Effects of a 1,3-diacylglycerol oil-enriched diet on postprandial lipemia in people with insulin resistance

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Abstract **Postprandial hypertriglyceridemia is common in individuals with insulin resistance, and diets enriched in 1,3-diacylglycerol (DAG) may reduce postprandial plasma triglycerides (PPTGs). We enrolled 25 insulin-resistant, nondiabetic individuals in a double-blind, randomized crossover trial to test the acute and chronic effects of a DAG-enriched diet on PPTG. Participants received either DAG or triacylglycerol (TAG) oil, in food products, for 5 weeks. Fasting lipids, and two separate postprandial tests, one with DAG oil and one with TAG oil, were performed at the end of each 5 week diet period. We found no acute or chronic effects of DAG oil on PPTG. Thus, neither the DAG oil PPTG (h/mg/dl) on a chronic TAG diet [area under the curve (AUC) = 503 ± 439] nor the TAG oil PPTG on a chronic DAG diet (AUC = 517 ± 638) was different from the TAG oil PPTG on a chronic TAG diet (AUC = 565 ± 362). Five weeks of a DAG-enriched diet had no acute or chronic effects on PPTG in insulin-resistant individuals. We suggest further studies to evaluate the effects of DAG on individuals with low and high TG levels.—**Reyes, G., K. Yasunaga, E. Rothenstein, W. Karmally, R. Ramakrishnan, S. Holleran, and H. N. Ginsberg. **Effects of a 1,3-diacylglycerol oil-enriched diet on postprandial lipemia in people with insulin resistance. *J. Lipid Res.* 2008.** 49: **670–678.**

Supplementary key words triglycerides • lipoproteins • hypertriglyceridemia

Increases in the age of the population and increasing rates of obesity, together with reduced levels of physical activity, have caused a dramatic increase in the prevalence of type II diabetes mellitus (T2DM) throughout the world (1, 2). The consequences of this cannot be underestimated; T2DM is a major cause of morbidity and mortality in the United States (1, 3). An essential component of T2DM is insulin resistance (IR) (4, 5), which is accompanied by disordered lipoprotein metabolism, including abnormal postprandial (PP) metabolism of both chylomicrons and VLDL (6–9). Indeed, PP hypertriglyceridemia is common in individuals with IR with or without concomitant T2DM and has been associated with the presence of both coronary and carotid artery atherosclerosis (10–12). Importantly, two recent studies indicate that nonfasting triglyceride (TG) concentrations are better predictors of future cardiovascular events than fasting TG levels (13, 14).

PP TG levels are governed by the assembly and secretion of chylomicrons from the small intestine, the rate of LPL-mediated lipolysis of chylomicron TG, and chylomicron remnant removal by the liver (15–17). In people with IR, LPL-mediated lipolysis may be reduced, either by the reduced availability of LPL (18) or the increased plasma concentrations of apolipoprotein C-III, an inhibitor of LPL (19). In addition, defective clearance of chylomicrons by the liver may be associated with abnormal hepatic proteoglycan synthesis (20). Recent studies suggest that in rodents (21) and humans (22), IR can be associated with an increased assembly and secretion of chylomicrons.

As a key step in chylomicron formation, fatty acids, generated by the lipolysis of TG in the intestinal lumen, are reesterified onto monoglycerides and diglycerides within enterocytes of the small intestine. The enzymes involved, monoacylglycerol acyltransferase and diacylglycerol acyltransferase, preferentially add FAs to the *sn*-1 or *sn*-3 position of the glycerol backbone and are more efficient in the presence of a FA in the *sn*-2 position (23, 24). Indeed, prior studies in rodents suggest that FAs released from dietary 1,3-diacylglycerol (DAG) oil are not efficiently incorporated into chylomicrons after absorption from the intestinal lumen, resulting in greater FA oxidation in the small intestine (25) and liver (26, 27). In support of these mechanistic studies, some studies in animals suggest lower postprandial plasma triglyceride (PPTG) levels (28, 29) and lower body weight (25, 30) with DAG-enriched diets. Lower PPTG levels have also been observed after acute administration of DAG to healthy humans (31–33), people with T2DM (34), and individuals with IR (35). On the other hand, several long-term studies in humans did not

Manuscript received 29 May 2007 and in revised form 17 December 2007.

Published, JLR Papers in Press, December 18, 2007.
DOI 10.1194/jlr.P700019-JLR200

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demonstrate any effects of DAG oil on fasting TG levels (36–39), even when there was a greater weight loss on the DAG diet treatment. None of these latter studies examined PPTG. Therefore, the current study was designed to address the acute and chronic effects of DAG oil on PPTG and PP remnant-like lipoprotein cholesterol (RLP-C) levels. We also compared the effects of the two diets on fasting levels of lipids and on both fasting and PP levels of glucose and insulin in subjects with IR.

METHODS

Subjects

Participants were recruited from the Columbia University Medical Center (CUMC) community and surrounding neighborhood by means of Institutional Review Board-approved flyers. Potential candidates were screened via telephone and asked to come to the General Clinical Research Center (GCRC) at CUMC for further evaluation of their eligibility. After obtaining signed informed consent, a study investigator obtained a medical history, performed a physical examination, and drew fasting blood. Eligibility for enrollment was based on these inclusion criteria: men and women 18 years of age and older, body mass index $> 23\ kg/m^2$, and fasting homeostasis model assessment (HOMA-R) [(glucose: mmol/l $\times$ insulin: μU/ml)/22.5] $\geq$ 2.5. Participants had to be normally active and in good health on the basis of their medical history, physical examination, and routine laboratory tests. Individuals were excluded if they had a previous diagnosis of diabetes, were on lipid-altering medications, had cardiac, renal, hepatic, gastrointestinal, or endocrine disorders, were pregnant, were breastfeeding, had a history of or a strong potential for substance abuse (allowable alcohol = 5 drinks or 75 g/week), or had a history of frequent change in smoking habits or smoking cessation within the past 6 months. Other exclusions included serum TG concentration $>$ 350 mg/dl, serum total cholesterol concentration $>$ 300 mg/dl, or systolic blood pressure $>$ 140 mmHg. Patients with a previous diagnosis of human immunodeficiency virus were also excluded from the study. Participants could be receiving medications for chronic conditions such as hypertension.

Eligible participants visited the GCRC for an enrollment visit, during which they read and signed a second informed consent form for the study protocol. Participants met with a registered dietitian, who provided nutritional counseling on diet requirements and study food consumption. They were also given an opportunity to taste study foods, and preferences were noted. In all, 110 individuals were screened, and 25 were recruited and completed the study. The study was approved by the Institutional Review Board of the CUMC.

Study design

This study was a randomized, double-blind, crossover trial. After enrollment, participants were instructed to follow diet guidelines defined by the National Cholesterol Education Program (NCEP) III and Therapeutic Lifestyle Change Diet guidelines for 1 week. This was followed by baseline blood tests and anthropometric measurements. Participants were then randomized to consume either a DAG- or triacylglycerol (TAG)-enriched diet for a period of 5 weeks. This was followed by a 1-week "rest period" before the subjects crossed over and received the opposite diet for an additional 5 weeks. During each diet period, participants visited the GCRC every week for diet compliance monitoring, diet review, and distribution of study foods. Blood samples and anthropometric measurements were obtained every 2 weeks. During the 3rd week of each diet period, postheparin plasma was obtained for measurement of LPL and HL activities. At the end of 5 weeks on each diet treatment, each participant had two PP studies 4–7 days apart (visits 5/6 and 9/10; **Fig. 1**).

Diet

The background diet was designed to fall within the recommendations of the NCEP Therapeutic Lifestyle Change Diet: saturated fat $<$ 7% of total calories, polyunsaturated fat up to 10% of total calories, monounsaturated fat up to 20% of total calories, total fat between 25% and 35% of total calories, carbohydrates between 50% and 60% of total calories, fiber between 20 and 30 g/day, protein $\sim$ 15% of total calories, and cholesterol $<$ 200 mg/day. Participants were instructed to avoid consuming any dietary supplements such as ω-3 fatty acids, plant stanols/sterols, or fiber, which could influence outcome variables. Caloric needs for weight maintenance were based on the weight obtained during the second visit to our center and were calculated for each participant using the Harris-Benedict equation and a physical activity factor of 1.3. Participants were required to maintain their weight within $\pm$5% of their initial body weight. The dietitian used a food exchange pattern in conjunction with the completed 3 day food records to develop specific dietary goals for the subjects to achieve. The participants were instructed to complete food records properly and were provided with measuring utensils for accurate portion size recording.

During the study, subjects were provided a variety of food products containing either DAG oil or TAG oil. The DAG oil was prepared by esterification of FA derived from natural plant edible oil with monoacylglycerol or glycerol in the presence of immobilized lipase (40). TAG was prepared by mixing rapeseed, safflower, and perilla oils to achieve a FA composition comparable to that of the DAG oil. There was no significant difference in FA composition between the oils (35). The ester distributions of acylglycerol and the FA compositions of TAG and DAG (by weight) were determined by gas chromatography.

The diets, including the food products, were developed for each subject to provide 15% energy for weight maintenance from DAG or TAG oil. Each serving of a food product had 10 g of either DAG or TAG oil. The DAG and TAG oil-containing food products were muffins, herbal and cranberry salad dressings, and mayonnaise. These products were developed and prepared in the GCRC bionutrition unit. In addition, for added variety, the oils were incorporated into commercially available fat-free products, such as fat-free pudding and yogurt. The DAG and TAG oil products were designed to be similar in taste and appearance. To meet each subject's daily study oil dose, subjects consumed between three and five servings of products per day (30–50 g of DAG or TAG oil per day). Food records were analyzed using the Nutrition Data System for Research software (version 4.05-33; University of Minnesota).

Study visits

Participants were asked to come to the GCRC after a 12 h overnight fast and after refraining from any alcoholic beverages for 48 h before each visit. Immediately after each blood draw, plasma and serum were isolated and kept in a $-80^{\circ}C$ freezer until analyzed.

Measurement of postheparin HL and LPL activity

During the 3rd week of each diet period, and after a 12 h overnight fast, a blood sample was obtained and 60 U/kg body weight (maximum of 5,000 units) of heparin was injected intra-

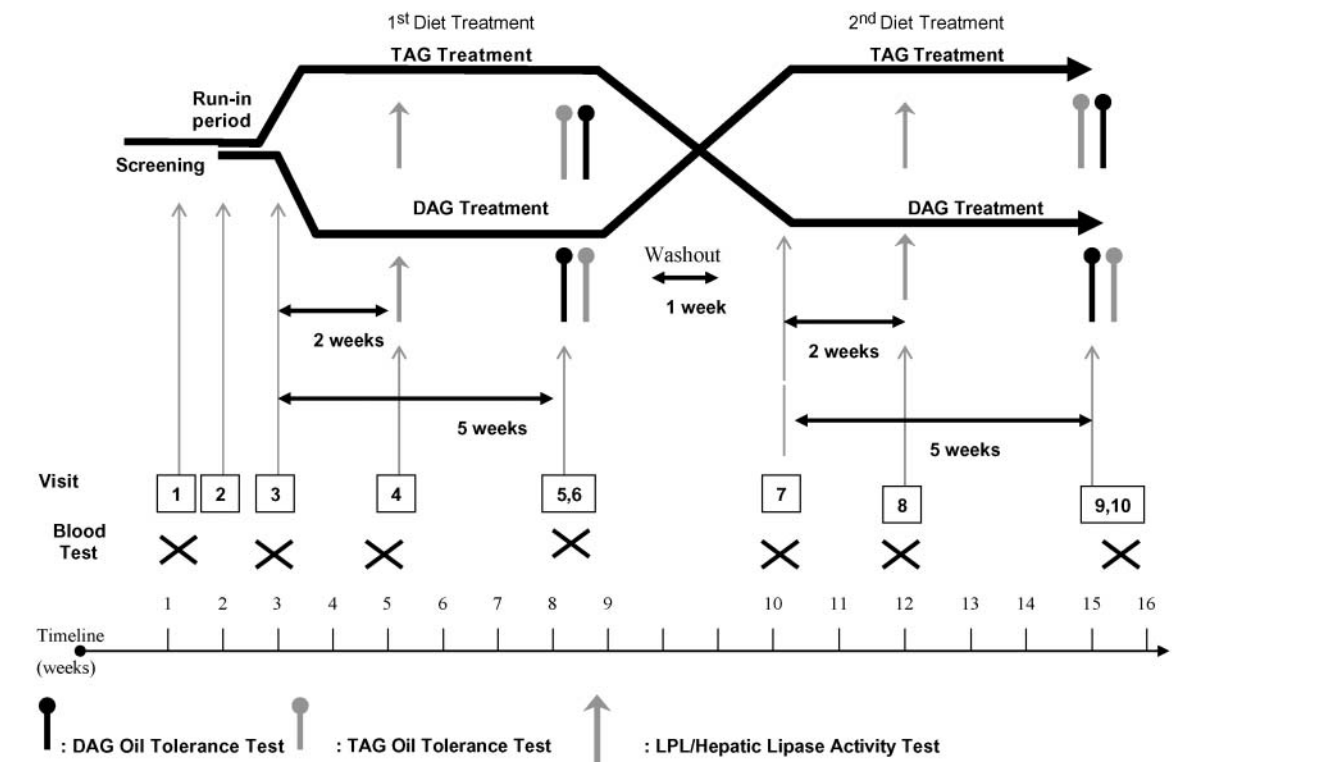

Fig. 1. Study design and time line. This was a randomized, double-blind, crossover design study. Each diet was consumed for 5 weeks, with a 1 week washout period. The postprandial (PP) studies were performed after the 5th week of each diet, while the subjects were still consuming those diets. DAG, 1,3-diacylglycerol; TAG, triacylglycerol.

venously. A second blood sample was obtained 15 min later for measurement of postheparin HL/LPL activity. Activities of HL and LPL were assayed in postheparin plasma in the laboratory of Dr. Ira Goldberg (41).

PP studies

During the 6th week of each diet period, subjects had two PPTG studies. The first PPTG study of each diet period used the same test oil that the participants had been eating the previous 5 weeks; the second PPTG study meal contained the other test oil (i.e., if a participant had been consuming a TAG chronic diet, the first PP study was a TAG challenge and the second was a DAG challenge). Participants were admitted to the GCRC after a 12 h overnight fast, and fasting blood was obtained. Immediately after, they consumed a liquid meal prepared by the GCRC bionutrition unit. The test meal was a liquid formula consisting of 75% fat, 15% carbohydrate, and 10% protein. The fat included DAG or TAG oil at a dose of 30 g oil/m^2 body surface area. The test formulas provided 360 kcal/m^2.

Participants remained in a semirecumbent position throughout the 8 h test unless they needed to stand or walk briefly. They were semirecumbent for at least 20 min before each blood sampling. Subjects were allowed to consume noncaloric, noncaffeinated beverages during the PP visit. All subjects were given a Lactaid® pill (McNeil Nutritionals, LLC) before consumption of the formula. They were allotted 15 min to consume the test formula. Blood samples were obtained at 2, 4, 5, and 8 h after the formula was consumed. The data are presented as area under the curve (AUC) of plasma TG concentrations above the baseline level. In previous studies from a multicenter feeding study (Dietary Effects on Lipoproteins and Thrombogenic Activity), the within-subject standard deviation for PPTG was <10% (L. Berglund et al., unpublished data).

Laboratory

Total cholesterol, TG, HDL cholesterol, and glucose were measured using standard enzymatic techniques on a Hitachi 912 chemistry analyzer in the CUMC GCRC as described previously (42). LDL cholesterol levels were calculated by the Friedewald method (43). The CUMC GCRC Core Laboratory participates in the Centers for Disease Control lipid standardization program, and both intra-assay and interassay coefficients of variation (CVs) are <3%. RLP-C concentrations were measured by an immunoseparation assay kit (Polymedco, Inc., Cortland Manor, NY); intra-assay CV was 3–5% and interassay CV was 6–11%. Serum insulin was measured using Coat-A-Count Insulin solid phase I125 radioimmunoassay (Diagnostic Products Corp., Los Angeles, CA); intra-assay CV was 5–9% and interassay CV was 7–10%. Hemoglobin A1c (HbA1c) levels were measured at CUMC's clinical laboratory (VARIANT II Hemoglobin A1c 1 Program; Bio-Rad Laboratories).

Statistical analysis

In earlier diet studies we have performed, in which we provided nearly all meals directly to the participants, within-subject standard deviation for PP TG (log of the AUC) was <10%.

Allowing for twice that standard deviation under conditions of looser diet control in the present protocol, we determined that 25 subjects would provide 80% power to detect a 12% effect using paired *t*-tests at $P = 0.05$.

Observed variables were first confirmed to be normally distributed. TG measurements were log-transformed, and square roots were taken of insulin levels to make their distributions normal and facilitate the use of parametric statistical procedures. Data are presented as means ± SD. Multiple fasting measurements during each diet period were averaged, and the DAG and TAG diet periods were compared by paired *t*-tests. The TG, RLP-C, insulin, and glucose responses to each fat load were analyzed by repeated-measures ANOVA followed by prespecified pairwise contrasts of interest. Statistical significance was tested at $P = 0.05$. Posthoc analyses in subgroups were done in the same manner. Multiple regression analysis was used to study the association between the AUC for PPTG and RLP-C and possible predictors such as fasting TG, HOMA, sex, glucose, insulin, and HbA1c.

TABLE 2. Energy intake and DAG and TAG oil composition

Item	Treatment	Mean ± SD[a]	*P*
Energy intake (kcal/day)	TAG	1,918 ± 471	NS
	DAG	1,968 ± 555	
Macronutrient composition (%)			
Fat[b]	TAG	32.8 ± 3.4	NS
	DAG	33.5 ± 3.6	
Saturated fat	TAG	6.3 ± 1.2	NS
	DAG	6.5 ± 1.5	
Carbohydrate	TAG	51.9 ± 3.9	NS
	DAG	50.7 ± 4.9	
Protein	TAG	17.0 ± 2.4	NS
	DAG	17.5 ± 2.6	
Cholesterol	TAG	157 ± 58	NS
	DAG	177 ± 62	

DAG, 1,3-diacylglycerol; TAG, triacylglycerol.
[a] SD was calculated using Excel.
[b] Percentage fat includes study oil consumed (both DAG and TAG).

RESULTS

The study group consisted of 7 males and 18 females aged 30–74 years. The clinical characteristics of the participants at screening are presented in **Table 1**. Body mass index and waist circumference were 34.6 ± 6 kg/m^2 and 105 ± 11 cm, respectively. All subjects had, based on inclusion criteria, IR (HOMA = 3.9 ± 2.2). Median screening TG levels were 152 mg/dl (131–206 interquartile range). HDL cholesterol levels were 47.3 ± 5.9 mg/dl in females and 34.9 ± 4.2 mg/dl in males. In addition, 44% of the females and 57% of the males met the NCEP Adult Treatment Panel III criteria for the metabolic syndrome (43).

All randomized participants completed the study. No serious adverse events were reported after the ingestion of the DAG or TAG oils. There were no significant differences in energy intake or macronutrient composition during the diet treatments of DAG versus TAG, as assessed with the 3 day diet record (**Table 2**). Weight changes did not differ significantly between DAG and TAG. Weight varied by <5% during the study. Mean fasting lipid, glucose, and insulin levels measured on the days of the PP studies on both diets were similar (**Table 3**). Thus, there was no effect of a DAG-enriched diet on basal metabolic parameters in this group of individuals with IR.

TABLE 1. Baseline subject characteristics

Data	Mean and SD	Range
Age (years)	43 ± 11	30–74
Body mass index [weight (kg)/height^2 (m^2)]	34.6 ± 6	24–45
Waist (cm)	105 ± 11	86–123
HOMA	3.9 ± 2.2	2.5–11.8
Total cholesterol (mg/dl)	202 ± 37	147–286
Triglyceride[a] (mg/dl)	152 (131–206)	85–479
HDL cholesterol	47.3 ± 5.9, females	37–60
	34.9 ± 4.2, males	30–41
LDL cholesterol (mg/dl)	124 ± 32	74–205
Insulin[a] (μU/ml)	15.4 (12.9–19.6)	9.7–53.1
Glucose (mg/dl)	86.2 ± 10	60–114
Hemoglobin A1c (%)	5.6 ± 0.4	5.0–6.3
Blood pressure (mmHg)	117/76 ± 14/10	

HOMA, homeostasis model assessment (glucose × insulin/22.3.) All data were collected during the screening visit. LDL cholesterol was estimated using the Friedewald equation. Waist was measured around the navel.
[a] Median and interquartile range are shown.

Our main hypothesis was that, compared with the ingestion of a TAG-enriched liquid meal after 5 weeks of a TAG chronic diet [TAG challenge on a TAG background diet (TT)], both a DAG challenge on a TAG background diet (DT) and a TAG challenge on a DAG background diet (TD) would result in smaller PP increases in plasma TG and RLP-C levels. The primary efficacy variables were the AUC of PP changes in plasma TG and RLP-C. The AUC for PPTG over 8 h (h/mg/dl; mean ± SD) were similar for the two major comparisons (**Table 4**): DT-PPTG, 503 ± 439; TD-PPTG, 517 ± 638; TT-PPTG, 565 ± 362. The comparison of DT versus TT is presented in graphic format in **Fig. 2**; it is clear that there was no difference in the response to an acute DAG challenge versus an acute TAG challenge during the 5th week of a TAG background diet. In **Fig. 3**, it is also clear that there was no difference in the response to an acute TAG challenge during the 5th week of a DAG background diet versus an acute TAG challenge during the 5th week of a TAG background diet.

Similar results were observed when we determined the AUC responses of RLP-C under these different diet conditions. Thus, RLP-C AUC over 8 h were similar for acute DAG or TAG challenges on a TAG background diet (**Fig. 4A**) as well as for an acute TAG challenge on a DAG background diet versus an acute TAG challenge on a TAG background diet (Fig. 4B).

We also examined the effects of the consumption of a DAG-enriched diet versus a TAG-enriched diet on PP levels of glucose and insulin; no differences were observed (Table 4). Finally, there were no effects of a DAG-enriched diet on postheparin plasma HL (DAG, 4.2 ± 2.2 mM FA/ml/h; TAG, 4.2 ± 2.4 mM FA/ml/h) and LPL (DAG, 4.1 ± 1.5 mM FA/ml/h; TAG, 4.4 ± 1.7 mM FA/ml/h).

In a multivariable analysis, which included fasting levels of TG, HOMA, sex, glucose, insulin, and HbA1c, fast-

TABLE 3. Fasting lipids, glucose, and insulin levels on DAG and TAG diets

Variable	DAG	TAG	Δ Mean ± SD	*P*
Total cholesterol (mg/dl)	183 ± 31	183 ± 29	−0.6 ± 14	NS
Triglycerides[a] (mg/dl)	160 (118–220)	159 (109–215)	1.8 ± 49	NS
HDL cholesterol (mg/dl)	40 ± 7	40 ± 7	0.9 ± 5	NS
LDL cholesterol (mg/dl)	108 ± 25	110 ± 22	−1.8 ± 12	NS
Glucose (mg/dl)	100 ± 12	99 ± 13	1 ± 8	NS
Insulin[a] (μU/ml)	13.3 (10.0–16.9)	16.5 (10.6–19.4)	−1.1 ± 6	NS

All values are means from postprandial study days.
[a]Median and interquartile range are shown.

ing plasma TG was the only factor predictive of PPTG AUC responses. Furthermore, there was a significant interaction between fasting plasma TG and the difference between the PPTG AUCs for DT and TT ($P = 0.005$) (**Fig. 5**). This result suggested a lower PPTG response to DT in subjects with lower fasting TG levels and led to a posthoc analysis in which subjects (n = 16) with fasting TG levels of <200 mg/dl were analyzed separately. This group had a lower PPTG in response to an acute DAG challenge on the TAG diet (DT) versus an acute TAG challenge on the TAG diet (TT) (**Fig. 6A**). A similar significant difference in the RLP-C AUC was observed on DT versus TT in the group with fasting TG levels of <200 mg/dl (Fig. 6B).

DISCUSSION

Hypertriglyceridemia, including PP lipemia, is a characteristic abnormality present in obese, IR individuals (9) and contributes to the risk for cardiovascular disease (10–12). Therefore, it would be helpful to find food choices that might improve PP lipemia. Recent interest relevant to this issue has focused on 1,3-DAG oil as such a diet alternative. This interest derives from the hypothesis that 1,3-DAG and 1-DAG are poor substrates for the reesterification of diet-derived FA in the enterocyte (23). Thus, after ingesting a DAG-enriched meal, less diet-derived FA would be incorporated into chylomicron TG, reducing the PP entry of dietary TG into the circulation. Indeed, several studies have demonstrated reduced PP lipemia in animals fed DAG oil (26–30, 44). Decreased chylomicron formation (44), increased FA oxidation in the intestine (25), and reduced lymphatic transport of 1,3-DAG compared with TAG (45) have all been implicated in the reduced PPTG in those studies. However, in very recent studies by our group, we did not observe reduced entry of chylomicrons into the circulation of normal C57BL/6J mice gavaged with DAG compared with TAG (46). In contrast, our data indicated that chylomicrons formed after the ingestion of DAG were better substrates for LPL-mediated lipolysis. Based on our results, we concluded that any beneficial effects of DAG on PP lipemia would be derived from more effective lipolysis of chylomicron TG, rather than from the reduced formation of chylomicrons. It should be noted, however, that a benefit of DAG oil has been demonstrated in a patient with homozygous LPL deficiency and severe hypertriglyceridemia (47).

Reduced PPTG has also been observed after acute DAG consumption in studies of healthy (31–33), IR (35), and diabetic (34) individuals. For example, Takase et al. (35) studied PPTG after consumption of a diet enriched with DAG in 18 Japanese subjects, 8 of whom were IR (HOMA > 2.0). They found that fasting TG and RLP-C levels correlated with HOMA and were significantly higher in the IR group compared with the insulin-sensitive group. PPTG and RLP-C levels were lower, particularly at the 4 h (last) time point after DAG ingestion compared with TAG ingestion; AUCs for either TG or RLP-C were not reported.

Because previous studies had been conducted almost exclusively in Japanese individuals, and to address a lack of data regarding PPTG responses to chronic DAG and TAG consumption, we conducted a study of the effects of acute and chronic DAG consumption in a population without diabetes but with IR. We expected that our IR population, which on average had mildly increased fasting TG levels, would show improved PPTG metabolism after a DAG challenge on the background of a TAG diet compared with a TAG challenge on the same background TAG diet. We also expected that a TAG challenge on a background DAG diet would result in lower PPTG than a TAG challenge on a background TAG diet. However, we found that an acute challenge of DAG on a TAG

TABLE 4. Postprandial TG, RLP, glucose, and insulin levels

Variable	TAG on TAG	DAG on TAG	TAG on DAG	DAG on DAG	DT-TT	TD-TT
PP TG	565 ± 362	503 ± 439	517 ± 638	505 ± 380	−62 ± 273	−49 ± 621
PP RLP	26 ± 49	17 ± 37	22 ± 50	25 ± 70	−9 ± 32	−4 ± 49
PP glucose	−73 ± 73	−87 ± 93	−91 ± 71	−79 ± 80	−14 ± 72	−18 ± 74
PP insulin	26.7 ± 47	23 ± 46	30.1 ± 47	32.4 ± 40	−4 ± 39	3.5 ± 54

Values are expressed as means ± SD (h/mg/dl). DT, diacylglycerol challenge on a TAG background diet; PP, postprandial; RLP, remnant lipoprotein; TD, TAG challenge on a DAG chronic background diet; TG, triglyceride; TT, TAG challenge on a TAG background diet.

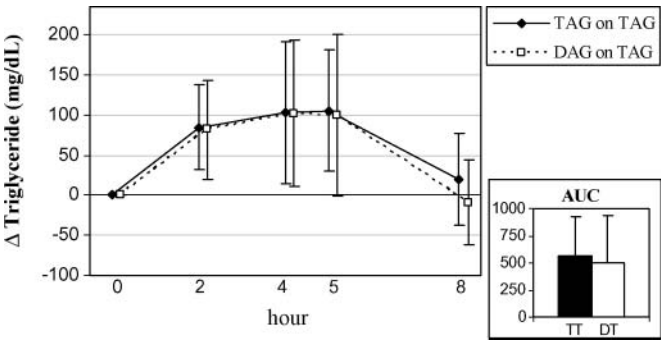

Fig. 2. Postprandial plasma triglyceride (PPTG) area under the curve (AUC) for DT versus TT. The inset shows a bar graph representation of AUC values. There was no significant difference in PPTG AUC when an acute challenge of DAG was compared with an acute TAG challenge after 5 weeks of a TAG diet. DT, diacylglycerol challenge on a TAG-enriched 5 week background diet; TT, TAG challenge on a 5 week TAG-enriched background diet. Data are presented as means ± SD.

background diet did not affect the PPTG or RLP-C AUC compared with an acute challenge of TAG on a TAG background diet. Thus, there was no evidence for either reduced chylomicron formation or improved LPL-mediated clearance of chylomicron TG after an acute challenge with DAG on the background of chronic TAG consumption. Furthermore, there were no differences in the AUC for PPTG or PP RLP-C with a TAG challenge on a DAG background diet versus a TAG challenge on a TAG background diet; a chronic DAG-enriched diet did not affect the metabolism of an acute TAG challenge. Consistent with the absence of any effects of acute or chronic DAG on PPTG, we did not see any effects of chronic DAG on HL or LPL activity. Tada et al. (32) found no effect of an acute DAG challenge on LPL mass.

Fasting lipid levels were also similar during the two diet periods. However, we did observe that the fasting TG levels on each diet were significantly correlated with the PPTG responses; this has been established previously (48).

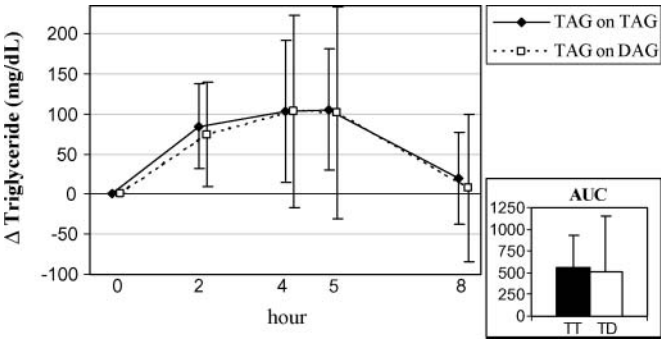

Fig. 3. PPTG AUC for TD versus TT. The inset shows a bar graph representation of AUC values. There was no significant difference in PPTG AUC when an acute challenge of TAG after 5 weeks of a DAG diet was compared with an acute TAG challenge after 5 weeks of a TAG diet. TD, TAG challenge on a diacylglycerol-enriched 5 week background diet. Data are presented as means ± SD.

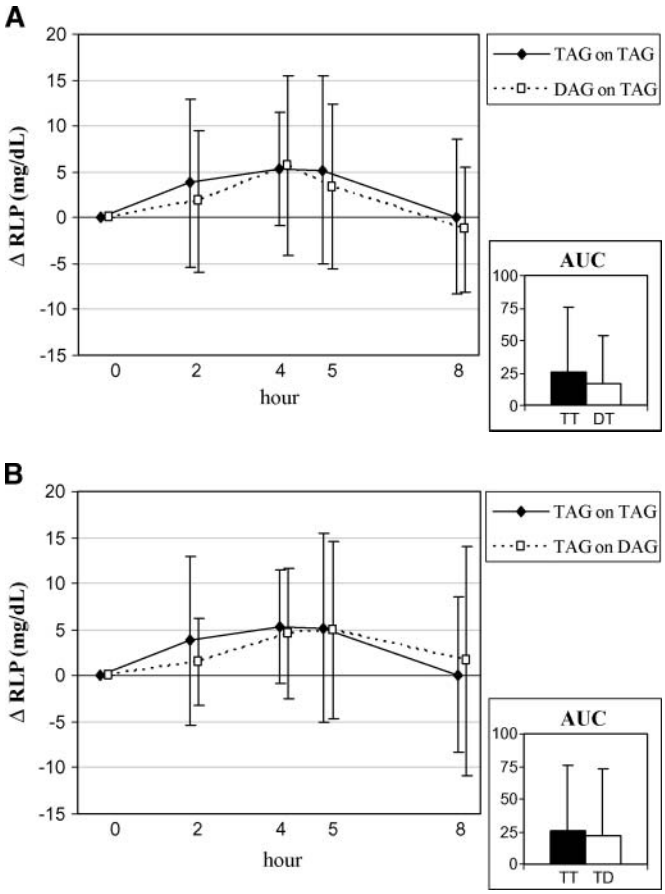

Fig. 4. Remnant lipoprotein cholesterol (RLP-C) AUC. The inset shows a bar graph representation of AUC values. A: DT versus TT. B: TD versus TT. PP RLP-C did not differ in the two main comparisons in this trial. Data are presented as means ± SD.

We were surprised to observe that there was a significant interaction between fasting TG and the difference in PPTG between DAG on TAG versus TAG on TAG, such that individuals with lower fasting TG levels had lower PPTG after the acute DAG challenge. This is consistent,

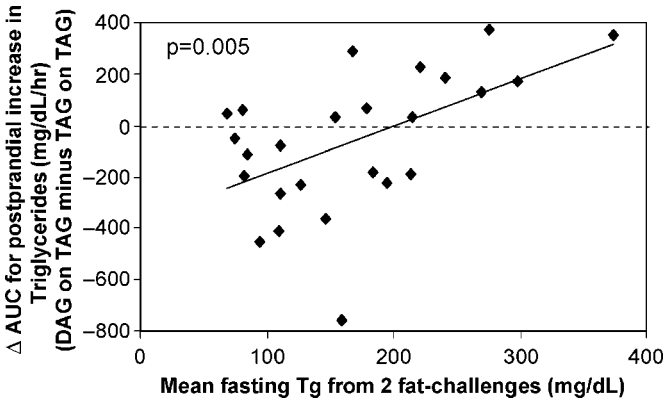

Fig. 5. Graphic representation of the relationship between fasting plasma TG and the difference between DT and TT. This graph suggests a lower PPTG response to DT in subjects with lower fasting TG levels.

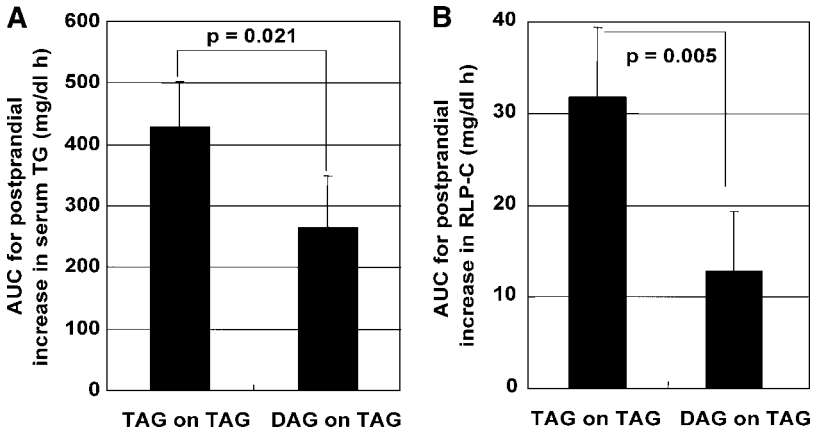

Fig. 6. Bar graphs representing AUCs for PPTG (A) and RLP-C (B) for DT versus TT in the subgroup (n = 16) of subjects with fasting TG levels of <200 mg/dl. In this subgroup, DT was associated with lower PPTG and RLP-C AUCs. Data are presented as means ± SD.

however, with the aforementioned studies, in which there was lower PPTG with DAG in healthy subjects with normal baseline TG levels (31–33). Furthermore, our posthoc analyses suggested that there were significantly lower PPTG and RLP-C AUCs after the DAG challenge on a TAG background in subjects with fasting TG < 200 mg/dl. One explanation for these posthoc findings could be that hypertriglyceridemic subjects, who already have increased VLDL secretion, might have an additional increase in the assembly and secretion of VLDL as a response to greater hepatic uptake of fatty acids generated by better lipolysis of DAG-enriched chylomicrons (46). In contrast to our result, Tomonobu, Hase, and Tokimitsu (33) reported that subjects who had fasting TG > 100 mg/dl had greater reductions in PPTG compared with subjects who had lower fasting TG levels. Further investigations will be needed to determine whether our unexpected posthoc analysis can be confirmed and, if so, to clarify the effects of DAG in subjects with lower or higher levels of plasma TG.

It is instructive to try and determine why we observed a different outcome from Takase et al. (35). In that study, the degree of IR, as estimated by HOMA criteria (2.07 ± 0.76 in the Takase study vs. 3.9 ± 2.1 in the present study) and baseline TG levels (165 ± 27 mg/dl in the Takase study vs. 174 ± 76 mg/dl in the present study), was lower than in our trial. In addition, the Takase study used a 1 day, acute study protocol in which participants consumed a mayonnaise-type food at a dose of 10 g/60 kg body weight. Our DAG and TAG oil challenges were performed on the background of 5 weeks of each diet (DAG- or TAG-enriched NCEP diets). Finally, the long-term, chronic background diets of the subjects studied by Takase et al. (35) were likely different from those of our subjects: in Western populations, the consumption of fats and oils accounts for at least 30% of total calories in the diet (23), whereas in Japan, the estimated percentage of fat calories is between 19.5% and 24% (50).

In our study, a DAG-enriched diet consumed for 5 weeks had no effect on fasting lipids levels. This result is consistent with several studies of chronic DAG consumption, from 4 to 24 weeks duration, in normal subjects (36–39). However, in patients with T2DM, Yamamoto et al. (51) found a significant reduction in fasting TG from baseline after 12 weeks of a DAG-enriched diet compared with a TAG-based diet. These same authors confirmed that finding in a more recent study of patients with diabetes (49). Fasting and PP levels of glucose and insulin were also unaffected by DAG in our study. Three studies of acute DAG administration in healthy humans (31–33) and two studies of chronic DAG consumption in patients with T2DM (49, 51) also reported no differences in fasting glucose or insulin between DAG and TAG. There have been no previously published studies of the potential effects of DAG on PP glucose or insulin.

In conclusion, 5 weeks of a diet enriched in 1,3-DAG had no effects on fasting or PP measures of lipid or glucose metabolism in a group of subjects with IR. Further studies will be required to confirm our posthoc finding of reduced PPTG on DAG in IR subjects with fasting TG < 200 mg/dl. Confirmation of this posthoc finding might be clinically relevant, because ~80% of the U. S. population has a fasting TG concentration below that level according to National Health and Nutrition Examination Survey data. Additionally, Miller et al. (52) identified fasting TG levels of >100 mg/dl as predictive of future cardiovascular events in men with angiographic evidence of coronary artery disease. Overall, therefore, a large proportion of the U. S. population might benefit from reductions in nonfasting TG concentrations (13, 14). It also remains to be determined whether longer periods of consumption of 1,3-DAG, in a setting in which spontaneous weight loss is allowed, will have a beneficial effect on PPTG in people with IR across a full range of plasma TG levels.

The authors acknowledge the contributions of Dr. Ira Goldberg, Dr. Mayumi Takahashi, and Ms. Marnie Rackmill in the analysis of the LPL and HL assay. The authors thank the Columbia

University GCRC inpatient unit, outpatient nursing, bionutrition unit, and Core Laboratory staff members for their clinical and technical support. This work was supported by an educational grant from the KAO Corporation and by funds from the National Institutes of Health (National Heart, Lung, and Blood Institute Grant T32 HL-07343).

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