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Effects of diacylglycerol administration on serum triacylglycerol in a patient homozygous for complete lipoprotein lipase deletion

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Abstract

We investigated postprandial and long-term effects of dietary diacylglycerol (DAG) on serum triacylglycerol (TAG) levels in a 34-year-old man homozygous for complete lipoprotein lipase deletion (LPL deletion). In study 1, Three different oils (DAG, TAG, or medium-chain fatty acid TAG [MCT]) were ingested to examine differences in the postprandial serum TAG response. Postprandial serum TAG levels after DAG oil ingestion were lower than those after TAG oil ingestion and similar to those after MCT oil ingestion. In study 2, the patient was allowed to ingest ordinary cooking oil for 2 months and then DAG oil (containing 80% DAG; target, 20 g/d) for the next 3 months. During the test period, serum TAG levels were measured and dietary evaluations were performed every month. The patient was provided with dietary instruction and consultation at each clinical visit. Serum TAG levels were 1939 to 2525 mg/dL when he used ordinary cooking oil, 1926 to 1173 mg/dL when he used ordinary cooking oil together with DAG oil, and 749 mg/dL when he used DAG oil alone. The TAG intake decreased from 86.9 to 43.0 g and the DAG intake increased from 0.9 to 12.4 g during the study period. Subsequently, 45 g DAG oil (equivalent to 36 g DAG) per day was consumed, and the serum TAG level increased to 2195 mg/dL. Although there was a positive correlation between the TAG intake and serum TAG levels during the period of DAG oil use ($P < .01$, $y = 33.7x - 583.1$), there was no such correlation between DAG oil intake and serum TAG levels. These results suggested that substitution of 12.0 g/d DAG (equivalent to 15 g DAG oil) for TAG oil had the same effect as reducing TAG oil consumption for controlling the serum TAG levels in an LPL-depleted patient with hypertriglyceridemia. In conclusion, the results of study 1 and study 2 demonstrate that DAG oil might be replaced by MCT oil as cooking oil for those with LPL deletion.

1. Introduction

Chylomicron triacylglycerol (TAG) is degraded and metabolized by lipoprotein lipase (LPL) in peripheral tissues [1]. In LPL deficiency, lack of this metabolism results in severe hyperchylomicronemia and hypertriglyceridemia often accompanied by severe hypercholesterolemia. This disease of the LPL gene, caused by autosomal dysgenic heredity, is rare with a frequency of 1 per million [2]. LPL deficiency, which is usually detected in childhood, is accompanied by splenohepatomegaly, xanthoma, and attacks of abdominal pain caused by pancreatitis. Although the relation between the serum TAG level and pancreatitis is unclear, the incidence of pancreatitis increases if the serum TAG level is higher than 1000 mg/dL and becomes almost 100% at a serum TAG level higher than 4000 mg/dL; however, it is almost 0% at a serum TAG level of less than 1000 mg/dL [3]. Therefore, it is necessary to maintain serum TAG levels of less than 1000 mg/dL to prevent pancreatitis. Fredrickson and Lees proposed a method to keep the daily intake of fat below 20 to 25 g for this purpose [2].

It is difficult, however, to consume ordinary meals with such a strict dietary control of fat content. Serum TAG levels are markedly reduced by substituting medium-chain fatty acid TAG (MCT) for long-chain fatty acid TAG [4] because MCT is absorbed without being reesterified to TAG. MCT is currently the only cooking oil available for patients with LPL deletion. Long-term use of MCT,

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Table 1
Fatty acid composition of test oils

Fatty acids	% by weight		
	DAG	TAG	MCT
C6:0	–	–	0.3
C8:0	–	–	81.6
C10:0	–	–	17.4
C12:0	–	–	0.4
C14:0	–	–	–
C16:0	5.68	3.16	–
C18:0	2.23	1.27	–
C18:1	35.67	37.49	–
C18:2	46.65	48.27	–
C18:3	6.94	6.38	–

however, might cause an insufficient intake of essential fatty acids, which are indispensable for growth [5]. In addition, MCT cooking oil has drawbacks such as fuming and bubbling when cooking at temperatures higher than 180°C [6] and a residual taste of coconut remains when meals are cooled [7]. There are also problems with MCT cooking oil concerning availability and cost. In contrast, diacylglycerol (DAG) cooking oils contain the essential fatty acids, can be used for cooking at high temperatures, and have no smell after cooking.

Recently, the beneficial effect of dietary DAG in lipid metabolism in animals and humans was reported [8-11]. Dietary DAG suppresses body fat accumulation as compared with TAG [8,9]. In addition, postprandial elevation of serum TAG levels after 1,3-DAG ingestion is smaller compared with TAG ingestion [12,13]. Although the mechanism for these effects is not fully elucidated, it might be based on the metabolic difference between 1,3-DAG and TAG in the small intestine [13,14]. Ingested fats containing 90% TAG [15] are hydrolyzed to 2-monoacylglycerol (2-MAG) and fatty acids and absorbed into the epithelial cells of the small intestine. Subsequently, 2-MAG is resynthesized to TAG via 1,2-DAG, and released as chylomicron TAG into the blood via the small intestinal lymph system. In contrast, 1,3-DAG is digested to 1-MAG in the small intestine. Consequently, less 2-MAG is formed, and less reesterification occurs to synthesize TAG as compared with TAG digestion [13,14].

In the present study, we investigated whether these metabolic characteristics of DAG improve lipid metabolism and overcome the problems of MCT oil in a patient with LPL deletion.

2. Patient and methods

2.1. Patient

The patient was a 34-year-old Japanese man (height, 172 cm; body weight, 55 kg; body mass index, 18.6 kg/m^2; blood pressure, 100/64 mm Hg; and pulse rate, 62 beats per minute). His chief complaint was hyperlipidemia. Physical examination revealed no splenohepatomegaly or xanthoma.

Table 2
Composition of test emulsions

Ingredients	% by weight
Oil (DAG or TAG or MCT)	5.0
Casein sodium	2.0
Fatty acid sucrose polyester	0.5
Lecithin from soybeans	0.36
Aspartame	0.2
Water	91.94

His plasma total cholesterol and TAG levels were 277 and 1818 mg/dL, respectively. His plasma LPL level, measured using a quantification reagent (Markit FLPL, Dainippon Pharmaceutical Co, Ltd, Osaka, Japan) after intravenous injection of 30 U/kg heparin (Novo heparin for injection 1000, Hoechst Marion Roussel Ltd, Tokyo, Japan), was less than 20 ng/mL. His plasma apolipoprotein C-II level, optically measured by AU800 (Olympus Corporation, Tokyo, Japan) using an antiserum reagent (Turbilinear-D "Eiken" apolipoprotein C-II, Eiken Chemical Co, Ltd, Tokyo, Japan), was 10.5 mg/dL. Analysis of the LPL gene indicated a nonsense mutation at base pair 438 in exon 3, corresponding to a substitution of the stop codon for tyrosine (TAT → TAA), which was diagnosed as a complete LPL deletion (homozygous). This type of mutation within the LPL gene was previously reported [16,17].

This study was carried out with sufficient respect for the spirit of the Helsinki Declaration. The procedures had been fully explained to the patient. Written informed consent was obtained from the patient before participation.

2.2. Study 1: effects of single administration of DAG on postprandial serum TAG responses

2.2.1. Experimental oils

DAG was prepared by esterifying glycerol with fatty acids from soybean and rapeseed oil according to Huge-Jensen's method [18]. TAG was prepared as a mixed oil with rapeseed, perilla, and safflower oil to make the fatty acid

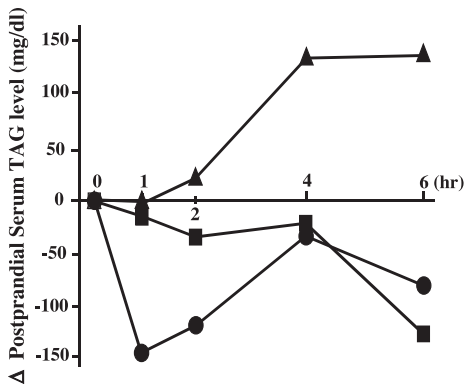

Fig. 1. Postprandial serum TAG responses after ingestion of 3 different oils in an LPL-deficient patient. Postprandial serum TAG levels after ingestion of each emulsified oil was expressed as Δpostprandial TAG (mg/dL), minus the initial TAG value from each time point value. Circles indicate DAG oil; triangles, TAG oil; and squares, MCT oil.

Table 3
Effect of long-term administration of DAG oil on serum lipid levels in an LPL-deficient patient

	Duration (mo)	TAG (mg/dL)	Total chol (mg/dL)	HDL chol (mg/dL)
	0	1939	205	21
	1	2525	293	34
DAG oil	2	1926	234	27
	3	1173	155	16
	4	749	142	17
	5	2195	269	29

DAG oil was used for the duration of 2 to 5 months. Chol indicates cholesterol; HDL, high-density lipoprotein.

composition the same as that of DAG. All of these material oils were obtained from Summit Oil Mills (Chiba, Japan) and Ohta Oil Mills (Aichi, Japan). DAG contained 86% of 1,3- and 1,2-DAG and 14% of TAG. The ratio of 1,3-DAG to 1,2-DAG was 68:32. The combustion energy of both the DAG and TAG measured with a bomb calorimeter was approximately 38 kJ/g (analyzed by the Japan Food Analysis Center, Tokyo, Japan). MCT was purchased from Kao Corporation (Tokyo, Japan). Table 1 shows the fatty acid compositions of DAG, TAG, and MCT.

2.2.2. Study design

On the day before the study, the patient ingested a meal before 9:00 PM, and thereafter, until the study day was over, he was allowed no food or drink except water. One of 3 emulsions (DAG, TAG, or MCT; Table 2) was ingested (within 1 minute) the next morning. Blood samples were collected before ingestion of the emulsion and 1, 2, 4, and 6 hours after ingestion. This study was performed on 3 different days. The amount of ingested oil was 10 g/m^2 body surface area.

2.3. Study 2: effects of long-term administration of DAG on serum TAG

2.3.1. Experimental oils

The DAG oil used in this study was purchased from Kao Corporation (Econa oil).

2.3.2. Study design

When we explained the information about study 2 to the patient for informed consent, the patient complained about taking more MCT oil because he had experienced previous MCT oil–induced nausea and gastric distension. Thus, in study 2 we examined the effect of long-term administration of DAG oil compared with that of TAG oil. The patient had meals prepared using ordinary cooking oil for the initial 2 months, and for the following 3 months, he had breakfast and supper prepared using DAG oil (target, 20 g/d). DAG

Table 4
Amount of TAG and DAG intake during the long-term administration of DAG oil in an LPL-deficient patient

	Duration (mo)	TAG intake (g/d)	DAG intake (g/d)
	0	86.9	0.9
	1	85.7	0.9
DAG oil	2	66.4	4.7
	3	53.1	12.7
	4	43.0	12.4
	5	80.6	37.1

DAG oil was used for the duration of 2 to 5 months.

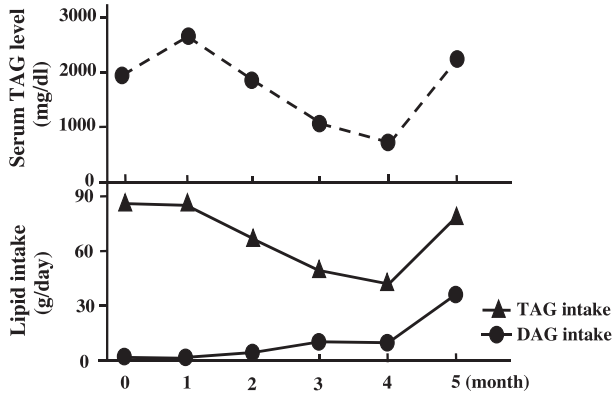

Fig. 2. Changes in serum TAG level (upper panel) and daily intake of dietary TAG and DAG (lower panel) during the study period in an LPL-deficient patient. Upper panel, Serum TAG level. Lower panel, Circles indicate DAG intake; triangles, TAG intake.

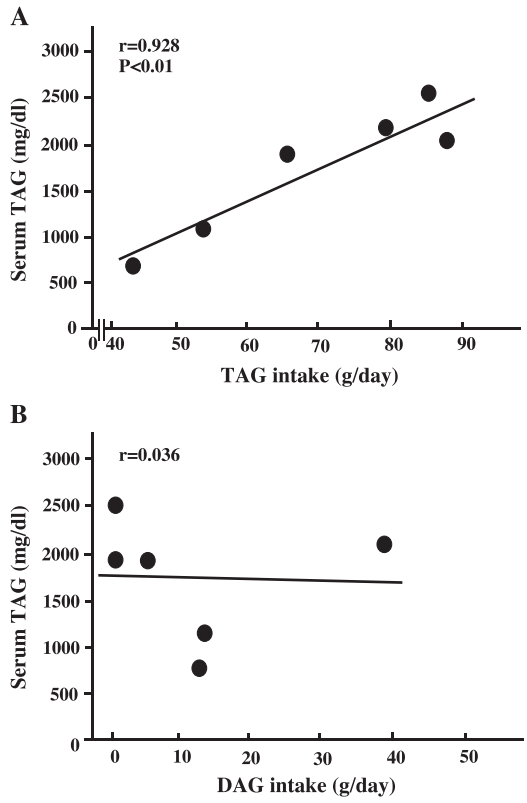

Fig. 3. Relation between serum TAG levels and dietary TAG (A) or DAG (B) intake. A simple linear regression equation was calculated using the least squares method. The correlation was tested for equality using Fisher's *Z* transformation.

Table 5
Nutrient intake amounts during the long-term administration of DAG oil in an LPL-deficient patient

	Duration (mo)	Energy (kJ/d)	Protein (g/d)	Lipid (g/d)	Oil (g/d)	Lipid to energy ratio (%)	Carbohydrate (g/d)
	0	11251	108.4	87.8	35	29	346.4
	1	9363	88.1	86.6	47	35	259.7
DAG oil	2	8790	83.4	71.1	37	30	273.2
	3	7789	73.9	65.8	27	32	234.4
	4	8677	94.0	55.4	15	24	283.2
	5	11154	96.6	117.7	80	40	292.5

DAG oil was used for the duration of 2 to 5 months.

oil was used as the cooking oil for deep-frying, frying, and as seasoning oil for dressing. During the study period, the patient visited our outpatient department once a month for blood sampling, which was performed under 12-hour fasting conditions, and for nutritional guidance and counseling, which was provided for the patient with his wife for 30 minutes. Nutritional guidance and counseling was conducted to evaluate his nutritional state based on two kinds of data obtained at every visit and to advise how to keep his conditions at a constant state based on the evaluation. Subjective data items are as follows: (1) physical conditions (health, appetite, sleep, and defecation), (2) workload, (3) frequencies of eating out, and (4) physical activity. Objective data items are as follows: (1) anthropometric measurements, (2) dietary record, and (3) blood analysis (TAG, total cholesterol, and high-density lipoprotein cholesterol). The same items were used at every visit. The daily intake of energy, lipid, protein, carbohydrate, and dietary oil were obtained from the dietary record of objective data.

2.4. Measurement of serum TAG level

Serum was obtained by centrifugation. The serum TAG level was determined by enzymatic methods using a highly specific kit for the measurement of TAG (Triglyceride E-HR; GPO DAOS method, glycerin elimination method; Wako Pure Chemical Industries, Ltd, Osaka, Japan).

2.5. Statistical analysis

Data were statistically examined by simple linear regression analysis, and a P value less than .05 was considered statistically significant.

3. Results

3.1. Study 1: effects of single administration of DAG on postprandial serum TAG responses

Study 1 was performed on 3 different days for ingestion of 3 different oils. On each day of ingestion, there was no apparent difference in the health of the patient. Differences in postprandial serum TAG responses after ingestion of the 3 different oils are shown in Fig. 1. The postprandial serum TAG level at each time point after DAG oil ingestion was lower than that after TAG oil ingestion. The postprandial serum TAG response after DAG oil ingestion was similar to that after MCT oil ingestion.

3.2. Study 2: effects of long-term administration of DAG on serum TAG

During the 2-month period in which ordinary cooking oil was used, the serum TAG levels in the patient with LPL deletion ranged from 1939 mg/dL at the beginning of the nutritional guidance and counseling to 2525 mg/dL at 1 month. During the study period when ordinary cooking oil and DAG oil were used, the serum TAG level was 1926 mg/dL at 2 months and decreased to 1173 mg/dL at 3 months. At 4 months, when DAG oil alone was used, the serum TAG level markedly decreased to 749 mg/dL (Table 3). The intake of TAG was halved from 86.9 g/d at the beginning of the nutritional guidance to 43.0 g/d at 4 months, whereas the intake of DAG increased from 0.9 to 12.4 g/d during the same period (Table 4). The serum TAG level in the patient was reduced, apparently due to decreasing the intake of TAG and increasing the intake of DAG during the 4-month period from the beginning of the nutritional guidance. The serum TAG level increased, however, to 2195 mg/dL at 5 months after starting dietary counseling. At this time, the intake of TAG had increased from 43.0 to 80.6 g/d, and the intake of DAG had also increased from 12.4 to 37.1 g/d (Fig. 2). The relation between the intake of TAG or DAG and serum TAG levels during the 5-month period was evaluated. There was a positive correlation between the intake of TAG and the serum TAG level ($P < .01$, $y = 33.7x - 583.1$) (Fig. 3A). There was no correlation, however, between the intake of DAG and the serum TAG level ($P = .946$) (Fig. 3B). The intake of energy (kJ/d), proteins (g/d), lipids (g/d), oils (g/d), and carbohydrates (g/d), and lipid to energy ratio (%) in this patient during the study period are summarized in Table 5. Serum TAG levels did not correlate with these parameters (data not shown).

4. Discussion

In study 1, the postprandial serum TAG level after DAG oil ingestion was lower than that after TAG oil ingestion, and similar to that after MCT oil ingestion. This result suggested that DAG oil ingestion might be useful for a patient homozygous for complete LPL deletion who needs

to be careful of elevated postprandial serum TAG levels. On the basis of this result, in study 2 we examined the effect of long-term administration of DAG oil compared with that of TAG oil.

The results of study 2 demonstrated that in this patient with LPL deletion and hypertriglyceridemia, there was a highly positive correlation between the serum TAG level and TAG intake. The serum TAG level decreased by 168 mg/dL with each 5-g decrease in TAG intake according to the equation ($y = 33.7x - 583.1$) estimated from the linear regression between the serum TAG level and TAG intake determined in Fig. 3. These results indicate that the decreased TAG intake induced a reduction in the serum TAG level, supporting the study by Fredrickson and Lees [2].

On the other hand, there was no correlation between DAG intake and serum TAG levels. These findings suggest that the daily intake of DAG oil might not affect serum TAG levels. The National Nutrition Survey in Japan (Ministry of Health, Labor and Welfare 2000) reported that the mean daily lipid intake in Japanese is 57.4 g. Thus, if the patient ingested 57.4 g lipid as TAG, the serum TAG level would be estimated to be 1351 mg/dL according to the equation, $y = 33.7x - 583.1$. Therefore, the serum TAG level was higher than the level (1000 mg/dL) that is considered to increase the incidence of pancreatitis [3]. When 12.0 g of TAG is replaced with DAG (equivalent to 15.0 g of TAG oil is replaced by DAG oil), the serum TAG level would be 947 mg/dL based on this equation. The present results demonstrate that use of 15.0 g DAG oil instead of the same amount of TAG oil as cooking oil might be useful for patients with LPL deletion.

Limitation of dietary fat intake is essential for patients with LPL deletion. Fredrickson and Lees [2] proposed a method to keep the daily intake of dietary fat below 20 to 25 g. This amount is approximately two fifths of the mean fat intake of Japanese (57.4 g) and one third of that for school-aged children (66.8 g) according to the National Nutrition Survey in Japan. These data suggest that it would be difficult for school-aged children during the growth period and adults during their working lives to strictly limit their dietary fat intake to under 20 to 25 g/d.

In this study, we demonstrated the following two results in the patient homozygous for complete LPL deletion. In study 1, single administration of DAG oil does not elevate postprandial serum TAG level as with that of MCT oil. In study 2, long-term administration of DAG oil hardly has any effects on serum TAG level compared with that of TAG oil. Therefore, these results suggested DAG cooking oil might be useful for maintaining the quality of life of patients with LPL deletion.

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