Size-Based Distributions of Postprandial Lipoproteins in Lymph and Serum after Oral Administration of Triacylglycerol and Diacylglycerol Oils in Rats

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Summary Several studies in humans and rodents suggest that postprandial serum triglyceride (TG) levels are decreased by a single oral administration of diacylglycerol (DAG) oil compared with administration of control triacylglycerol (TAG) oil. To gain further insight into the mechanisms underlying the metabolic properties of DAG in a postprandial state, we analyzed the size-based distributions of postprandial lipoproteins in the lymph and serum using gel filtration-based high-performance liquid chromatography. In thoracic duct lymph pooled for 3 h after oral administration of TAG or DAG, the size-based distributions of postprandial lymphatic lipoprotein-TG and -cholesterol levels did not differ significantly, suggesting that DAG did not affect the size of lipoprotein particles secreted from the small intestine. Serum lipoprotein-TG (60%) and -cholesterol levels (90%), however, were significantly different among fractions with a diameter of greater than 80 nm 1 to 2 h after the administration of DAG compared to TAG. In addition, there was a considerable, but nonsignificant, reduction in lipoprotein-TG levels (~40%) in fractions with a diameter of 80 to 30 nm, suggesting that DAG-derived chylomicrons as well as DAG-derived chylomicron remnants were catabolized rapidly. In conclusion, dietary DAG reduced the amount of large-size lipoproteins in the serum, but did not affect the size distribution of lipoproteins produced in the small intestine. Thus, compared with TAG, dietary DAG may reduce the postprandial serum total TG levels.

Key Words diacylglycerol, triacylglycerol, lipoprotein, HPLC, rat

Diacylglycerol (DAG), a naturally occurring oil present at levels ranging from 0.8 to 9.5% in vegetable oils (1), has long been used for human consumption. DAG is used as a food additive in small amounts, but the 1,3-specific lipase-catalyzed reverse reaction currently allows for large-scale production of DAG, which is commercially available in the United States and Japan as cooking oil and in fat products. DAG occurs in two isoforms, 1,2 (or 2,3)-diacyl-sn-glycerol (1,2-DAG) and 1,3-diacyl-sn-glycerol (1,3-DAG) (2). In most natural edible oils and manufactured DAG oil, the 1,3- and 1,2-isoforms are present in a 7:3 ratio (w/w) (3). DAG and conventional triacylglycerol (TAG) oil have a similar fatty acid composition as well as similar taste, appearance, stability against oxidation and heating, and cooking properties (3, 4). Thus, DAG can be easily incorporated into food products such as mayonnaise, spreads, and salad dressing.

Postprandial hyperlipidemia is a characteristic abnormality in obese and insulin-resistant individuals, and contributes to the risk for cardiovascular disease (5, 6).

Therefore, dietary alternatives may be useful to improve postprandial hyperlipidemia. Recent studies suggest that compared to control TAG, DAG administration results in lower postprandial serum triglyceride (TG) levels in healthy subjects (7–9), insulin-resistant subjects (10), and subjects with type II diabetes mellitus (11). A possible explanation for the effect of DAG on postprandial hyperlipidemia was provided by animal studies in which the lymphatic transport of radiolabeled fatty acids in DAG was significantly delayed and reduced (12), presumably as a result of poor reesterification into TAG in the intestinal mucosa (13–15). A novel mechanism was also suggested by the results of a recent study in mice showing that radiolabeled DAG produced less TG, but more monoacylglycerol (MAG) and 1,3-DAG in secreted chylomicrons compared with TAG, which led to the more rapid clearance of DAG-derived chylomicrons than of TAG-derived chylomicrons by lipoprotein lipase (LpL)-mediated lipolysis (16). In these studies, the balance of production and catabolism of DAG- or TAG-derived lipoproteins was estimated by the recovery rate of administered radioactivity, but it is not known how particle size-based metabolism of
lipoproteins occurs.

In the present study, to gain further insight into the effects of DAG on the intestinal production and catabolism of lipoproteins, size-based distributions of lipoprotein-TG and -cholesterol in the lymph and serum were analyzed after oral administration of DAG or control TAG using gel filtration-based high-performance liquid chromatography (HPLC) with component peak analysis and a Gaussian curve-fitting method (17, 18).

**MATERIALS AND METHODS**

**Test oil.** DAG oil was prepared from rapeseed oil in the presence of immobilized lipase using the method of Watanabe et al. (19). The control TAG oil, with a fatty acid composition similar to that of DAG oil, was prepared by mixing rapeseed oil, soybean oil, and safflower oil (Nissinh Oil Mills Ltd., Tokyo, Japan). Table 1 shows the fatty acid compositions of the TAG and DAG oils. The DAG oil comprised 90% DAG (1,3-DAG and 1,2-DAG [2,3-DAG] in a 7:3 ratio) and 10% TAG.

**Experimental animal.** Male Sprague-Dawley rats (7 wk old: 230–250 g) were obtained from Charles River Japan, Inc. (Kanagawa, Japan). The rats were raised under a 12-h light/dark light cycle (lights on 0700–1900, lights off 1900–0700), and water and a stock diet were given ad libitum. All rats were used for experimentation after a 1-wk adaptation period.

Rat experiments were performed with the approval of the Ethics Committee for Experimental Animals of the Kao Corporation. The rats were cared for in accordance with the principles for the use of animals for research and education, following the Statement of Principles adopted by the Federation of American Societies for Experimental Biology Board.

**Lipid concentration.** Particle size-based lipoprotein-TG and -cholesterol profiles in the lymph and serum were analyzed by Skylight Biotech Inc. (Akita, Japan) using a dual detection HPLC system with two tandem-connected TSKgel LipoproatXXL columns (300×7.8 mm: Tosoh Co., Tokyo, Japan) according to the method of Usui et al. (17). The lipoprotein subclasses were identified using component peak analysis with the Gaussian curve-fitting method described by Okazaki et al. (18), in which the fractions were separated into 20 component peaks on the basis of particle size. Using this analytical method, the fractions were classified into major lipoprotein subclasses as follows: particles with a mean diameter greater than 80 nm corresponded to chylomicrons; those with a mean diameter of 80 to 30 nm corresponded to very low-density lipoprotein (VLDL); those with a mean diameter of 30 to 16 nm corresponded to low-density lipoprotein (LDL); and those with a mean diameter of 16 to 8 nm corresponded to high-density lipoprotein (HDL). Total serum TG and cholesterol levels were measured enzymatically using commercial kits purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Each experiment to measure the total lipids and the lipids in the lipoprotein fractions described above were conducted separately in different rats.

**Lipid oral loading test.** TAG and DAG emulsions for ingestion were prepared by mixing 300 mg of sodium taurocholate (Nakalai Tesque, Inc., Kyoto, Japan) and 50 mg of bovine serum albumin fraction V (Sigma Chemical Co., St. Louis, MO, USA) as emulsifiers. 200 mg of TAG or DAG oil, and 2.45 mL of distilled water, and emulsifying the mixture with a SONIFIER 450 (Branson Ultrasonic Corporation, Danbury, CT, USA).

After a 14-h fasting period, blood was obtained from rats (270–300 g) in both groups at hour 0, and then the rats were immediately administered an oral lipid emulsion (6.67 g lipid/kg body weight) by oral gavage. Blood samples were obtained from the jugular vein 1, 2, 3, 4, and 6 h later: serum was obtained by centrifugation at 1,500 ×g for 15 min at 4°C, and the serum lipoprotein-TG and -cholesterol levels were analyzed as described above (see “Lipid concentration”).

**Lymph recovery.** Rats (280–300 g) were anesthetized with pentobarbital (Nembutal), and cannulae were inserted into the thoracic duct and stomach using an improved modification of the method of Bollman et al. (20, 21). Thereafter, until the end of lymph collection, a 139 mmol/L glucose–85 mmol/L NaCl solution was continuously instilled into the gastric tubing at a flow rate of 2.4 mL/h. A glucose-normal saline solution was given ad libitum until the end of the experiment. Rats were placed in a Bollman’s cage after surgery until the end of the experiment, and administered a lipid emulsion the next morning after surgery. Thoracic duct lymph was pooled for 3 h on ice after TAG or DAG emulsion administration. To prevent lymph coagulation, 2Na-EDTA (final concentration 10 mg/mL) was placed in a graduated cylinder, and NaN3 (final concentration 0.01 mg/mL) and gentamicin (0.05 mg/mL) were added to prevent degradation. The collected lymph was stored at 5°C until lipoprotein measurement. The lymphatic lipoprotein-TG and -cholesterol levels were analyzed as described above (see “Lipid concentration”).

**Statistical analysis.** The means and standard deviation (SD) are presented. Statistically significant differences (i.e., p<0.05; two-tailed) in the mean values between the two groups were assessed by Student’s t test and repeated measures two-way analysis of variance (ANOVA; effect of interaction between time and oil).
Effects of DAG on Postprandial Lipoprotein Metabolism

RESULTS

Size-based distributions of lymphatic lipoprotein-TG and -cholesterol levels after oral administration of TAG or DAG

Thoracic duct lymph was pooled for 3 h after oral administration of TAG (n=4) or DAG (n=4), and size-based distributions and total lymphatic lipoprotein-TG and -cholesterol levels were measured using gel filtration-based HPLC. The total concentrations of lymphatic TG and cholesterol after DAG administration were reduced by approximately 20% compared with those after TAG administration, but the distributions of lipoprotein-TG and -cholesterol in lymph were not significantly different (Fig. 1).

Changes in postprandial total serum TG and cholesterol levels

The total serum TG and cholesterol levels after oral administration of TAG (n=6) or DAG (n=6) were measured enzymatically using a commercial kit and are shown in Fig. 2. As reported in previous studies in rodents and humans (7–16), in the present study, there was a significant reduction in the postprandial total serum TG level after DAG administration compared with that after TAG administration (Fig. 2, top panel. p=0.028 (time×oil) by repeated measures two-way ANOVA, p<0.05 at the 1 and 2-h time-points by Student’s t-test). There was no significant difference in the postprandial total serum cholesterol level, however, between the TAG and DAG groups (Fig. 2, bottom panel).

Changes in serum TG levels in size-based lipoprotein fractions

Serum TG levels in the size-based lipoprotein fractions after oral administration of TAG (n=6) or DAG (n=6) were analyzed by gel filtration-based HPLC. The serum lipoprotein-TG level in the fraction with a diameter of more than 80 nm was significantly reduced after DAG administration compared with that after TAG administration, assessed by repeated measures two-way ANOVA (Fig. 3, p=0.007, time×oil). At the 1 and 2-h time points, the reductions were approximately 60% statistically significant (Student’s t test, p<0.001 and p<0.05, respectively). In addition, there were considerable, although nonsignificant, reductions (approximately 40%, p=0.073 by Student’s t test) in lipoprotein-TG levels among fractions with a mean diameter of 80 to 30 nm at the 2-h time point after DAG administration compared with that after TAG administration. There were no significant differences among fractions with mean particle diameters of 30 to 16 nm or 16 to 8 nm between the DAG and TAG groups.

Changes in serum cholesterol levels in size-based lipoprotein fractions

The serum cholesterol levels in the size-based lipoprotein fractions after oral administration of TAG (n=6) or DAG (n=6) were analyzed by gel filtration-based HPLC.

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Fig. 1. Lymphatic lipoprotein-triglyceride (top panel) and -cholesterol (bottom panel) levels pooled for 3 h after oral ingestion of TAG (white bar, n=4) or DAG (black bar, n=4) in the fractions separated based on particle size (diameter). Values are expressed as mean±SD. There were no significant differences in the analysis performed using Student’s t-test.

Fig. 2. Changes in serum total-triglyceride (top panel) and -cholesterol (bottom panel) levels after oral ingestion of TAG (white circles, n=6) or DAG (black circles, n=6). Values are expressed as mean±SD. Repeated measures two-way ANOVA was performed on the overall data set (time×oil). The asterisk denotes significant differences between the TAG and DAG groups at individual time points (*p<0.05, assessed by Student’s t-test).
Fig. 3. Changes in serum lipoprotein-triglyceride levels after oral ingestion of TAG (white circles, \( n=6 \)) or DAG (black circles, \( n=6 \)) in the fractions separated based on particle size (diameter). Values are expressed as mean±SD. Repeated measures two-way ANOVA was performed on the overall data set (time×oil). The asterisks denote statistically significant differences between the TAG and DAG groups at individual time points (***\( p<0.001 \), *\( p<0.05 \), assessed by Student’s t-test).

Fig. 4. Changes in serum lipoprotein-cholesterol levels after oral ingestion of TAG (white circle, \( n=6 \)) or DAG (black circle, \( n=6 \)) in the fractions separated based on the particle size (diameter). Values are expressed as mean±SD. Repeated measures two-way ANOVA was performed on the overall data set (time×oil), but there was no statistically significant difference. The asterisk denotes statistically significant differences between the TAG and DAG groups at individual time points (*\( p<0.05 \), assessed by Student’s t-test).
The serum lipoprotein-cholesterol level among fractions with a mean particle diameter of more than 80 nm was significantly reduced (by approximately 90%) at the 2-h time point after DAG administration compared with that after TAG administration, assessed by Student’s t test (Fig. 4, p<0.05). There were no significant differences among fractions with a mean particle diameter smaller than 80 nm between the DAG and TAG groups.

**DISCUSSION**

In the present study, we assessed the effects of dietary DAG on lymphatic and serum lipoprotein profiles separated on the basis of particle diameter using a novel gel filtration-based HPLC system with component peak analysis. Chylomicrons are secreted from the small intestine to the lymph duct after lipid ingestion, and then transported to the bloodstream. The lymphatic lipoprotein profile reflects lipoprotein production and secretion from the small intestine. The serum lipoprotein profile also reflects the lipoprotein transport from the lymph duct, clearance of lipoproteins in the vascular walls, and the secretion of lipoproteins from the liver, suggesting that a detailed analysis of lipoprotein profiles of both the lymph and serum will provide important information on the effect of DAG on lipoprotein metabolism, such as the size distribution of lipoproteins produced in the small intestine and particle size-associated clearance of lipoproteins in the vascular wall.

Our main observation in this study was that DAG administration, compared with TAG administration, significantly reduced the serum lipoprotein-TG and -cholesterol levels in the fraction with a particle diameter of more than 80 nm (Figs. 3 and 4), although the size distributions of lymphatic lipoprotein-TG and -cholesterol did not differ significantly (Fig. 1). These findings suggest that dietary DAG enhances the rapid clearance of large-size lipoproteins in the blood or reduces chylomicron transport from the lymph ducts to bloodstream, but does not affect the size distribution of lipoprotein particles secreted from the small intestine. Yasunaga et al. reported that oral DAG-derived chylomicrons contained more 1,3-DAG and MAG, but less TG than oral TAG-derived chylomicrons, leading to a more rapid LpL-mediated clearance of DAG-derived chylomicrons than of TAG-derived chylomicrons (16), suggesting that the DAG administration-induced reduction of postprandial serum large-size lipoproteins was associated with the rapid clearance of DAG-derived chylomicrons.

Based on the gel filtration-based HPLC analysis (18), particles with a diameter of more than 80 nm and 80 to 30 nm correspond to chylomicrons and VLDLs, respectively. Chylomicron remnants are formed on the vascular wall by the action of LpL on chylomicrons (22) and can be included in the VLDL fraction as a result of gel filtration separation. Because we did not measure the density of the lipoprotein particles, we could not determine whether DAG affected the clearance of chylomicron remnants or VLDL secretion from the liver. Tada et al. reported that a single oral ingestion of DAG, compared with TAG, reduced the amount of postprandial serum remnant-like particles-TG (8), suggesting that the reduction in postprandial large-size lipoproteins in the serum after DAG ingestion is associated with the rapid clearance of remnants as well as chylomicrons. Indeed, TG levels were quantitatively higher in the VLDL/chylomicron remnant fraction (80–30 nm) than in the chylomicron fraction (>80 nm) (Fig. 3). This factor may have a greater impact on the total level of serum TG and, as such, be the reason for the difference between TAG and DAG.

On the other hand, there was no significant difference in the postprandial total serum cholesterol level despite the significant reduction of cholesterol in the chylomicron fraction after DAG administration (Fig. 2 bottom and Fig. 4, >80 nm). As shown in Fig. 4, the HDL-cholesterol level was greater, likely due to the lack of cholesteryl ester transfer protein activity (23) in rats, which promotes the transfer of cholesteryl esters from HDL to apolipoprotein B-containing particles (i.e., VLDL and LDL) in exchange for TG (24). The level of chylomicron-cholesterol was much lower than that of HDL-cholesterol. The constant change in the postprandial HDL-cholesterol levels seemed to cancel the impact of the reduction of chylomicron-cholesterol after DAG administration.

Total TG and total cholesterol amounts in lymphatic lipoproteins pooled for 3 h after oral administration of DAG were approximately 20% lower than the amounts detected after oral administration of TAG, but this difference was not significant (Fig. 1). DAG and TAG with a similar fatty acid composition have similar energy values and undergo similar absorption in rats (25), suggesting that these properties are not associated with the reductions observed in the DAG group in the present study. Yanagita et al. reported a lower recovery rate of dietary radiolabeled 1,3-DAG in the lymph compared with dietary radiolabeled TAG (12). Additionally, Kondo et al. reported that 1,3-DAG was little utilized as a substrate for TG synthesis mediated by DAG acyltransferase, which is a microsomal enzyme that catalyzes the acylation of DAG into TG (26, 27), in intestinal mucosal cells (14). Thus, a possible mechanism underlying the 20% reduction in total TG and cholesterol in lymphatic lipoproteins observed in the present study is the poor reesterification of dietary DAG to TG in the small intestine.

Test DAG oil was 90% pure. The ratio of 1,3-DAG and 1,2-DAG was 7:3 due to acyl migration during storage and heating during the production process (2). Thus, the final composition of the test DAG oil was 10% TAG, 27% 1,2-DAG, and 63% 1,3-DAG. As mentioned above, oral administration of 1,3-DAG has unique digestive and metabolic properties compared with TAG, whereas the properties of pure 1,2-DAG have not been investigated. It is speculated that oral administration of 1,2-DAG with concomitant TAG is digested and absorbed normally like TAG because 1,2-DAG is a digestive inter-
mediate of TAG, which is transiently generated in the intestinal mucosal cells of the digestive tract and is utilized as substrate for TG synthesis mediated by DAG acyltransferase. Nevertheless, further investigations are required to clarify the metabolic properties of pure 1,2-DAG.

In conclusion, our findings suggest that dietary DAG suppresses the postprandial increase in serum total TG by reducing the amount of large-size TG-rich lipoproteins, specifically chylomicrons and possibly chyli-micron remnants, but does not affect the size distribution of lipoproteins secreted from the small intestine, and might thereby contribute to reducing the risk for cardiovascular disease.

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