	37.1 ± 4.1*
Energy intake[c] [kcal/(cage·d)]	54.8 ± 8.9***	65.3 ± 3.8	62.2 ± 8.7
Epididymal WAT (g)	0.98 ± 0.23***	1.81 ± 0.47	1.38 ± 0.56*
Perirenal WAT (g)	0.11 ± 0.03***	0.31 ± 0.16	0.14 ± 0.09**
Interscapular WAT (g)	0.24 ± 0.08***	0.62 ± 0.4	0.38 ± 0.14
Interscapular BAT (g)	0.13 ± 0.02***	0.29 ± 0.11	0.16 ± 0.11***
Glucose (mg/dL)	67.64 ± 12.65***	111.65 ± 25.18	83.28 ± 13.20**
Insulin (ng/mL)	0.60 ± 0.13*	1.45 ± 1.04	0.64 ± 0.57*
Leptin (ng/mL)	3.47 ± 1.23***	24.00 ± 18.67	7.40 ± 8.62**

[a]Values are means ± SD, n = 5. *Source*: Ref. 5.
[b]Mice were killed after 8 mo of consuming the respective diets and body fat weight was then determined.
[c]Energy intake was measured on a per-cage basis over the course of 24 h 1 d/wk.
Different from the High-TAG group: *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

ALA, was the focus of interest, and the antiobesity effect of ALA-DAG was examined in C57/BL6J mice. ALA-DAG was prepared from perilla oil in the presence of immobilized lipase. The DAG content in the ALA-DAG oil was >80%, and the ratio of 1,2-DAG and 1,3-DAG was 3:7.

In the first study, male C57BL/6J mice (7 wk old) were randomly assigned to 1 of 5 study groups (19). This experiment included a low-fat (LF) diet, a high-fat (HF) diet, and a HF diet with the addition of incremental percentages of ALA-DAG oil (1, 2, and 4%, w/w). After a 7-d acclimatization, the mice were allowed free access to food and water. During the 4 wk in which the mice were fed these diets, they were monitored weekly for body weight. The amount of food consumed over a 24-h period was measured and the energy intake was calculated once a week. At the end of the experiment, the visceral fats [epididymal white adipose tissue (WAT), mesenteric WAT, retroperitoneal WAT, and perinephric WAT] were dissected and weighed.

No significant differences in the initial body weights or mean energy intakes during the test period were observed within each group. All of the groups showed gains in body weight by wk 4. The HF group had a significantly greater weight gain than the LF group. Conversely, the ALA-DAG-added group had a smaller body weight gain than the HF group. At the end of the 4 wk, the ALA-DAG-4%-added group had the lowest body weight and weight gain. The visceral fat weight of the HF group was significantly higher than that of the LF group. The ALA-DAG-added groups had significantly lower values than the HF group for total visceral fat weight, epididymal WAT, mesenteric WAT, retroperitoneal WAT, and perinephric WAT (Table 9.4). The visceral fat weight tended to decrease with linearly increasing ALA-DAG content.

TABLE 9.4
Effects of α-Linolenic Acid–Rich Diacylglycerol (ALA-DAG) Diet on Body Weight at 4 wk[a]

	Dietary treatment				
			High fat + ALA-DAG		
	Low fat	High fat	ALA-DAG 1%	ALA-DAG 2%	ALA-DAG 4%
Body weight (g)					
Initial	21.6 ± 1.3	21.6 ± 1.3	21.6 ± 0.9	21.6 ± 1.2	21.6 ± 1.2
Final	26.3 ± 1.2*	29.0 ± 1.9	26.4 ± 0.3*	26.6 ± 1.3*	25.9 ± 2.0**
Gain (4 weeks)	4.8 ± 0.4***	7.4 ± 1.5	4.9 ± 0.7***	5.0 ± 0.7***	4.3 ± 0.8***
Visceral-fat weight (g)					
Total	0.95 ± 0.10**	1.46 ± 0.37	1.11 ± 0.18*	1.00 ± 0.15**	0.94 ± 0.16**
Epididymal	0.49 ± 0.06**	0.76 ± 0.21	0.56 ± 0.12*	0.51 ± 0.08**	0.49 ± 0.10**
Mesenteric	0.32 ± 0.04*	0.41 ± 0.08	0.34 ± 0.03*	0.33 ± 0.05*	0.30 ± 0.03**
Retroperitoneal	0.10 ± 0.03***	0.24 ± 0.07	0.16 ± 0.05*	0.12 ± 0.02***	0.11 ± 0.03***
Perinephric	0.04 ± 0.00	0.05 ± 0.02	0.04 ± 0.01	0.04 ± 0.01	0.04 ± 0.01
Energy intake [kcal/(cage·d)]	55.8 ± 7.1	62.9 ± 4.2	59.4 ± 5.5	57.2 ± 4.7	57.9 ± 5.1

[a]Values are means ± SD, n = 5. *Source*: Ref. 21.
Different from High Fat: *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

In the second study, male C57/BL6J mice (7 wk old) were fed a low-fat (LF) diet, a high-fat (HF) diet, or a HF diet with the addition of 3% ALA-DAG for 20 wk (21). As above, C57/BL6J mice were used as a model for diet-induced obesity and diabetes mellitus. In this study, serum insulin and leptin levels in addition to body weight and the weight of the visceral fats were also examined. The effects on body weight gain and visceral fat weight were similar to the results in the first study (21) (Table 9.5). Serum insulin and leptin concentrations for the HF group were higher than those for the LF group. Serum insulin and leptin concentrations of the 3% ALA-DAG group were significantly lower than those of the HF group (Table 9.5). These results suggest the possibility that ALA-DAG may be effective against diabetes mellitus.

In addition to the two studies discussed above, Murase *et al.* (22) examined the effect of ALA-DAG in genetically obese C57BL/KsJ-db/db mice, which develop severe obesity because of their leptin signaling deficiency (23). Three oils were used in this study, i.e., a TAG oil mixture that was prepared by combining soybean and rapeseed oils, an α-linolenic acid–rich TAG oil (ALA-TAG oil), and an α-linolenic acid–rich DAG oil (ALA-DAG oil) that was prepared by esterifying glycerol with fatty acids from the linseed oil. The ALA-DAG and ALA-TAG oils had similar fatty acid compositions. Female C57BL/KsJ-*db*/*db* mice (5 wk old) were randomly assigned to 1 of 3 study groups in which they were had free access to water and 1 of the 3 synthetic diets: containing 14% (w/w) TAG oil, 4% (w/w) ALA-TAG oil with 10% TAG oil, or 4% (w/w) ALA-DAG oil with 10% TAG oil. The mice were fed these diets for 1 mo and monitored for variables of body weight and food intake. There were no significant

TABLE 9.5
Effects of ALA-DAG on Body Weight, Liver Weight, Visceral-Fat Weight, and Serum Insulin and Leptin at 20 wk[a]

	Dietary treatment		
	Low fat	High fat	High fat + ALA-DAG
Body weight (g)			
Initial	22.0 ± 0.6	22.5 ± 0.9	22.0 ± 0.7
Final	27.6 ± 1.7***	36.4 ± 2.3	32.0 ± 3.7*
Gain (20 wk)	5.6 ± 1.2***	13.8 ± 1.9	10.0 ± 3.1*
Visceral-fat weight (g)			
Total	1.264 ± 0.380***	3.180 ± 0.623	2.284 ± 0.873
Epididymal	0.578 ± 0.194***	1.620 ± 0.328	1.188 ± 0.516
Mesenteric	0.454 ± 0.101***	0.874 ± 0.161	0.634 ± 0.156*
Retroperitoneal	0.162 ± 0.069***	0.488 ± 0.094	0.340 ± 0.151*
Perinephric	0.070 ± 0.022**	0.198 ± 0.068	0.122 ± 0.056*
Energy intake [kcal/(cage·d)]	57.4 ± 2.8**	68.6 ± 1.3	66.5 ± 4.2
Insulin (pg/mL)	176.40 ± 73.95**	897.60 ± 505.26	287.30 ± 72.05**
Leptin (ng/mL)	1.59 ± 1.07***	16.72 ± 7.66	6.75 ± 5.27*

[a]Values are means ± SD, n = 5. *Source*: Ref. 21. See Table 9.4 for abbreviations.
Different from High Fat: *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

TABLE 9.6
Body Weight, Weight Gain, and Energy Intake in Mice Fed TAG, ALA-DAG, or ALA-TAG Diets for 1 mo[a]

	TAG	ALA-DAG	ALA-TAG
Body weight (g)	42.3 ± 1.6[d]	38.6 ± 2.7[c]	40 ± 2.5[c,d]
Body weight gain (g/mo)	12.3 ± 1.7[d]	8.6 ± 2.4[c]	10.2 ± 1.7[c,d]
Energy intake[b] (kJ/d)	296.9 ± 28.4	296.9 ± 31.7	288.6 ± 38.4

[a]Values are means ± SD, n = 8; means without a common letter differ, $P < 0.05$. *Source*:Ref. 22. See Table 9.4 for abbreviations.
[b]Energy intake: 4 mice/cage, n = 2.

differences in energy intake during the test period among the three groups. Compared with the mice fed the diet supplemented with the ALA-TAG, the ALA-DAG diet reduced body weight gain, indicating that dietary ALA-DAG more effectively suppresses increases in body weight than ALA-TAG (Table 9.6).

As shown in the second study (21), the intake of 3% ALA-DAG oil (in 30% fat in diet) significantly suppressed body weight gain and hyperinsulinemia induced by high-fat and high-sucrose diets; however, the effects are not directly comparable to the effects of 30% DAG oil (in 30% fat in diet). ALA-DAG may be more effective in suppressing body fat accumulation than DAG. Although further studies will be required, these data suggest that ALA-DAG might be an excellent oil having the characteristics of both a DAG structure and a n-3 PUFA.

Summary

The results in this chapter show that the continuous consumption of DAG suppresses body fat accumulation in experimental animal models. The antiobesity effect as the result of the long-term consumption of DAG was clearly observed in obese animals models, compared with the normal experimental animals. In Chapter 10 and 11, these effects are also examined in humans. The results of this collective research indicate the possibility that dietary intervention with DAG may contribute to the prevention and management of obesity as well as various lifestyle–related diseases such as diabetes, hyperlipidemia, and arteriosclerosis in humans. Further research to understand the nutritional characteristics of dietary DAG and its molecular mechanisms may provide new insights for the management of obesity and lipid nutrition.

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