NOTE

Alpha Linolenic Acid-enriched Diacylglycerol Consumption Enhances Dietary Fat Oxidation in Healthy Subjects: A Randomized Double-blind Controlled Trial

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Abstract: Consumption of alpha linolenic acid-enriched diacylglycerol (ALA-DAG) reduces visceral fat area. In this study, we performed a randomized, placebo-controlled, double-blind, crossover intervention trial to investigate the effect of ALA-DAG on dietary fat oxidation in comparison with control triacylglycerol (TAG). Each subject (n=16) consumed either 2.5 g/d of ALA-DAG or TAG for 14-d, separated by a 21-d washout period. At the end of each consumption period, we assessed dietary fat oxidation. ALA-DAG consumption significantly enhanced dietary fat utilization as energy compared to TAG consumption.

Key words: ALA-DAG, alpha-linolenic acid, diacylglycerol, dietary fat oxidation, human

1 INTRODUCTION

Visceral obesity is strongly associated with metabolic risk factors, such as hyperglycemia, hypertension, and hyperlipidemia¹⁻³. The development of obesity is related to genetic background, physical activity, and diet composition. In particular, a chronic imbalance between fat intake and fat expenditure is an important regulator of body fat⁴. Moreover, dietary fat ingestion may be related to fat oxidation⁵⁻⁷.

Alpha linolenic acid-enriched diacylglycerol (ALA-DAG) is a minor natural component of many edible oils and has long been consumed by humans. ALA-DAG mainly occurs with the chemical structure 1,3-diacyl-*sn*-glycerol (1,3-DAG) and alpha-linolenic acid as the fatty acid. Previous human studies demonstrated that the long-term consumption of ALA-DAG significantly decreases body weight and visceral fat area compared to consuming the control triacylglycerol (TAG)⁸⁻¹⁰. ALA-DAG consumption enhances both fat oxidation and energy expenditure in healthy humans¹¹. Thus, ALA-DAG consumption could be useful for controlling body weight by maintaining or improving fat

and energy metabolism. This underlying mechanism is supported by reports showing enhanced expression of β -oxidation related enzymes and genes in the small intestine¹² and in the liver¹³ in rodents. Based on these reports, ALA-DAG is expected to enhance oxidation of diet-derived fat as well as body fat. Indeed, in rodents, dietary fat oxidation is enhanced after repeated ingestion of ALA-DAG compared to TAG¹⁴. Its effect in humans, however, remains unclear. Therefore, this study evaluated whether repeated ALA-DAG consumption affects oxidation of diet-derived fat in healthy human subjects.

2 EXPERIMENTAL

2.1 Ethics and registration

This study was performed in accordance with the principles of the Declaration of Helsinki and was approved by the clinical ethics committee of Oriental Ueno Kenshin Center (Tokyo, Japan). The study protocol was registered in advance with the University hospital Medical Information

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Network Clinical Trials Registry (registration no. UMIN000021181), and the execution of this study was outsourced to TES Holdings Co., Ltd (Tokyo, Japan). After receiving an explanation of the study, all subjects provided written informed consent to participate in the study.

2.2 Subjects

Twenty subjects were enrolled in the study according to the inclusion and exclusion criteria. Inclusion criteria were 1) age 35 to 64 years and 2) body mass index 23.0 to 29.9 kg/m². Exclusion criteria included the presence of severe disease, surgery within 2 months, taking medication, taking supplements or foods with health claims, premenopausal woman, and food allergy. The sample size was estimated based on our previous study showing that ALA-DAG significantly enhanced postprandial total fat oxidation in 19 subjects¹¹. Because the dose and the consumption duration of ALA-DAG in this present study were the same as the previous study, we estimated that a similar sample size was appropriate.

2.3 Design and protocol

The study had a randomized, double-blind, placebo-controlled crossover design with two 14-d consumption periods of ALA-DAG or control TAG, separated by a 21-d washout period. The subjects were assigned to each order of the consumption period by stratified block randomization using computer-generated random numbers under blinded condition. During each consumption period, the subjects consumed shortbreads containing 2.5 g/d of ALA-DAG or TAG each day. During the consumption period, the subjects were instructed to maintain their habitual lifestyle, including their usual physical activity and dietary intake. The subjects recorded their meals for 3-d before the measurements, and nationally registered dietitians analyzed the dietary records. Alcohol intake and strenuous exercise were not allowed for the 3-d before each measurement. One day before the measurements, the subjects ingested specified meals for breakfast, lunch, and dinner (total calories: 2173 kcal/day for men, 1818 kcal/day for women). At the end of the consumption period, we measured the subjects' body composition and dietary fat oxidation, and collected serum samples after they had fasted for at least 12 h.

2.4 Test diet

We used a previously reported method¹⁵ to prepare the ALA-DAG 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). Each 2.5 g of ALA-DAG contained 0.9 g DAG-bound ALA. Rapeseed oil was used as the source of the TAG (The Nisshin OilliO Group, Ltd, Tokyo, Japan). **Table 1** shows the components of the TAG and

Table 1 Glycerides and fatty acids composition of TAG and ALA-DAG.

	TAG	ALA-DAG
Glyceride (g/100 g)		
DAG	1.5	80.2
DAG-bound ALA	0.1	35.3
Monoacylglycerol	0.0	0.5
Free fatty acid	0.0	0.1
TAG and others	98.5	19.4
Fatty acid (wt, %)		
C16:0	4.1	2.6
C18:0	1.9	1.5
C18:1	61.0	26.9
C18:2	20.4	16.9
C18:3	9.3	50.7
C20:0	0.6	0.1
C20:1	1.1	0.4
C22:0	0.4	0.3
Others	1.1	0.8

ALA-DAG. We produced a cooking oil by mixing the ALA-DAG with rapeseed oil, anti-oxidants, and emulsifying agents. We made a test shortbread (hard flour, soft flour, superfine sugar, salt, egg, pullulan, water, and the prepared cooking oil) to ensure accurate ingestion of the ALA-DAG or TAG. Each shortbread (60 g) contained 291 kcal (protein:fat:carbohydrate = 8:39:53) per serving (2.5 g ALA-DAG or TAG) and were individually packaged. The shortbreads containing TAG and ALA-DAG could not be distinguished from each other by appearance, taste, or odor.

2.5 Dietary fat oxidation assessment

We synthesized the ¹³C-labelled triolein probes from [1-¹³C]oleic acid (purity > 99%, ¹³C > 99%; Isotec, Miamisburg, OH, US) and free glycerol using an enzymatic method and purified the probes using silica gel liquid chromatography. The assessment of dietary fat oxidation was performed as reported previously^{16–18}. Briefly, before and after ingestion of the test meal (555 kcal, protein: fat: carbohydrate = 17:30:52 as energy value) containing ¹³C-labelled triolein (400 mg), we collected breath samples in aluminum bags (GL Science Inc., Tokyo, Japan) every hour for 6 h. As the primary outcome, oxidation of dietary fat was assessed by measuring recovery rate of ingested ¹³C-labelled triolein to ¹³CO₂ in the breath. Recovery rate of ¹³C was assessed by the combined use of an indirect calorimeter (Arco Systems Inc., Chiba, Japan) and a stable isotope ratio mass spectrometer (ANCA-GSL; Sercon, Crewe, UK).

2.6 Statistical analysis

Data are expressed as mean $\pm$ SD. Comparisons of the difference in two periods between the ALA-DAG treatment followed by the TAG treatment and the TAG treatment followed by the ALA-DAG treatment were performed using a two-sample t-test. A p-value of less than 0.05 was considered statistically significant.

3 RESULTS

3.1 Subjects and characteristics

Sixteen subjects (11 men and 5 women) completed the measurements and were included in the analyses, and 4 subjects did not complete the measurements. The baseline physical characteristics, including body composition, serum triglyceride, glucose, insulin, non-esterified fatty acid, total-, low-density-, and high-density-cholesterol did not significantly differ between the treatment orders. Table 2 shows the physical characteristics of the subjects after the treatments. Body fat ratio and fat mass were significantly lower after the ALA-DAG treatment compared with the TAG treatment. The serum parameters did not significantly differ after the treatments between ALA-DAG and TAG (data not shown).

3.2 Outcomes

The ^{13}C recovery at the 1 h time-point (Fig. 1A) and the cumulative ^{13}C recovery at 6 h (Fig. 1B) were significantly higher in the ALA-DAG treatment compared to the TAG treatment. The cumulative recovery of ^{13}C was $14.8 \pm 4.3\%$ in the TAG treatment and $17.1 \pm 4.0\%$ in the ALA-DAG treatment (Fig. 1B).

Table 2 Physical status of the subjects after the treatment with either TAG or ALA-DAG.

Parameter	TAG	ALA-DAG
Sex (M/F)	11/5	
Age (year)	49 $\pm$ 9	
Body weight (kg)	70.8 $\pm$ 8.9	70.4 $\pm$ 8.6
Body mass index (kg/m^2)	26.0 $\pm$ 2.0	25.8 $\pm$ 2.0
Body fat ratio (%)	29.2 $\pm$ 7.5	28.9 $\pm$ 7.6*
Fat mass (kg)	20.4 $\pm$ 4.4	20.0 $\pm$ 4.3*
Fat-free mass (kg)	50.4 $\pm$ 9.7	50.4 $\pm$ 9.7
Waist (cm)	92.4 $\pm$ 6.2	92.1 $\pm$ 6.3

Mean $\pm$ SD; n = 16. *, $p < 0.05$ between TAG and ALA-DAG.

4 DISCUSSION

In the present study, to gain further insight into the mechanism underlying the anti-visceral obesity effect of ALA-DAG, the recovery rate of ^{13}C -labelled dietary fat to $^{13}\text{CO}_2$ in the breath was assessed as an indicator of dietary fat oxidation during either ALA-DAG or TAG treatment. The ALA-DAG treatment enhanced dietary fat utilization as energy compared to the TAG treatment in humans. Taken together with our previous data showing increased postprandial total fat oxidation and energy expenditure after treatment with ALA-DAG¹¹, these findings suggest that ALA-DAG treatment induces up-regulation of dietary fat metabolism and contributes to prevent visceral obesity. Indeed, significantly decreased fat mass (Table 2) and significantly increased dietary fat oxidation (Fig. 1) were both observed in this present study.

Repeated consumption of ALA-DAG enhances β -oxidation in the small intestine¹² and liver¹³ in rodents.

Fig. 1 ^{13}C recovery in the breath for up to 6 h (A), and its cumulative value (B). Time zero corresponds to the ingestion time of the test meal containing ^{13}C -triolein. Data are presented as means $\pm$ SD. *, $p < 0.05$ between TAG and ALA-DAG.

Although the precise mechanism by which ALA-DAG consumption stimulates gene expression is not clear, it may be related to the metabolic pathway of ALA-DAG. In general, the TAG structure is hydrolyzed by lipase to 2-monoacylglycerol (MAG) and fatty acids and then resynthesized into TAG in the intestinal mucosa cells. In contrast, the DAG structure is hydrolyzed to 1-(or 3)MAG and fatty acids. Because 1-MAG is not easily resynthesized to TAG in the small intestine^{19, 20)}, the 1-MAG and free fatty acids may remain in the intestinal mucosa cells after DAG consumption²¹⁾. Omega 3 fatty acids, such as ALA, eicosapentaenoic acid, and docosahexaenoic acid increase the gene expressions including farnesoid X receptor and peroxisome proliferator-activated receptor compared to other fatty acids, such as saturated and omega 6 fatty acids²²⁾, suggesting that the free ALA derived from ALA-DAG activates fat oxidation and suppress fat synthesis, particularly in the small intestine and the liver. Thus, DAG and ALA can synergistically affect fat oxidation. Indeed, Murase *et al.* suggested that β -oxidation is higher in rodents fed ALA-DAG than in those fed ALA-TAG¹²⁾. Additionally, ALA-DAG can enhance fat oxidation at 25% of the dose compared to oleic and linolenic acid-rich DAG¹⁶⁾.

A limitation of the present study is that the ALA-DAG shortbread contained more ALA than the control TAG shortbread because we did not adjust the fatty acids component. Therefore, we cannot determine whether the DAG structure or the fatty acid composition was the major factor inducing the ALA-DAG effects. Additional studies with a similar fatty acid composition are required.

5 CONCLUSION

Treatment with ALA-DAG for 14-d enhanced the utilization of dietary fat as energy in healthy humans. This result indicates that ALA-DAG consumption has beneficial effects for preventing visceral obesity by upregulating dietary fat metabolism.

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CONFLICT OF INTEREST

This study was financially supported by Kao Corpora-

tion. ALA-DAG was prepared using equipment owned by Kao Corporation. Study management, sample collection, and data analysis were performed independently, and thus there are no conflicts of interest that would affect the study results.

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