Chapter 3

Digestion and Absorption of Diacylglycerol

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Introduction

Most edible oils contain diacylglycerol (DAG) as a minor constituent. Although the isoform of DAG that occurs in the process of triacylglycerol (TAG) digestion by lingual or pancreatic lipase is 1,2- or 2,3-DAG, a substantial fraction of DAG in edible oils is converted to 1,3-DAG by acyl migration (1). We reported previously that the serum triglyceride concentration of rats fed DAG oil that is composed mainly of 1,3-DAG was significantly lower than that of rats fed common TAG oil of a similar fatty acid composition (2). We also showed that the rate of lymphatic transport of triglyceride as chylomicrons was significantly retarded in rats that had been intragastrically infused with a DAG oil emulsion compared with a TAG oil emulsion (3). We reported recently that the long-term ingestion of dietary DAG oil, in contrast to TAG oil, reduces the accumulation of body fat in humans (4,5). In the digestive tract, 1,3-DAG is hydrolyzed either to 1 (or 3)-monoacylglycerol (MAG) and fatty acids or to glycerol and fatty acids *via* the intermediate 1-MAG (6), whereas TAG is hydrolyzed to 2-MAG and fatty acids. On the basis of these results, we hypothesized that the limited availability of 2-MAG for reesterification retards chylomicron-triglyceride transport in rats that have been infused with the DAG oil emulsion.

Before progressing to further studies on the possible mechanisms for these phenomena, it is essential to investigate precisely the energy value and bioavailability of DAG compared with those for TAG with the same fatty acid composition. When the overall absorption rate of DAG and TAG oil is similar, it is still possible that the processes of digestion and assimilation of DAG differ from those of TAG. A detailed analysis of the digestion products and metabolites originating from DAG or TAG might provide further mechanistic insights into the functions and role of DAG.

The altered digestion and absorption of DAG, on the other hand, may have some influence on the absorption of fat-soluble vitamins. Because the physicochemical properties of DAG are similar to those of TAG, it has the potential to be used in cooking, frying, shortening, and for dressings. Edible oils are carriers of fat-soluble vitamins, which are essential for growth and normal functions of the human body. These vitamins are generally absorbed from the gastrointestinal tract only when bile is present. Fat-soluble vitamins and dietary fat are emulsified by bile acids. During the diges-

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tion of dietary fats, micelles containing lipophilic compounds are formed. The absorption rate of fat-soluble vitamins and lipophilic compounds depends on the degree to which they are partitioned between oil and water (7). Therefore, it is important to determine the effect of DAG oil consumption on the fat-soluble vitamin status although the contribution of the altered digestion products of 1,3-DAG in these micellar properties might be small.

This chapter summarizes the results of studies on energy values and the absorption coefficients of DAG and TAG oils. The effects of dietary DAG consumption on the absorption of fat-soluble vitamins and on gastric emptying time are also discussed because they are intimately related to the digestion process of dietary fats.

Energy Values of DAG and TAG Oils with a Similar Fatty Acid Composition

Fats and oils provide ~9 kcal/g metabolizable energy compared with 4 kcal/g for proteins and carbohydrates (8,9). These values have been approved and are widely used to calculate energy intake from various foodstuffs. These values are generalized, and the precise amount of energy contained in an individual food may vary depending on its composition and structure. In situations such as specific experiments, therapeutics, and special diet programs, precise determination of food energy values is required. These include experiments directed at the development of foods that involve less fat accumulation in the body.

It is thus necessary to determine the energy value of the fat and its digestibility when the effect of a dietary fat on energy metabolism is studied. Taguchi *et al.* (10) compared the energy values of DAG and TAG oils by calculation and by means of a bomb calorimeter, and also compared the absorption coefficient or digestibility of DAG and TAG oils to assess the contribution of energy and digestibility differences to the functional differences observed in these dietary oils, as described in other chapters of this book.

The fatty acid composition and acylglycerol composition of each of the oils used in the studies for the first two sections in this chapter are shown in Table 3.1. The fatty acid composition of the DAG oil was very similar to that of the blended TAG oil. The DAG concentration of the DAG oil was 87.0 g/100 g, and the ratio of 1(3), 2- to 1,3-DAG was 32:68. Using the approach outlined in the literature (11,12), Taguchi *et al.* (10) calculated the heat energy values of both DAG oil and TAG oil.

The energy values for the fatty acids were estimated using the equation $-\Delta Hc = 0.653n - 0.166d - 0.421$, where $-\Delta Hc$ is the heat of combustion in MJ/mol, n is the number of carbon atoms/molecule fatty acid, d is the number of double bonds/fatty acid, as described by Livesey (11). The energy value used for glycerol was 18.0 kJ/g (12). The heat of esterification was neglected because it amounts to only 3.8 kJ/mol of ester bond as determined with methyl stearate (13). In the case of tristearin, this value corresponds to 13 J/g.

The theoretical energy values calculated from the amount of fatty acids and glycerol released from 1 g of test oil and from the energy value for each fatty acid are

TABLE 3.1
Data Used to Calculate the Energy Values for Triacylglycerol and Diacylglycerol Oils

Component	Weight fraction	
	DAG oil	TAG oil
Fatty acid	(g/100 g)	
16:0	2.4	6.0
18:0	0.7	2.2
18:1	28.0	29.1
18:2	60.3	57.8
18:3	5.6	2.5
20:0	<0.05	0.4
20:1	0.2	0.6
22:0	<0.05	0.3
22:1	<0.05	0.2
24:1	<0.05	0.2
Other	2.8	0.7
Acylglycerol		
TAG	10.7	97.2
DAG	87.0	1.1
1(3), 2-DAG	27.8	ND[a]
1, 3-DAG	59.2	ND
Monoacylglycerol	0.82	<0.05
Free fatty acid	ND	ND

[a]ND, not determined.

shown in Table 3.2. The energy values determined using a bomb calorimeter for the DAG oil and the TAG oil are also shown in Table 3.2. The combustion energies measured by bomb calorimetry were in good agreement with the calculated values. The energy value of the DAG oil was ~98% of the TAG oil. Because the fat energy content in a practical diet is only a part of the total energy, this energy difference (2%) between the oils would be expected to result in only a negligible difference in the total energy value of the diet. Because the animal diet used in the experiment of the next section contained 38.6 energy% of fat, this difference in energy value (2%) between the oils will produce an 0.8% difference in the energy value of the diet in the animal experiment. The 0.8% difference in the energy value between the DAG diet and the TAG diet corresponds to 3.6 kcal/100 g. This difference is far less than the energy required to detect any differences in the growth of rats at the maximum sensitivity. In the human trial by Nagao *et al.*, for example (4), the contribution of the energy difference to the results was much less (<0.1%) because the amount of the test oil substituted was only 10 of 42 g of the daily total fat consumed. Our results in conjunction with the previous study suggest that the energy difference between DAG and TAG is negligible.

Absorption Coefficient of DAG Oil (Animal Study)

The rat is the most frequently used animal model to predict the digestibility of various food components. Bach Knudsen *et al.* (14) demonstrated that the digestibility of non-

TABLE 3.2
Theoretical and Experimental Energy Values of Diacylglycerol and Triacylglycerol Oils

		Diacylglycerol oil		Triacylglycerol oil	
Fatty acid	Gross energy (kJ/g)	Weight fraction (g/g oil)	Contribution to energy value (kJ/g oil)	Weight fraction (g/g oil)	Contribution to energy value (kJ/g oil)
16:0	39.1	0.023	0.9	0.058	2.3
18:0	39.9	0.007	0.3	0.021	0.8
18:1	39.5	0.263	10.4	0.280	11.1
18:2	39.3	0.567	22.2	0.557	21.8
18:3	38.9	0.053	2.0	0.024	0.9
20:0	40.5	0.000	0.0	0.004	0.2
20:1	40.2	0.002	0.1	0.006	0.2
22:0	41.0	0.000	0.0	0.003	0.1
22:1	40.7	0.000	0.0	0.002	0.1
24:1	40.9	0.000	0.0	0.002	0.1
Fatty acids		0.913	35.9	0.956	37.6
Glycerol	18.0	0.145	2.6	0.105	1.9
Total		1.000	38.5	1.000	39.5
Experimental			38.9		39.6

starch polysaccharides and most of their monosaccharide residues was higher in humans than in rats, whereas the digestibility values for protein, energy, and fat in general were very similar for the two species. Wisker *et al.* (15) also reported that the digestibilities of protein, fat, and nonstarch polysaccharides in a low-fiber diet and diets containing coarse or fine whole-meal rye were comparable in rats and humans. It should, therefore, be reasonable to compare the digestibility of DAG oil and TAG oil using a rat model.

Taguchi *et al.* (10) calculated the absorption coefficient of DAG and TAG oils from a lipid analysis of the diet and 3-d fecal excretion. Male Sprague-Dawley rats (n = 16; 5 wk old) were divided into 2 groups of 8 so that the body weight of each group was approximately equal; they were then transferred to individual metabolic cages. Rats in one group were fed a DAG oil diet [20 g/100 g DAG] and the others were fed a TAG oil diet [20 g/100 g TAG] (DAG group and TAG group), respectively. Feces were collected and pooled during the last 3 study days. The digestibility coefficient is defined as the percentage of ingested fat that is not excreted in the feces (16–18).

The overall movement of fats in the rat during the last 3 d of the experiment are shown in Table 3.3. No significant differences between the diet groups were found for the following variables: food intake, fat intake, dried feces mass, fat excretion, fat content of dry feces, and fat absorption coefficients. To determine the effect of dietary DAG on the lipid composition of feces, they analyzed the fecal lipid profile. The groups did not differ with respect to the pattern of the fecal lipids, as evidenced by GC.

In addition, Murase *et al.* determined the digestibility of dietary DAG in mice that accumulated less body fat during a period of 5 mo compared with the TAG diet

TABLE 3.3
Fat Intake and Excretion over 3 d in Rats Fed Either the Diacylglycerol or Triacylglycerol Diet[a]

Variable	Diacylglycerol group	Triacylglycerol group
	[g/(3 d·rat)]	
Diet intake	49.99 ± 1.23	50.12 ± 1 .74
Fat intake	9.67 ± 0.24	9.67 ± 0.33
Dried feces mass	3.15 ± 0.17	3.35 ± 0.14
Fat excretion	0.354 ± 0.043	0.352 ± 0.022
	(g/100 g)	
Fat content of dry feces	10.49 ± 0.38	11.18 ± 1.03
	(%)	
Fat absorption coefficient	96.3 ± 0.42	96.3 ± 0.26

[a]Values are means ± SE (n = 8).

(19). They measured the amount of lipid in the feces of mice fed a high-fat (30 g/100 g) diet. Although the amount of fecal lipid in mice fed a high-fat diet increased in both the DAG and TAG groups, compared with that of the low-fat diet–fed mice, the amount of fecal lipid in the high-DAG–fed mice was nearly equivalent to that in the high-TAG–fed mice. These results indicate that the rate of absorption of dietary DAG oil does not differ significantly from that of TAG oil.

These results suggest that the physiologic differences between the DAG oil and TAG oil observed in animals and humans described in other chapters are caused by differences in metabolic fate after absorption by gastrointestinal epithelial cells. This encourages further studies on the mechanism of the function of dietary DAG as it relates to the reduction of body fat accumulation in animals and humans.

Digestion of DAG and the Fate of the Digestion Products (Animal Study)

TAG is digested into 2-MAG and free fatty acids (FFA) in the intestinal lumen and then resynthesized to triglyceride in the intestinal mucosal cells. It was shown previously that triglyceride is resynthesized in the mucosa mainly *via* the 2-MAG pathway through two steps of enzyme action, the acylation of 2-MAG and the subsequent acylation of 1,2(2,3)-DAG (20,21). However, the metabolic pathway of 1,3-DAG, a major constituent of the dietary DAG oil, in the small intestine has not been clarified.

Therefore, Kondo *et al.* (22) investigated the metabolic features of DAG in the small intestine with regard to the digestion pathway in the lumen and the triglyceride-synthesis pathway in the mucosa using a rat model to clarify the mechanisms underlying the beneficial effects of the DAG oil described in other chapters of this book.

An intraduodenal infusion was performed according to the method of Mansbach and Nevin, using male Wistar rats at 6–10 wk of age (23). When the rats were infused duodenally with [carboxyl-^{14}C] trioleoylglycerol (TO), 76.0% (mean, n = 4) of the labeled TO was digested in the 10 min after infusion, and 1,2(2,3)-dioleoylglycerol (DO) (9.9%), 2-monooleoylglycerol (MO) (28.2%), and oleic acid (OA) (36.8%)

were generated in the intestinal lumen as the major products of digestion (Fig. 3.l). In contrast, when 1,3-[carboxyl-^{14}C]DO was infused, the degree of DO digestion (mean: 79.8%, n = 4) did not differ from that of TO, and 1(3)-MO (25.6%) and oleic acid (50.6%) were generated mainly as the digestion products in the lumen (Fig. 3.1). The high oleic acid/MO ratio in the lumen infused with DAG suggests that substantial amounts of l(3)-MAG were further converted to FFA and glycerol in the lumen after the infusion of DAG.

Furthermore, lipid metabolites in rat intestinal mucosal cells infused with DAG or TAG were determined. To achieve a steady state of digestion, emulsions of TAG oil or DAG oil were infused into the duodenum for 5 h. After 5 h, an emulsion supplemented with ^{14}C-labeled linoleic acid was infused and the incorporation of the ^{14}C label into the mucosal lipids was determined. Ten minutes after the ^{14}C-infusion, 59.8 ± 9.1% (n = 6) of the ^{14}C was incorporated into the mucosal lipids of the TAG-infused rats, whereas a smaller amount of ^{14}C label was incorporated in the case of DAG-infused rats [42.9 ± 7.8% (n = 6), $P < 0.00$l].

The incorporation of the ^{14}C label into the mucosal triglyceride was decreased in the DAG-infusion group compared with the TAG-infusion group. In the TAG-infusion group, 91.5 ± l.2% of the incorporated radioactivity was detected in triglyceride, whereas 86.2 ±1.4% was detected in triglyceride in the DAG-infusion group (Fig. 3.2). This finding suggests that DAG digestion products were less efficiently synthesized into triglyceride in the intestinal mucosa compared with TAG digestion products.

In contrast to the lower incorporation of the ^{14}C label into the mucosal triglyceride in the DAG-infusion group, a higher proportion of radioactivity was detected in 1,3-DAG, MAG, and FFA in the DAG-infusion group compared with the TAG-infusion group. The radioactivity of 1,3-DAG accounted for 1.1% of the total amount of

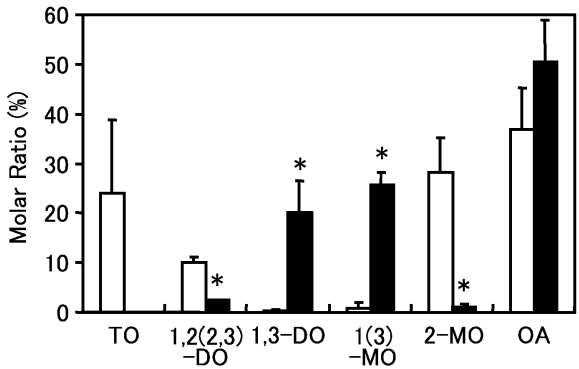

Fig. 3.1. Analysis of digestion products of [carboxyl-^{14}C] trioleoylglycerol (TO) and 1,3-[carboxyl-^{14}C] dioleoylglycerol (DO) in the rat intestinal lumen. Rats were intraduodenally injected with a TAG emulsion containing [carboxyl-^{14}C] TO (□) or a DAG emulsion containing 1,3-[carboxyl-^{14}C] DO (■). MO, monooleoylglycerol; OA, oleic acid. Values are means ± SD; n = 4. *Different from TAG infusion, $P < 0.001$.

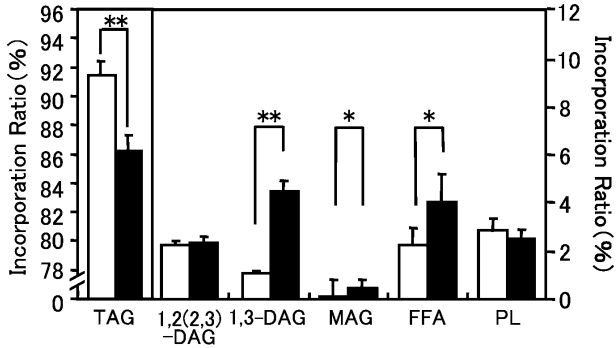

Fig. 3.2. Incorporation of [1-^{14}C] linoleic acid into lipids in rat intestinal epithelial cells after the infusion of DAG or TAG. A lipid emulsion containing 30 mmol/L TAG (□) or 45 mmol/L DAG (■) was infused at a rate of 4.5 mL/h into the duodenum in rats. After 5 h, [1-^{14}C] linoleic acid (2.2×10^6 dpm) was added to the infusion emulsion. At 10 min after the initiation of [1-^{14}C] linoleic acid infusion, the intestinal epithelial cells were collected. The percentages of radioactivity in TAG (triglyceride), DAG, MAG, FFA, and phospholipids (PL) were determined. Values are means ± SD; n = 4, $^*P < 0.01$, $^{**}P < 0.001$.

incorporated radioactivity in the TAG-infusion group, whereas that in the DAG-infusion group accounted for 4.5% (Fig. 3.2), resulting in a 4.4-fold increase in the ratio ($P < 0.001$).

The incorporation of label into MAG was also significantly higher in the DAG-infusion group (DAG infusion: 0.4%; TAG infusion: 0.1%) (Fig. 3.2). The separation of MAG isomers by TLC using boric acid–impregnated plates revealed that 57.3 ± 2.1% of MAG in the mucosal lipids was l(3)-MAG in the DAG-infusion group. However, l(3)-MAG was not detected in the mucosal lipids of the TAG-infusion group.

Moreover, the amounts of labeled glycerides in the mucosal cells were strikingly different between the treatment groups. Compared with an infusion with TAG, a 4.5-fold higher amount of 1,3-DAG accumulated in the intestinal mucosa after a DAG infusion (Fig. 3.3), whereas the amount of 1,2(2,3)-DAG did not differ between the TAG and DAG infusion groups.

To identify the pathway for the synthesis of 1,3-DAG, everted sacs prepared from a rat duodenum were cultured with labeled FFA in the presence or absence of MAG. When the everted intestinal sacs were cultured with [1-^{14}C]linoleic acid in the presence of 1-MO, 1,3-DAG synthesis in the mucosa of the everted sacs accounted for 39.6% of the total DAG synthesis (Fig. 3.4). In contrast, when the everted sacs were incubated in the presence of 2-MO and labeled linoleic acid, mucosal 1,3-DAG synthesis accounted for only 11.0% of the total DAG synthesis. In the absence of MO (a culture with glycerol, oleic acid, and labeled linoleic acid), on the other hand, the synthesis of 1,3-DAG was 2.8% of the total DAG synthesis. This indicates that after the

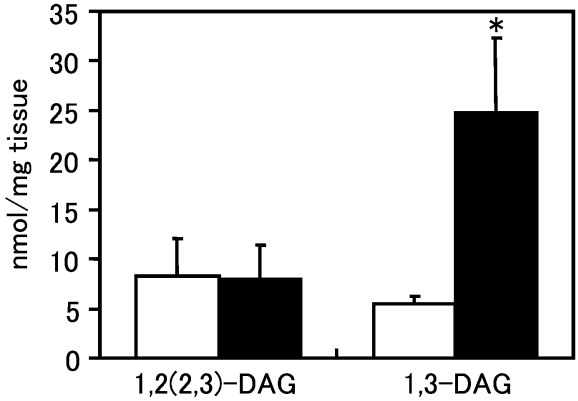

Fig. 3.3. Analysis of the amount of 1,2-DAG or 1,3-DAG in rat intestinal epithelial cells after intraduodenal infusion. Rats were intraduodenally infused with TAG (open bar) or DAG (solid bar) as described in the legend for Figure 3.2. Values are means ± SD; n = 4; *different from TAG infusion, $P < 0.05$.

intraduodenal infusion of DAG, some of the 1(3)-MAG is absorbed from the lumen into the mucosal cells without further hydrolysis and then esterified to 1,3-DAG. A substantial amount of 1,3-DAG was also produced in mucosal cells after TAG infusion (Figs. 3.2, 3.3). The 1,3-DAG might be synthesized via the acylation of 1(3)-MAG generated by the isomerization of 2-MAG.

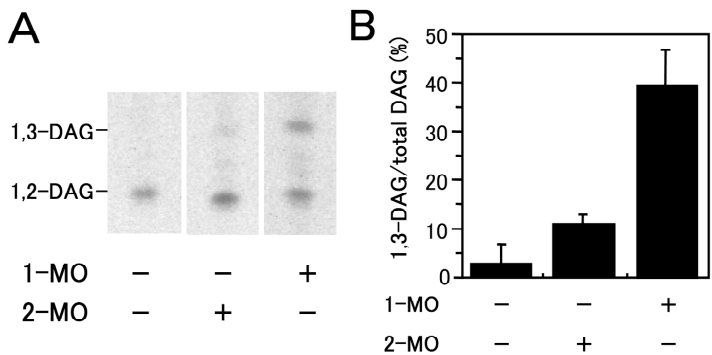

Fig. 3.4. Incorporation of [^{14}C] linoleic acid into 1,3-DAG in epithelial cells of rat intestinal everted sacs. Everted sacs from a rat intestine were incubated with [1-^{14}C] linoleic acid in the presence or the absence of *sn*-1-MO (1-MO) and *sn*-2MO (2-MO). The extracted lipids were fractionated by TLC using chloroform/acetone (95:4) as the developing solvent. A representative TLC pattern is shown in panel A. The percentage of radioactive 1,3-DAG to the total radioactivity of DAG is shown in panel B. Values are means ± SD; n = 3. For abbreviations, see Figure 3.1.

A measurement of diacylglycerol acyltransferase (DGAT) activity in the microsome fraction prepared from intestinal mucosa revealed that the isomeric 1,3-DO was not utilized substantially as a substrate for triglyceride synthesis compared with 1,2-DO (Fig. 3.5). The amount of triglyceride produced in the presence of 1,3-DO was ~10% or less of that in the presence of 1,2-DO.

DGAT is a microsomal enzyme that catalyzes the last step in the synthesis of triglyceride (24,25). The reaction involves the acylation of DAG, which is supplied mainly by the esterification of 2-MAG in the intestine after TAG ingestion. Thus far, two species of DGAT, DGAT-1 and DGAT-2, have been identified, and the small intestine expresses both of these (26,27). The low substrate specificity of mucosal DGAT for 1,3-DAG may be a factor that is responsible for the accumulation of 1,3-DAG and the decrease in triglyceride synthesis in intestinal mucosal cells after a DAG infusion. These findings suggest that the nature by which 1,3-DAG is digested and assimilated in the intestine may be responsible for the reduction in postprandial serum triglyceride levels by dietary DAG.

Effect of DAG Oil Consumption on Absorption of Fat-Soluble Vitamins (Human Study)

Fats are digested in three different and coordinated processes: emulsification, hydrolysis of the substrate, and micellar solubilization of the hydrolysis products in the aqueous medium of the intestinal contents (28). Therefore, the absorption of fat-soluble vitamins and other lipophilic compounds by enterocytes may be influenced by various dietary factors. A decrease in serum 25-hydroxyvitamin D, for example, was reported for humans who ingest large amounts of dietary fiber (29). The type of dietary fat, consisting of hydrolyzable TAG, can also affect the extent of the absorption of cholesterol and, presumably, other lipophilic compounds as well (30). Nonabsorbable

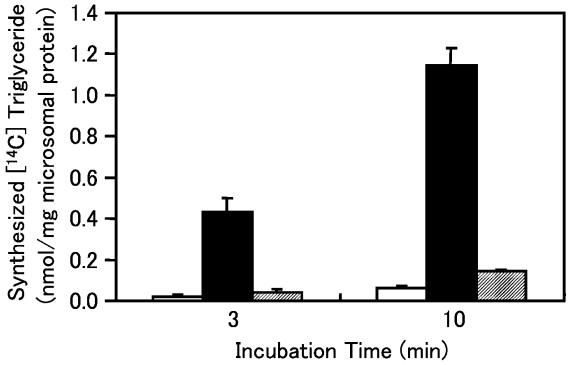

Fig. 3.5. Utilization of diacylglycerol isomers for triglyceride synthesis. The microsomal fraction (0.5 mg/mL) obtained from the rat intestinal mucosa was incubated with *sn*-1,2-DO (solid bar) or 1,3-DO (hatched bar). The open bar represents the control incubated without DO. Values are means ± SD; n = 3. For abbreviations, see Figure 3.1.

lipophilic substances such as sucrose polyesters affect the absorption of fat-soluble molecules (7).

Thus, Watanabe *et al.* (31) examined the issue of whether the DAG oil, when administered over a long period to humans, has an altered effect on the bioavailability of fat-soluble vitamins, compared with the TAG oil. Serum concentrations of fat-soluble vitamins were used as indicators. This study was a parallel, placebo-controlled, double-blind design. Healthy men aged 27–47 y participated in this study.

Subjects were randomly assigned to two groups; one group was given DAG oil (n = 15) and the other group was given TAG oil (n = 12). The dose of the test oil was 20 g/d in mayonnaise or an emulsion drink. The subjects were instructed not to change their daily energy intake or physical activity, to avoid excessive intakes of alcohol and food, and not to take any medication or vitamin supplements during the study. The physical characteristics of the subjects at the beginning of the study are shown in Table 3.4. According to the diet record analysis, no significant differences in dietary intake were observed between the baseline and test periods in either group. All laboratory values remained within the normal ranges during the study. Body weight, waist circumference, hip circumference, and serum parameter changes indicative of test food–related effects were not observed during the study.

At 4, 8, and 12 wk, blood samples were drawn from fasting subjects and serum concentrations of vitamins A, E, and D were determined. Vitamin A was measured as retinol by the method of Thompson *et al.* (32). Vitamin E levels were calculated as the sum of α-, β-, and γ-tocopherol by the method of Abe *et al.* (33). Three species of vitamin D were measured by the modified method of Shepard *et al.* (34).

Changes in serum concentrations of fat-soluble vitamins are shown in Tables 3.5–3.8. Although there were some fluctuations from the initial values during the study period in the serum concentrations of vitamin A, vitamin E, 25-hydroxyvitamin D, and 1-α, 25-dihydroxyvitamin D, differences between the two groups at each point were not significant. From these results, they concluded that DAG has no effect on the absorption of fat-soluble vitamins.

The absorption of fat-soluble vitamins during the digestion of dietary lipids is inhibited by the ingestion of the gastrointestinal lipase inhibitor, orlistat (35), and a noncaloric fat substitute, olestra (36). These observations indicate that the role of edi-

TABLE 3.4
Baseline Characteristics of Study Subjects and Their Responses After the Experiment[a]

	Baseline		12 wk	
n	DAG group 15	TAG group 12	DAG group 15	TAG group 12
Age (y)	33.8 ± 4.5	33.3 ± 5.7		
Weight (kg)	65.6 ± 2.2	66.0 ± 2.6	65.7 ± 2.2	66.5 ± 2.6
Height (cm)	169.4 ± 1.4	171.9 ± 1.3		
Serum triglyceride (mg/dL)	90.8 ± 10.6	79.3 ± 9.2	85.4 ± 11.2	75.0 ± 10.3
Serum total cholesterol (mg/dL)	188.4 ± 6.7	184.6 ± 8.4	184.6 ± 8.4	184.8 ± 6.7

[a]Values are means ± SE.

TABLE 3.5
Mean Serum Concentration of Vitamin A During the Experiment[a]

					P^b		
	0 wk	4 wk	8 wk	12 wk	Lipid	Time	Interaction
DAG	665 ± 105	629 ± 77	640 ± 88	630 ± 92			
Group	(100)	(95.6 ± 12.2)	(97.5 ± 15.1)	(95.7 ± 14.2)			
TAG	641 ± 117	606 ± 161	616 ± 130	609 ± 180	0.679	0.332	1.000
Group	(100)	(94.2 ± 16.8)	(96.9 ± 16.8)	(94.3 ± 19.4)			

[a]Values are means ± SE (ng/mL serum); n = 15 for DAG and 12 for TAG. The percentage of the initial value is given in parentheses.
[b]*P*-values are for main effects and interactions by a two-way ANOVA between two groups.

TABLE 3.6
Mean Serum Concentration of Vitamin E During the Experiment[a]

					P^b		
	0 wk	4 wk	8 wk	12 wk	Lipid	Time	Interaction
DAG	1.27 ± 0.26	1.10 ± 0.15**	1.13 ± 0.20**	1.26 ± 0.18			
Group	(100)	(87.9 ± 9.3)	(89.8 ± 12.7)	(100.4 ± 11.7)			
TAG	1.21 ± 0.24	1.15 ± 0.30*	1.10 ± 0.29**	1.17 ± 0.22	0.155	0.619	0.759
Group	(100)	(94.5 ± 11.0)	(90.5 ± 12.4)	(97.2 ± 7.5)			

[a]Values are means ± SE (mg/100 mL serum); n = 15 for DAG and 12 for TAG. The percentage of the initial value is given in parentheses. Asterisks indicate different from wk 0: $*P < 0.05$, $**P < 0.01$.
[b]*P*-values are for main effects and interactions by a two-way ANOVA between two groups.

TABLE 3.7
Mean Serum Concentration of 25-Hydroxyvitamin D During the Experiment[a]

					P^b		
	0 wk	4 wk	8 wk	12 wk	Lipid	Time	Interaction
DAG	27.4 ± 8.7	29.4 ± 6.6	30.8 ± 6.9	26.8 ± 7.9			
Group	(100)	(115.5 ± 43.0)	(123.4 ± 55.7)	(106.2 ± 50.6)			
TAG	24.4 ± 7.1	26.5 ± 4.8	29.0 ± 6.2*	24.8 ± 4.5	0.090	0.072	0.986
Group	(100)	(113.0 ± 24.7)	(125.2 ± 42.9)	(105.6 ± 24.2)			

[a]Values are means ± SE (ng/mL serum); n = 15 for DAG and 12 for TAG. The percentage of the initial value is given in parentheses. *Different from wk 0, $P < 0.05$.
[b]*P*-values are for main effects and interactions by a two-way ANOVA between two groups.

ble oils in the absorption of fat-soluble vitamins is very important as a nutritional element, and DAG, in contrast to these lipid metabolism modifying agents, was shown not to affect the absorption of fat-soluble vitamins.

Faster Gastric Emptying of Food Containing DAG Oil (Human Study)

Before the health benefits of DAG were discovered in the early stages of the study of dietary DAG, information was available indicating that DAG had a light taste, was

TABLE 3.8
Mean Serum Concentration of 1-α, 25-Dihydroxyvitamin D During the Experiment[a]

					P^b		
	0 wk	4 wk	8 wk	12 wk	Lipid	Time	Interaction
DAG	30.9 ± 7.7	29.8 ± 7.6	41.0 ± 6.6**	34.9 ± 7.3			
Group	(100)	(100.7 ± 34.0)	(141.3 ± 45.7)	(120.9 ± 48.0)			
TAG	30.9 ± 8.4	30.6 ± 9.6	44.4 ± 9.1**	40.9 ± 9.1	0.100	0.109	0.523
Group	(100)	(102.9 ± 34.4)	(150.7 ± 46.5)	(139.5 ± 44.6)			

[a]Values are means ± SE (pg/mL serum); n = 15 for DAG and 12 for TAG. The percentage of the initial value is given in parentheses. **Different from wk 0, $P < 0.01$.
[b]*P*-values are for main effects and interactions by a two-way ANOVA.

less heavy on the stomach, and had a less oily texture. Through these sensory evaluations, we hypothesized that the gastric emptying time of DAG oil might be shorter than that of TAG oil. To test this hypothesis, Yasunaga *et al.* (37) measured the halftime for gastric emptying of the ingested test food cooked with DAG or TAG oil.

This study was a double-blind and crossover trial with six subjects (5 men and 1 woman, 28–34 y old). All subjects were healthy with no history of gastrointestinal tract disease and were not taking any medications. They fasted for 4 h before starting the experiments and ingested the test food within 5 min. The test food was a scrambled egg containing 50 g of whole egg and test oil and a loaf of bread. The dose of test oil was 30 g/60 kg body weight. The DAG oil contained ~88% DAG by weight and the remainder was TAG with a trace of monoglycerides and sterols. The TAG oil was prepared by mixing rapeseed and safflower oil so that the fatty acid composition was similar to that of DAG oil. The control test food was prepared without adding the test oil. To measure gastric emptying time, technetium 99 human macroaggregated albumin (Tc-99 MAA) was incorporated into the scrambled egg so as to locate by a scintigram the position of ingested food in the gastrointestinal tract. Immediately after ingestion, the subjects reclined on their backs in a bed with a scintillation detector for 180 min. The scintigram was taken from both forward and backward of the gastric region at 10-min intervals.

The radioactivity in the stomach compensated for the disintegration of technetium, whose half-life is 6.01 h. The time course for gastric emptying after the ingestion of the test food is shown in Figure 3.6. All of the ingested radioactivity was detected in the stomach during the initial 30 min after ingestion. Approximately 40 min after ingestion, the test food passed into the duodenum, and the amount of food in the stomach decreased linearly for a period of up to 3 h. The control food (no oil) passed most rapidly from the stomach. The amount of food with DAG oil remaining in the stomach was significantly less than that of TAG oil at several time points, suggesting that the food with DAG oil was emptied from the stomach faster than that of TAG oil. The average halftime ($t_{1/2}$) of gastric emptying of the ingested food was calculated. The halftime values for DAG oil, TAG oil, and the control were 125, 155, and 98 min, respectively. The halftime of the DAG-containing food was significantly less than that of TAG-containing food ($P < 0.05$).

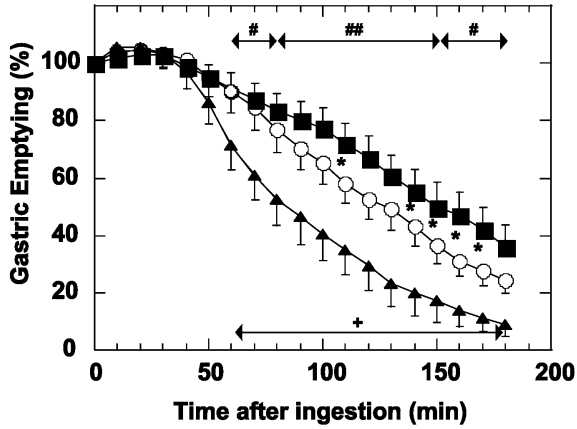

Fig. 3.6. Time course for gastric emptying after the ingestion of test foods with DAG oil (○), TAG oil (■) and control food without oil (▲). Gastric emptying was measured by a scintigram using 99Tc human macroaggregated albumin incorporated into the test food. Each value was expressed as percent of initial value and compensated for disintegration of Tc. *Significant difference between DAG oil and TAG oil ($P < 0.05$); #significant difference between TAG oil and Control ($\#P < 0.05$; $\#\#P < 0.01$); +significant difference between DAG oil and Control ($+P < 0.05$).

The complaint of heaviness on the stomach was well correlated with the slow gastric emptying time, indicating that the subjects with shorter gastric emptying time had fewer gastric complaints after ingestion of the test food. This suggests that the gastric emptying time is related to the sensation of satiety and supports, at least in part, the hypothesis that DAG oil is light in the stomach because of its faster gastric emptying.

Summary

In this chapter, we discussed the characteristics of DAG in terms of energy value, absorption coefficient, digestion products, effect on the absorption of fat-soluble vitamins, and stomach-emptying time compared with those of TAG. The energy value of DAG oil containing 87 wt% DAG and TAG oil with a similar fatty acid composition was 38.9 kJ/g (9.29 kcal/g) and 39.6 kJ/g (9.46 kcal/g), respectively. The difference was <2%. Therefore, the difference in the energy value in the practical diet was considered to be negligible, and the nutritional differences between DAG oil and TAG oil appear to reside in the metabolic fate of DAG after absorption by enterocytes. DAG oil and TAG oil did not differ in other features including apparent absorption coefficients and the effect on fat-soluble vitamin status. However, the digestion products of DAG oil, containing mainly 1,3-DAG, were distinct from those of TAG oil with regard to 1-MAG production as a major intermediate during the process of DAG digestion. These different metabolic processes after the ingestion of 1,3-DAG oil in

enterocytes may be one of the mechanisms responsible for the suppressed postprandial increase in triglyceride-rich lipoproteins and for the suppressed body fat accumulation as a long-term effect compared with TAG ingestion. In addition, faster gastric emptying time was another characteristic feature of DAG oil. Although this effect may be related to the sensory evaluation that DAG is light in the stomach, the mechanism for this is not known at present.

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