Alpha-Linolenic Acid-Enriched Diacylglycerol Oil Suppresses the Postprandial Serum Triglyceride Level —A Randomized, Double-Blind, Placebo-Controlled, Crossover Study

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Summary  This study investigated the effect of a single oral ingestion of alpha-linolenic acid-enriched diacylglycerol (ALA-DAG) on postprandial serum triglyceride (TG) levels. A randomized, double-blind, controlled, crossover study was performed in subjects with normal or moderately high fasting serum TG levels. Subjects ingested 0.00 g [control: triacylglycerol; TAG (rapeseed oil)], 1.25 g (1.25-g: mixture of 1.25 g ALA-DAG and 1.25 g TAG), or 2.50 g (2.50 g) of ALA-DAG in random order with a 6-d washout period. Serum TG levels were evaluated in the fasting state, and at 2, 3, 4, and 6 h after the test meal. Thirty-eight subjects completed the study and were defined as the per protocol set. As the primary outcome, postprandial serum TG levels were significantly lower in the 2.50-g treatment compared with the control. The TG level did not differ significantly between the 1.25-g and control. The suppressive effect of ALA-DAG on the serum TG level correlated significantly with the body mass index and fasting insulin level. ALA-DAG at a dose of 2.50 g had greater effects on serum TG and apolipoprotein B levels in subjects with a higher body mass index (≥25 kg/m2) and higher fasting serum insulin levels (>10 μU/mL). Our findings suggest that ingesting 2.50 g ALA-DAG suppresses the postprandial serum TG level in people with normal and moderately high fasting serum TG levels, presumably as a result of poor reesterification of dietary fat into TG in the intestinal mucosa.

Key Words  alpha-linolenic acid, diacylglycerol, human, postprandial triglyceride

Coronary heart disease and stroke were among the top 10 causes of death reported by the World Health Organization (WHO) from 2000 to 2012 (1). Both diseases are caused by the progression of arteriosclerosis. The Vital Statistics in Japan of 2013 (2) indicated that heart disease and cerebrovascular disease accounted for more than 30% of the total causes of death in Japan—comparable with the percentage of death caused by malignant neoplasm.

Hypertriglyceridemia is an independent factor for coronary artery disease (3), and postprandial hypertriglyceridemia is highly correlated with ischemic heart disease, particularly myocardial infarction precipitated by coronary artery disease (4, 5). Excessive intake of saturated fatty acids and other types of fat is considered a factor in hypertriglyceridemia, and therefore prevention and improvement by changing the diet is important.

Diacylglycerol (DAG), which is widely present in nature and has a long history of human consumption, occurs in two isoforms, 1,2 (or 2,3)-diacyl-sn-glycerol (1,2-DAG) and 1,3-diacyl-sn-glycerol (1,3-DAG). In most natural edible oils and manufactured DAG oil, ~70% (w/w) of the DAG is present as the 1,3-isoform which has different characteristics from triacylglycerol (TAG) in the digestion and absorption processes in the small intestine and the subsequent TAG synthesis pathway (6–8). DAG ingestion inhibits the increase in postprandial blood triglyceride (TG) levels in cases of mild hyperlipidemia (9, 10), insulin resistance (11) and abnormal glucose tolerance (12). It was reported that the minimum effective dose was 2.73 g of DAG compared to general edible oils in mild hyperlipidemia (13). The fatty acid composition of the DAG and TAG used in these reports was the same, and the inhibitory effects on the increase in blood TG are thought to be due to the difference in the DAG structure.

Alpha-linolenic acid (ALA) is easily utilized as energy in animals (14, 15) and humans (16) compared to other fatty acids, such as stearic, oleic, and linoleic acids. Therefore, DAG containing mainly ALA (ALA-DAG) is expected to increase the fat oxidation rate compared to DAG mainly containing other fatty acids, and to induce visceral fat reduction in an animal model (17) and humans (18, 19). The effect of ALA-DAG on postprandial blood TG levels, however, has not been investigated. Additionally, the effect of a lower dose (<2.73 g) of DAG

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on blood TG levels is unclear.

Therefore, in the present study, ALA-DAG was administered at a dose of 0.00, 1.25, or 2.50 g to subjects with fasting blood TG levels to determine the minimum effective dose of ALA-DAG to suppress postprandial serum triglyceride (TG) levels.

MATERIALS AND METHODS

Subjects. Study subjects that were screened and provided written informed consent, fulfilled the inclusion criteria, and did not fulfill the exclusion criteria were enrolled in the study. Inclusion criteria were male and female between 35 and 64 yr of age with fasting blood TG levels of 120 to 199 mg/dL, and a body mass index (BMI) of 23 to less than 30 kg/m². Exclusion criteria included chronic disease; taking medicine affecting glucose metabolism, fat metabolism, or blood pressure; surgery for disease or injury within 2 mo before the study; allergic symptoms to the ingredients in the test diets; regular consumption of Food with Health Claims; a feeling of unwellness or adverse physical effects from blood collection; donation of 200 mL or more of blood within 1 mo before the study; heavy smoking (≥21 cigarettes a day); being a shift-worker; business trip or travel planned for 6 consecutive days or more during the study period; inability to record a diet for 9 d (3 d for 6 consecutive days or more during the study period; day); being a shift-worker; business trip or travel planned for 6 consecutive days or more during the study period; inability to record a diet for 9 d (3 d×3 times); lack of consent to view past medical records; plan to participate in another clinical study during the study period; planned pregnancy or lactation during the study period; and unsuitability as judged by the physician in charge based on medical exams or other factors.

The sample size was determined based on preliminary examination conducted prior to this study (preliminary examination results have not yet been disclosed). In the preliminary examination, an inhibitory effect on an increase in postprandial serum TG levels was observed during fasting, and at 2, 3, 4, and 6 h after ingesting the test diets. Subjects recorded their lifestyles, including diets and activities, for 3 d prior to the study, and a registered dietitian used nutritional calculation software (Healthy Maker Pro 501 R8, nutritional counseling version, Mushroomsoft Co., Ltd., Okayama, Japan) to calculate the nutritional level. Moderate and intense activity and consumption of alcohol were prohibited, and the subjects ingested the identical diet for their evening meal by 9:00 pm on the day before the study. On the day of the study, the subjects were prohibited from consuming any foods or drinks and from smoking after waking, and blood samples were collected after at least 12 h of fasting, and at 2, 3, 4, and 6 h after ingesting the test diets. Subjects recorded their lifestyles, including diets and activities, for 3 d prior to the study, and a registered dietitian used nutritional calculation software (Healthy Maker Pro 501 R8, nutritional counseling version, Mushroomsoft Co., Ltd., Okayama, Japan) to calculate the nutritional level. Moderate and intense activity and consumption of alcohol were prohibited, and the subjects ingested the identical diet for their evening meal by 9:00 pm on the day before the study. On the day of the study, the subjects were prohibited from consuming any foods or drinks and from smoking after waking, and blood samples were collected after at least 12 h of fasting, and at 2, 3, 4, and 6 h after ingesting the test diets.

Test diets. ALA-DAG was prepared using the method reported by Watanabe et al. (20), from flaxseed oil (Summit Oil Corporation, Chiba, Japan) and rapeseed oil (The Nisshin OilliO Group, Ltd., Tokyo, Japan) using equipment owned by Kao Corporation (Tokyo, Japan). The w/w ratio of 1.3-DAG and 1.2-DAG in ALA-DAG was approximately 2 : 1. The control TAG was a rapeseed oil (produced by The Nisshin OilliO Group, Ltd.). The composition of the test oils is shown in Table 1.

The ALA-DAG preparation was mixed with rapeseed oil, antioxidants, and emulsifying agents to produce a cooking oil for heating. The test shortbread was prepared for accurate and certain ingestion of ALA-DAG or TAG and contained the cooking oil, hard flour, soft flour, superfine sugar, salt, egg, pullulan, and water. The test diets comprised the shortbread administered together with a yogurt product (Bulgaria Yogurt, 0% fat, Meiji Dairies Corporation, Tokyo, Japan) and a dairy product (low-fat milk, Yotsuba Milk Products Co., Ltd., Hokkaido, Japan). Total energy of the test diets was 467 kcal; P (protein) : F (fat) : C (carbohydrate)=15.8 : 27.8 : 55.1 as the energy ratio.

Study design. The study was a randomized, double-blind, controlled, crossover study. Subjects were administered a single dose of the test diet containing 0.00 g (control), 1.25 g, or 2.50 g ALA-DAG in random order with a 6-d washout period. Changes in the serum TG level were observed during fasting, and at 2, 3, 4, and 6 h after ingesting the test diets. Subjects recorded their lifestyles, including diets and activities, for 3 d prior to the study, and a registered dietitian used nutritional calculation software (Healthy Maker Pro 501 R8, nutritional counseling version, Mushroomsoft Co., Ltd., Okayama, Japan) to calculate the nutritional level. Moderate and intense activity and consumption of alcohol were prohibited, and the subjects ingested the identical diet for their evening meal by 9:00 pm on the day before the study. On the day of the study, the subjects were prohibited from consuming any foods or drinks and from smoking after waking, and blood samples were collected after at least 12 h of fasting, and at 2, 3, 4, and 6 h after ingesting the test diets.

The subjects were prohibited from ingesting any medications. Food with Functional Claims, nutritional food supplements, supplements, or other substances that could affect this study during the study period, including the washout periods. The subjects were also instructed to avoid excessive eating and drinking, and to maintain their usual level of diet, exercise, and other lifestyle habits.

This study was conducted in accordance with the Declaration of Helsinki (2013), and with the approval of the Oriental Ueno Health Center Ethics Committee. The study protocol was registered with the University Hospital Medical Information Network (UMIN) Center (UMIN-CTR, http://www.umin.ac.jp/ctr/index-j.

### Table 1. Compositions of TAG and ALA-DAG.

<table>
<thead>
<tr>
<th>Constituents</th>
<th>TAG</th>
<th>ALA-DAG</th>
</tr>
</thead>
<tbody>
<tr>
<td>DAG, g/100 g</td>
<td>1.5</td>
<td>80.2</td>
</tr>
<tr>
<td>DAG bound ALA, g/100 g</td>
<td>0.1</td>
<td>35.3</td>
</tr>
<tr>
<td>MAG, g/100 g</td>
<td>0.0</td>
<td>0.5</td>
</tr>
<tr>
<td>TAG and others, g/100 g</td>
<td>98.5</td>
<td>19.4</td>
</tr>
<tr>
<td>FFA, g/100 g</td>
<td>0.0</td>
<td>0.1</td>
</tr>
<tr>
<td>Fatty acid, %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C16:0</td>
<td>4.1</td>
<td>2.6</td>
</tr>
<tr>
<td>C18:0</td>
<td>1.9</td>
<td>1.5</td>
</tr>
<tr>
<td>C18:1</td>
<td>61.0</td>
<td>26.9</td>
</tr>
<tr>
<td>C18:2</td>
<td>20.4</td>
<td>16.9</td>
</tr>
<tr>
<td>C18:3</td>
<td>9.3</td>
<td>50.7</td>
</tr>
<tr>
<td>C20:0</td>
<td>0.6</td>
<td>0.1</td>
</tr>
<tr>
<td>C20:1</td>
<td>1.1</td>
<td>0.4</td>
</tr>
<tr>
<td>C22:0</td>
<td>0.4</td>
<td>0.3</td>
</tr>
<tr>
<td>Others</td>
<td>1.1</td>
<td>0.8</td>
</tr>
</tbody>
</table>

1. Monoacylglycerol.
2. Free fatty acid.
Primary endpoint (change in postprandial serum TG)

There was no significant difference in the initial TG values among the study treatments (control, 1.25-g, and 2.50-g treatments: 158±7.7, 169±8.1, and
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161±8.7 mg/dL, respectively).

The postprandial serum TG level was significantly lower in the 2.50-g treatment at the 3-h point compared to the control when assessed by Dunnett’s test (Fig. 1A), but the iAUC was not significantly different (Fig. 1B). The suppressive effects of 2.50 g ALA-DAG on the iAUC of the postprandial serum TG level was significantly correlated with the BMI (Fig. 2A) and fasting serum insulin level (Fig. 2B). BMI was also significantly correlated with the fasting serum insulin level \( R = 0.441, p < 0.01 \), data not shown. In the stratified analysis, subjects in the 2.50-g treatment with a BMI ≥ 25 kg/m\(^2\) or higher, which is categorized as obesity in Japan, had significantly lower postprandial serum TG levels at the 2, 3, and 4-h points (Fig. 3A) and a smaller iAUC (Fig. 3B). Additionally, in subjects with an abnormal fasting insulin level (>10 \( \mu U/mL \)), the postprandial serum TG level was significantly decreased at the 3 and 4-h points in the 2.50-g treatment (Fig. 3C). The iAUC also tended to decrease (Fig. 3D). The ALA-DAG effect, however, did not significantly correlate with the other factors, including the fasting serum TG level (data not shown).

In the two-treatment comparison between the 2.50-g and control without consideration of multiplicity (paired \( t \)-test or Wilcoxon signed rank test), the TG levels at the 2 and 3-h points and the iAUC were significantly decreased in the 2.50-g treatment compared with the control in the overall PPS samples (data not shown).

Secondary endpoint (change in postprandial serum ApoB48)

The initial ApoB48 values were not significantly different among the control, 1.25-g, and 2.50-g treatments (9.6±0.8, 10.8±1.1, and 10.8±0.8 \( \mu g/dL \), respectively). Although no significant differences in ApoB48 were detected among the treatments in the PPS samples (Fig. 4A and B), the decrease in ApoB48 at the 3-h time-point in the 2.50-g treatment was significant when analyzed in subjects with a BMI of at least 25 kg/m\(^2\) by Dunnett’s test (Fig. 4C). The decrease in iAUC was not significant, even when analyzed in subjects with a BMI of at least 25 kg/m\(^2\) by Dunnett’s test (Fig. 4D). ApoB48 was also not significantly different among the treatments in subjects with abnormal fasting serum insulin levels higher than 10 \( \mu U/mL \) (data not shown).

Other blood parameters

None of the other parameters measured in this study (lipoprotein fraction, insulin, free fatty acids, ketone body fraction, total GIP, and active GLP-1) were significantly different among the treatments (data not shown).

DISCUSSION

In the present study, we compared the effects of different dosages of ALA-DAG on postprandial serum TG levels. Ingestion of 2.50 g ALA-DAG suppressed the increase in postprandial serum TG levels compared to ingestion of the control oil (Figs. 1A and 3). Ingestion of 1.25 g ALA-DAG did not have a significant suppressive
Fig. 3. Changes in postprandial serum TG (A) and iAUC (B) in subjects with higher BMI ($\geq 25$ kg/m$^2$, $n=22$). Change in postprandial serum TG levels (C) and iAUC (D) in subjects with higher fasting serum insulin levels ($>10$ µU/mL, $n=19$). A and B show the control (solid line, open circle), the 1.25-g (dotted line, closed circle), and the 2.50-g (solid line, closed circle). Values represent mean±SE. Significant differences compared to the control were assessed by Dunnett’s test, ** $p<0.01$, * $p<0.05$.

Fig. 4. Changes in postprandial serum ApoB48 and iAUC in the overall PPS samples ($n=38$, A and B) and in subjects with higher BMI ($\geq 25$ kg/m$^2$, $n=22$, C and D) after ingestion of control (solid line, open circle), 1.25 g ALA-DAG (dotted line, closed circle), or 2.50 g ALA-DAG (solid line, closed circle). Values represent mean±SE. Significant differences compared to the control were assessed by Dunnett’s test, * $p<0.05$. 
effect on postprandial TG levels, suggesting that 1.25 g ALA-DAG—half the dose that provides a visceral fat decreasing effect—is not sufficient to suppress the postprandial serum TG level.

Saito et al. (11) reported that postprandial serum TG levels are significantly suppressed by administering 2.7 g DAG (mainly containing oleic acid and linoleic acid) in 10 g of fat. Although the difference between the report by Saito et al. (11) and the present study is the type of fatty acids bound to DAG, the dosage of ALA-DAG was similar. Therefore, the suppressive effect on postprandial serum TG levels observed in this study was presumably due more to the contribution of the DAG structure, predominantly 1,3-DAG, which leads to poor re-esterification of dietary fat into TG in the intestinal mucosa, than to the contribution of the types of fatty acids. On the other hand, TG-rich lipoproteins are poorly formed after a DAG diet compared to a TAG diet (21). In addition, chylomicrons are rapidly cleared after ingesting DAG compared to TAG (22). These mechanisms are potentially different depending on the type of fatty acids bound to DAG. Therefore, additional studies are required to explain the relationship between DAG and the type of fatty acids bound to it. Moreover, the type of fatty acids comprising the fat and their binding sites (i.e., the sn positions) may also affect the increase in postprandial serum TG levels (23). Fatty acids comprising DAG and the binding sites of those fatty acids could have different effects on postprandial serum TG. ApoB48 is an indicator of chylomicron quantity, but our observation did not reveal an obvious effect of ALA-DAG on ApoB48 (Fig. 4). ALA-DAG may have more of an effect on the size, rather than on the number, of chylomicron particles. Further studies are required to investigate the relationship between the DAG structure and fatty acid composition.

The TG-lowering effect of ALA-DAG correlated significantly with both BMI and insulin (Fig. 2). Moreover, the effect was more pronounced in obese subjects with a BMI of at least 25 kg/m² than in the overall PPS analysis. In addition, the TG-lowering effect of DAG is associated with the DAG structure, type of fatty acid composition, and we presume that this TG-lowering effect is most likely related to the anti-obesity effect. Moreover, the TG-lowering effect of DAG is readily expressed in cases of an increased visceral fat in obese people has been reported (18, 19), and we presume that this TG-lowering effect is most likely related to the anti-obesity effect. Moreover, the TG-lowering effect of DAG is readily expressed in cases of insulin resistance and glucose intolerance abnormalities (12, 13), and thus it is possible that the effects of ALA-DAG are more easily detected in cases of an increased insulin level. A high insulin level is likely to be induced by obesity (24), and the concomitant insulin resistance may be associated with postprandial hypertriglyceridemia due to lower lipoprotein lipase activity and the hydrolysis rate of chylomicron remnant (25). DAG was reported to induce rapid clearance of remnants as well as chylomicrons (21), suggesting that improved clearance of chylomicron remnants by ALA-DAG could account for the clear effect in subjects with high BMI and insulin in this study.

A limitation of the present study is that fatty acid compositions differed among the test oils (Table 1). Therefore, we cannot conclude whether the suppressive effect of ALA-DAG on the postprandial serum TG level is associated with the DAG structure, type of fatty acid bound to DAG, or synergistic actions.

Conclusion

Our observation suggests that ingesting 2.50 g ALA-DAG has suppressive effects on the postprandial serum TG level in people with normal and moderately high fasting serum TG levels, presumably due to poor re-esterification of dietary fat into TG in the intestinal mucosa.

Acknowledgments

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REFERENCES


