Metabolites of Dietary Triacylglycerol and Diacylglycerol During the Digestion Process in Rats

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ABSTRACT: The present study investigated the metabolic fate of dietary TAG and DAG and also their digestion products in the stomach and small intestine. A diet containing 10% TAG or DAG oil, enriched in 1,3-DAG, was fed to Wistar rats *ad libitum* for 9 d. After 18 h of fasting, each diet was re-fed *ad libitum* for 1 h. The weights of the contents of the stomach and small intestine were measured, and the acylglycerol and FFA levels were analyzed by GC at 0, 1, and 4 h after the 1-h re-feeding. The amounts of re-fed diet ingested and the gastric and small intestinal content were not different between the two diet groups. In the TAG diet group, the main products were TAG and DAG, especially 1(3),2-DAG. In addition, 1,3-DAG and 1(3)-MAG were present in the stomach, and the 1,3-DAG levels increased over time after the re-feeding period. In the DAG diet group, the main products in the stomach were DAG, MAG, FFA, and TAG. There were significantly greater amounts of 1,3-DAG, 1(3)-MAG, and FFA in the DAG diet group in the stomach compared with the TAG diet group. The amount of FFA in the stomach relative to the amount of ingested TAG plus DAG in the DAG diet group was higher than that in the TAG diet group. Acylglycerol and FFA levels were considerably lower in the small intestine than in the stomach. These results indicate that, in the stomach, where acyl migration might occur, the digestion products were already different between TAG and DAG oil ingestion, and that DAG might be more readily digested by lingual lipase compared with TAG. Furthermore, almost all of the dietary lipid was absorbed, irrespective of the structure of the acylglycerol present in the small intestine.

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Although the majority of the digestion of dietary lipids is completed in the small intestine, the digestion starts in the stomach. From 10 to 30% of ingested lipid is digested in the stomach by gastric lipase, and, as a result, gastric lipid digestion has an important role in humans (1,2), especially in neonates and in patients with cystic fibrosis or pancreatitis (3–6). In rats, the digestion of lipids in the stomach is catalyzed by lingual lipase (7–11), which has considerable homology with the amino acid sequence of human gastric lipase (12,13). This enzyme preferentially cleaves the ester bonds at the *sn*-3 position relative to the *sn*-1 position under acidic conditions (pH 3.0 to 6.5) (7,8). Hamosh *et al.* (11) measured gastric TAG levels after feeding a diet containing corn oil to rats *ad libitum* for 10 or 20 min. They reported that approximately 20 and 29% of the ingested TAG was digested after 10- and 20-min feeding periods, respectively. Lai and Ney (14) reported that the gastric TAG levels were approximately 38 to 50%, 12 to 17%, and 2 to 4% of the ingested TAG at 2, 5, and 9 h, respectively, after a diet containing corn oil or palm oil was fed to rats. In these studies, only changes in the ingested TAG levels were determined, and changes in makeup of digestion products (DAG, MAG, and FFA) were not investigated. Differences in the rate and extent of digestion of lipids by gastric and pancreatic lipases depend on the nature of the FA present. Medium-chain TAG that contain FA such as octanoic and decanoic acids were reported to be digested more rapidly than long-chain TAG (15). However, differences in the digestion of lipids with respect to the number and position of FA attached to the glycerol backbone have not been examined extensively.

TAG constitutes the greater portion of dietary lipids of animal and vegetable origin as a high energy source, whereas DAG, a type of natural structured lipid contained in many edible oils and fats, makes up a few to approximately 10% of the dietary lipids from animals and vegetables (16,17). A dietary DAG oil rich in 1,3-DAG decreases serum TAG levels (18,19), prevents postprandial hyperlipidemia (20–22), and suppresses visceral fat accumulation (23–26) in animals and humans in comparison with TAG oil with a similar FA composition. Taguchi *et al.* (27) also reported that the absorption coefficient of DAG oil was 96%, similar to that of TAG oil. However, a detailed understanding of the digestion products of DAG oil compared with TAG oil is limited.

In this study, we administered a diet containing TAG or DAG oil with a similar FA composition to rats and investigated the metabolic features of the dietary lipids, which differed in structure, by analyzing the digestion products in the stomach and small intestine, the main locations where digestion occurs.

EXPERIMENTAL PROCEDURES

Test oil. TAG oil was prepared by mixing rapeseed oil, safflower oil, and perilla oil to conform to the FA composition of DAG oil. DAG oil was prepared by esterifying glycerol with FFA from rapeseed oil and soybean oil by the method of

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Abbreviation: HSL, hormone-sensitive lipase.

TABLE 1
FA and Acylglycerol Compositions of TAG Oil and DAG Oil

Component	TAG	DAG
FA	g/100 g total FA	
14:0	0.1	0
16:0	5.4	3.1
16:1	0.2	0
18:0	2.0	1.2
18:1	37.1	39.2
18:2	46.0	47.5
18:3	7.3	8.6
20:0	0.5	0.2
20:1	0.9	0.2
22:0	0.2	0
22:1	0.1	0
Acylglycerols	g/100 g oil	
TAG	97.7	13.8
DAG	2.3	85.8
1(3),2-DAG	1.1	27.9
1,3-DAG	1.2	57.9
MAG	0	0.4

Huge-Jensen *et al*. (28). The FA and acylglycerol composition of each oil, as analyzed by GC, is shown in Table 1. The FA composition of the TAG oil was very similar to that of the DAG oil. The DAG concentration of the DAG oil was 85.8 g/100 g and the ratio of 1(3),2- to 1,3-DAG was 32.5:67.5.

Diet. The test diet contained 10 g/100 g of either TAG or DAG oil. Ingredients other than the test oil were casein (20 g/100 g), cellulose (4 g/100 g), AIN-76 mineral mixture (29) (4 g/100 g), AIN-76 vitamin mixture (29) (1 g/100 g), and potato starch (61 g/100 g). Each diet was prepared in one batch for the entire experimental period and was stored at 5°C.

Animals and experimental design. Male Wistar rats (7 wk old, 189 ± 7.8 g), obtained from CLEA Japan (Tokyo, Japan), were housed in metal cages and had free access to commercial rodent diet CE-2 (CLEA Japan) and drinking water. They were maintained in a temperature-controlled environment (23 ± 2°C) under a 12-h light/dark cycle. After a 7-d acclimatization period, they were divided into two groups so that the body weight of each group was approximately equal and transferred to individual metabolic cages. The rats in one group were fed the TAG oil diet, and the others were fed the DAG oil diet (TAG diet group and DAG diet group, respectively) for 9 d to accustom the animals to eating each test diet. Food intake was recorded every 3 or 4 d. The body weights of all rats were recorded at day 1 and 9. After ingestion of the test diets for 9 d, the rats were fasted for 18 h and then re-fed each diet *ad libitum* for 1 h. At the defined time points, 0, 1, and 4 h after refeeding the test diets, the rats were anesthetized with diethyl ether and killed by withdrawing blood from the abdominal aorta. The stomach and small intestine (60 cm from the pylorus) were removed and washed three times with 5 mL of ice-cold 150 mM NaCl, respectively. Each content was mixed with 15 mL of ethanol and stored at −70°C until measurement. The groups that were killed at 0, 1, and 4 h after re-feeding of the test diets are referred to hereafter as the 0 h, 1 h, and 4 h groups, respectively. The group that was not re-fed is referred to as the fasting group. The animal experiments were performed with the approval of the Ethics Committee for Experimental Animals of the Kao Corporation.

Lipid extraction. The lipids were extracted from aliquots of the freeze-dried gastric and small intestinal contents homogenized using a mortar, and a chloroform solution of pentadecanoic acid methyl ester (Sigma Chemical Co., St. Louis, MO), as an internal standard, was added using a modification of the method of Folch *et al.* (30).

Lipids analysis by GC. The lipid contents of the extracted total lipids were analyzed by GC. The lipids were purified using Sep-Pak silica (Waters, Milford, MA). The lipids were silylated with trimethylchlorosilane TMSI-H (GL Science, Tokyo, Japan) by a modification of the American Oil Chemists' Society official method (31) and the method of Taguchi *et al.* (27). The trimethylsilyl esters dissolved in hexane were separated on a GC-18A gas chromatograph (Shimadzu, Kyoto, Japan) connected to a FID and fitted with a DB-1 capillary column (15 m × 0.25 mm × 0.1 μm; J&W Scientific, Folsom, CA). Operating conditions were: initial temperature, 80°C; rate of temperature increase, 10°C/min; final temperature, 335°C (held for 44.5 min); injection and detector temperature, 350°C; carrier gas, helium at 1.78 mL/min. Peak detection was performed using a GC work station CLASS-GC 10 (Shimadzu) programmed for peak identification.

Materials for GC. Palmitic, stearic, oleic, linoleic, and linolenic acids as the FFA standards, 1-monooleoyl-glycerol as the 1(3)-MAG standard, and 2-monooleoyl-glycerol as the 2-MAG standard were purchased from Sigma Chemical Co. 1-Oleoyl-2-palmitoyl-glycerol and 1-oleoyl-3-palmitoyl-glycerol were purchased from Funakoshi (Tokyo, Japan). 1,2-Dioleoyl-glycerol and 1,3-dioleoyl-glycerol were purchased from Sigma Chemical Co. 1-Oleoyl-2-palmitoyl-glycerol and 1,2-dioleoyl-glycerol were used as the 1(3),2-DAG standards, 1-oleoyl-3-palmitoyl-glycerol and 1,3-dioleoyl-glycerol were used as the 1,3-DAG standards. 1,3-Dioleoyl-2-palmitoyl-glycerol, from Sigma Chemical Co, and trioleoyl-glycerol, from Wako (Osaka, Japan) were used as the TAG standards.

Statistical analyses. Data are expressed as the means ± SD. Statistical significance of the differences ($P < 0.05$) between the two diet groups were determined by Student's *t*-test and a two-way ANOVA using StatView for Windows version 5.0 (SAS Institute, Inc., Cary, NC).

RESULTS AND DISCUSSION

Food intake and body weight. The amounts of food intake in the TAG and DAG diet groups for 9 d were 152.1 ± 9.2 and 147.7 ± 10 g/rat, respectively. Body weights of the fasting, 0, 1, and 4 h groups in the TAG diet group after ingestion for 9 d were 234.2 ± 10.2, 247.6 ± 7.5, 236.7 ± 9.7, and 248.5 ± 6.5g, respectively. The values for the fasting, 0, 1, and 4 h groups in the DAG diet group after ingestion for 9 d were 234.2 ± 8.9, 240.8 ± 6.4, 235.9 ± 12.8, and 244.5 ± 11.5 g, respectively. The

TABLE 2
Amounts of Gastric and Small Intestinal Contents[a] (dry wt)

Tissue	Test group	TAG diet group		DAG diet group	
		(g/stomach)	(%)[b]	(g/stomach)	(%)
	Fasting	0.16 ± 0.12		0.15 ± 0.04	
Stomach	0 h	3.81 ± 1.24	(87.8 ± 7.8)	4.43 ± 1.60	(87.7 ± 18.9)
	1 h	2.77 ± 1.81	(63.3 ± 12.9)	2.74 ± 1.06	(64.0 ± 17.2)
	4 h	1.82 ± 0.82	(34.1 ± 9.3)	1.53 ± 0.71	(34.1 ± 4.8)
		(g/intestine)	(%)	(g/intestine)	(%)
	Fasting	0.16 ± 0.12		0.15 ± 0.04	
Small intestine	0 h	0.22 ± 0.03	(5.4 ± 2.2)	0.20 ± 0.05	(4.1 ± 1.5)
	1 h	0.27 ± 0.13	(7.1 ± 3.2)	0.28 ± 0.07	(7.1 ± 1.6)
	4 h	0.22 ± 0.07	(4.5 ± 1.7)	0.21 ± 0.04	(4.2 ± 1.1)

[a]Values are the mean ± SD (n = 7).
[b]Mean ± SD are shown as percentages of the amounts of ingested test diet for 1 h.

amounts of food intake during the re-feeding period (for 1 h) of the 0, 1, and 4 h groups in the TAG diet group after 18 h of fasting were 4.3 ± 1.2, 4.1 ± 1.9, and 4.7 ± 1.2 g, respectively. The values for 0, 1, and 4 h groups in the DAG diet group after 18 h of fasting were 5.0 ± 1.4, 4.1 ± 1.1, and 5.0 ± 1.3 g, respectively. There were no differences at any point between the two diet groups in the amounts of food consumed for 9 d, body weights, and amounts of food intake during the re-feeding period . All rats remained healthy during the study period.

Gastric/intestinal contents. The dried gastric and small intestinal (60 cm from the pylorus) contents at 0, 1, and 4 h after the re-feeding period in the TAG and DAG diet groups and the fasting group are shown in Table 2. The gastric contents decreased over time after the re-feeding period in both the TAG and DAG diet groups. At 4 h after the re-feeding period, the gastric contents in the TAG and DAG diet groups were 34.1 ± 9.3 and 34.1 ± 4.8% of the weight of the re-fed diet, respectively. In the two diet groups, the small intestinal contents were highest at 1 h after the re-feeding period. At 1 h after the re-feeding period, the small intestinal contents in the TAG and DAG diet groups were 7.1 ± 3.2 and 7.1 ± 1.6% of the weight of the re-fed diet, respectively. There were no differences in the gastric and small intestinal contents between the two diet groups.

Acylglycerols and FFA in the gastric content. Acylglycerol and FFA levels in the gastric contents of the TAG and DAG diet groups are shown in Table 3. The gastric contents in the fasting group were small, and there were no differences in the acylglycerol and FFA levels between the two diet groups.

In the TAG diet group, the main products were TAG and DAG, especially 1(3),2-DAG. The TAG and 1(3),2-DAG levels decreased over time after the re-feeding period, whereas the 1,3-DAG level increased over time after the re-feeding period. The 1(3)-MAG, 2-MAG, and FFA levels were highest at 1 h after the re-feeding period. The TAG intakes, which were calculated from the food intake and the TAG content in the TAG

TABLE 3
Amounts of Acylglycerols and FFA in the Stomach[a] (mg/gastric content)

	Test group	TAG[b]	1(3),2-DAG[c]	1,3-DAG[d]	1(3)-MAG[e]	2-MAG[f]	FFA[g]	FFA/ingested TAG + DAG (%)[h]
	Fasting	0.06 ± 0.12	0.00 ± 0.01	ND	0.01 ± 0.03	0.07 ± 0.17	0.05 ± 0.14	—
TAG diet	0 h	218.85 ± 97.38	51.98 ± 14.58	0.54 ± 0.70	0.63 ± 0.83	4.15 ± 2.41	12.09 ± 5.49	2.72 ± 1.05
group	1 h	154.89 ± 73.09	46.86 ± 24.49	8.71 ± 6.94	1.22 ± 0.83	4.38 ± 2.41	20.07 ± 11.38	4.44 ± 1.60
	4 h	154.85 ± 50.94	32.53 ± 9.86	16.89 ± 8.71	0.90 ± 0.50	0.92 ± 1.08	13.84 ± 5.30	2.46 ± 0.69
	Fasting	0.14 ± 0.20	ND	ND	0.06 ± 0.16	0.04 ± 0.08	0.18 ± 0.47	—
DAG diet	0 h	67.94 ± 49.18**	78.58 ± 39.05	127.48 ± 59.02**	16.78 ± 7.88**	6.99 ± 4.03	26.19 ± 22.85	4.62 ± 2.96
group	1 h	42.45 ± 17.76**	49.49 ± 15.81	85.33 ± 33.11***	11.24 ± 3.75***	5.97 ± 3.22	23.70 ± 7.96	5.57 ± 1.02
	4 h	28.67 ± 6.85***	44.39 ± 13.01	71.87 ± 17.35**	9.25 ± 3.09**	4.19 ± 1.88*	20.12 ± 4.56*	3.64 ± 0.65**
	Diet	<0.0001	NS	<0.0001	<0.0001	=0.0037	=0.0251	=0.017
Two-way	Time	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	=0.028
ANOVA	Diet × time	=0.0014	NS	<0.0001	<0.0001	NS	NS	NS

[a]Values are means ± SD (n = 7). ND, not determined; NS, not significant. Significantly different from TAG diet group: *P < 0.05; **P < 0.01; ***P < 0.001.
[b]TAG standard.
[c]1(3),2-DAG standard.
[d]1,3-DAG standard.
[e]1(3)-MAG standard.
[f]2-MAG standard.
[g]FFA standard.
[h]Gastric FFA contents relative to the amount of ingested fat (sum of TAG + DAG).

TABLE 4
Amounts of Acylglycerols and FFA in the Small Intestine[a] (mg/small intestinal content)

	Test group	TAG[b]	1(3),2-DAG[c]	1,3-DAG[d]	1(3)-MAG[e]	2-MAG[f]	FFA
	Fasting	ND	0.01 ± 0.02	ND	ND	0.01 ± 0.02	0.16 ± 0.24
TAG diet	0 h	0.26 ± 0.24	0.25 ± 0.11	0.14 ± 0.09	ND	0.07 ± 0.06	0.75 ± 1.17
group	1 h	0.30 ± 0.44	0.15 ± 0.15	0.03 ± 0.06	ND	0.15 ± 0.10	0.52 ± 0.53
	4 h	0.04 ± 0.07	0.09 ± 0.13	0.03 ± 0.04	ND	0.04 ± 0.06	1.33 ± 1.57
	Fasting	ND	0.02 ± 0.03	ND	ND	0.02 ± 0.03	0.06 ± 0.15
DAG diet	0 h	1.11 ± 0.60*	0.29 ± 0.08	0.18 ± 0.08	0.03 ± 0.02	0.06 ± 0.04	1.78 ± 1.64
group	1 h	0.48 ± 0.60	0.20 ± 0.19	0.13 ± 0.19	ND	0.06 ± 0.06	0.73 ± 0.70
	4 h	0.10 ± 0.10	0.10 ± 0.05	0.01 ± 0.02	ND	ND	1.06 ± 0.92
	Diet	= 0.0047	NS	NS	NS	NS	NS
Two-way	Time	<0.0001	<0.0001	<0.0001	NS	= 0.0013	= 0.0135
ANOVA	Diet × time	= 0.0132	NS	NS	NS	NS	NS

[a]For footnotes see Table 3.

diet, were 452 ± 126, 430 ± 199, and 540 ± 110 mg/rat in the 0, 1, and 4 h groups, respectively. The TAG levels in the gastric contents in the TAG diet group were 219 ± 97, 155 ± 73, and 155 ± 51 mg/rat in the 0, 1, and 4 h groups, amounting to 47 ± 9, 36 ± 5, and 28 ± 6% of the TAG intakes, respectively. Lai and Ney (14) reported that the gastric TAG levels were approximately 38 to 50% and 12 to 17% of the TAG intakes at 2 and 5 h, respectively, after a diet containing 16% corn oil was fed to rats. Therefore, the results of the present study were consistent with those of Lai and Ney. TAG is digested into DAG, MAG, or FFA by lingual lipase or gastric lipase under acidic conditions (pH 3.0–6.5) (1,2,11). The main products of digestion of TAG in the stomach include 1,2-DAG and 2-MAG, since these lipases preferentially cleave the ester bond at the *sn*-3 position relative to the *sn*-1 position (7,8). In the present study, DAG and MAG, detected in the stomach of the TAG diet group were mainly 1(3),2-DAG and 2-MAG, as has been previously reported (8); in addition, 1,3-DAG and 1(3)-MAG digestion products were present. It is noteworthy that the 1,3-DAG levels increased over time after the re-feeding period. This finding raises the possibility that 1(3),2-DAG, a digestion product of TAG, might have been converted to 1,3-DAG in the stomach under acidic conditions as the result of acyl migration, as heat and acid treatment promotes the migration of acyl groups in the DAG molecule (32). Meanwhile, 1(3)-MAG might be produced as a digestion product of 1,3-DAG.

In the DAG diet group, the main products were DAG, MAG, FFA, and TAG. The TAG, 1(3),2-DAG, 1,3-DAG, 1(3)-MAG, 2-MAG, and FFA levels decreased over time after the re-feeding period. There were no differences in the 1(3),2-DAG level in the gastric contents between the TAG diet group and the DAG diet group. The 1,3-DAG and 1(3)-MAG levels in the DAG diet group were higher than those in the TAG diet group. The levels of 2-MAG and FFA, and the gastric FFA contents relative to the amount of ingested fat (TAG plus DAG) in the DAG diet group were higher than those in the TAG diet group. Murase *et al*. (25) and Kondo *et al*. (33) reported that the levels of 1(3)-MAG and FFA, as digestion products in the small intestinal lumen after the administration of a 1,3-[carboxyl-^{14}C]DAG emulsion into the small intestine, were higher than those after the administration of a [carboxyl-^{14}C]TAG emulsion. The data presented herein confirm 1(3)-MAG and FFA as metabolic features of DAG oil, also in the stomach, in the case of rats fed a DAG diet. We hypothesize that the 1(3)-MAG levels in the DAG diet group were higher than those in the TAG diet group because an ester bond in 1,3-DAG, a major constituent of the DAG diet, was cleaved at the *sn*-3 position by lingual lipase, as was demonstrated for TAG (7,8). The FFA levels and the gastric FFA contents relative to the amount of ingested fat [FFA/ingested TAG plus DAG (%)] in the DAG diet group were higher than those in the TAG diet group. These findings indicate that DAG might be more readily digested by lipase compared with TAG in the stomach. The rate constant (k_1) in the first step, in which TAG is digested into 1(3),2-DAG and FFA by pancreatic lipase, was lower than that (k_2) in the second step, in which 1(3),2-DAG is digested into 2-MAG and FFA, suggesting that pancreatic lipase more markedly promotes digestion of DAG (34). In adipose tissue, hormone-sensitive lipase (HSL) has a high affinity for DAG compared with TAG (35). To our knowledge, there are no analogous reports of differences between lingual and gastric lipase; however, the results reported herein suggest that lingual and gastric lipase promote the preferential digestion of DAG rather than the digestion of TAG, as demonstrated for pancreatic lipase and HSL.

Acylglycerols and FFA in the small intestinal content. The acylglycerol and FFA levels in the small intestinal contents of the TAG and DAG diet groups are shown in Table 4. In the fasting group, there were no differences between the two diet groups in the acylglycerol and FFA levels in the small intestinal contents. Nor were there differences in the 1(3),2-DAG, 1,3-DAG, 1(3)-MAG, 2-MAG, and FFA levels between the two diet groups. The TAG level in the DAG diet group was significantly higher than that in the TAG diet group at 0 h after the re-feeding period; however, there were no differences at 1 and 4 h after the re-feeding period. As just above, DAG might be more readily digested by lipase compared with TAG in the stomach. Thus, in the DAG diet group, the digestion of TAG in the presence of DAG might be delayed compared with DAG digestion, and the TAG content in the small intestine might be transiently higher than that in the TAG diet group. In both the TAG and DAG diet groups, the acylglycerol and FFA levels in the small intestine were markedly lower than those in the stomach. The amounts of

acylglycerols plus FFA in the small intestinal contents were 0.4% or less of the amounts of ingested TAG or DAG oil. Lai and Ney (14) reported that the amount of TAG in the small intestinal contents was less than 0.7% of the total amount of ingested TAG oil. Therefore, the results of the present study are consistent with those reported by Lai and Ney. Our results also demonstrated that both TAG and DAG oils are nearly completely digested and absorbed in the small intestine.

Our results suggest that, in the stomach of the rat, acyl migration from 1,2-DAG to 1,3-DAG may occur and the digestion products of TAG and DAG oil ingestion are different. Moreover, DAG oil is more readily digested than TAG oil. Therefore, DAG oil might be useful for neonates with underdeveloped digestion and absorption, and patients with cystic fibrosis or pancreatitis.

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