Original Article
CLINICAL TRIALS AND INVESTIGATIONS

Obesity

Diacylglycerol Enhances the Effects of Alpha-Linolenic Acid Against Visceral Fat: A Double-Blind Randomized Controlled Trial

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Objective: To investigate the effect of alpha-linolenic acid-rich diacylglycerol (ALA-DAG) compared with alpha-linolenic acid-rich triacylglycerol (ALA-TAG) on visceral fat area (VFA) in people with overweight.
Methods: Subjects with overweight were recruited to a randomized, double-blind, controlled, parallel-group designed trial and randomly allocated to two groups that consumed either 2.5 g/d ALA-TAG or ALA-DAG for 12 weeks. Two 4-week nontreatment periods were placed before and after the treatment period. One hundred fourteen subjects ($n = 57$ in the ALA-TAG group, $n = 57$ in the ALA-DAG group) were enrolled into the analysis set for efficacy evaluation.
Results: The VFA and BMI were significantly decreased by the ALA-DAG treatment with a treatment-by-time interaction compared with the ALA-TAG treatment ($P < 0.05$). Additionally, the change from baseline of the fasting serum TAG concentration at week 12 was significantly decreased by ALA-DAG treatment compared with ALA-TAG treatment ($P < 0.05$). Safety parameters such as urinary measurements, hematologic parameters and blood biochemistry, and the incidence of adverse events did not differ significantly between groups, and no ALA-DAG–associated adverse effects were detected.
Conclusions: Incorporation of ALA-DAG in a regular diet for 12 weeks may lead to a reduction in VFA, BMI, and serum TAG in men and women with overweight.

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Introduction

Visceral fat is a well-known risk factor for mortality, probably independent from subcutaneous fat and waist circumference (WC) (1-3). Higher visceral fat area (VFA) is more strongly associated with the clustering of metabolic risk factors such as hypertension, high blood glucose, high triacylglycerol (TAG), and low high-density lipoprotein cholesterol than higher subcutaneous fat, WC, or BMI (4,5). These findings suggest that not only BMI but also VFA should be reduced to decrease the risk of metabolic syndrome-induced coronary heart disease. Lifestyle improvements, particularly dietary habits, are well established as important first-line therapy against visceral fat (6).

Alpha-linolenic acid (ALA) is a plant-derived polyunsaturated n-3 (omega-3) fatty acid that is considered an essential fatty acid because it cannot be produced in the human body. Studies in rodents have revealed that an ALA-rich oil upregulates fatty acid metabolism in the liver (7) and reduces liver fat and body weight (8,9) compared with high-oleic or -linoleic oils. In humans, however, ALA-rich oil does not affect energy metabolism (10,11) or body weight (12) compared with high-oleic or -linoleic oils. Therefore, we hypothesized that conventional ALA-rich oils have the potential to partially improve fat metabolism and obesity at a dose range appropriate for consumption by humans. While TAG is the dominant lipid structure, diacylglycerol (DAG) and monoacylglycerol are minor constituents making up ~10% of conventional edible oils that have a long history of human consumption. Dietary DAG is not easily resynthesized to TAG in the intestinal mucosa in animals (13-15) and thus reduces postprandial hyperlipidemia in humans compared with conventional edible oils containing TAG as the major constituent (16,17). Additionally, our recent studies have demonstrated that

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long-term repeated consumption of ALA-rich DAG (ALA-DAG) enhances energy metabolism in humans (18,19) and reduces VFA compared with oleic acid-rich rapeseed oil in people with obesity (20). In these previous reports, we could not determine whether the DAG structure enhanced the ALA effects against visceral fat because of a different fatty acid composition between the control oil (high-oleic TAG; rapeseed oil) and ALA-DAG. Therefore, in the present study, we investigated the effect of ALA-DAG on VFA in overweight individuals in comparison with ALA-rich TAG (ALA-TAG).

Methods

Ethics and management

This study was performed in accordance with the tenets of the Declaration of Helsinki (2013) and approved by the Ethics Review Board of Oriental Ueno Kenshin Center (Tokyo, Japan). After receiving a full explanation of the study, all participants provided written informed consent. The study was conducted at Oriental Ueno Kenshin Center (Tokyo, Japan) and Tohto Bunkyo Hospital (Tokyo, Japan) and was managed by a contract research organization (CRO), TES Holdings Co., Ltd. (Tokyo, Japan). The CRO managed the random allocation, enrollment, and assignment of participants and the blinding of the assignments and assessed outcomes under the supervision of the physician in charge.

Design and protocol

This was a randomized, double-blind, controlled, parallel-group designed study with a 12-week treatment period and a 4-week nontreatment period before and after the treatment period (−4 and +4 weeks). Randomized allocations to two groups consuming either ALA-TAG or ALA-DAG were conducted during the first 4-week nontreatment period (between weeks −4 and 0). The primary outcome was VFA, and secondary outcomes were BMI, WC, and safety parameters. Based on the mean global intake of ALA (1.4 g/d) reported in 2014 (21) and our previous human studies that have demonstrated positive effects (18-20), the dose of the test oils was set at 2.5 g/d, which contained 1.3∼1.4 g/d of ALA. One serving (2.5 g/d) of the test oils was packed into individual plastic bottles and consumed with the habitual diet of the subjects with no menu limitations (e.g., the test oils were allowed to be mixed with salad, yogurt, soup, cooked meat, bread, rice, or their favorite food item). The subjects were instructed to consume one full serving with one meal. Additionally, subjects were not allowed to add the oil to snacks between meals and were prohibited from cooking with it such as in a stir-fry. Treatment allocation was concealed throughout the study (from screening to finalizing the data set) among the people involved, including subjects, caregivers, physicians, CRO members, test diet manufacturer, and person in charge of allocation and outcome assessors. Based on the dietary records for the 3 days before the measurements, the amounts of nutrients consumed were analyzed. The subjects were instructed to limit their alcohol consumption to less than 30 g/d and to maintain their usual exercise and smoking habits during the study. To confirm their physical activity level, the subjects recorded their number of daily steps measured using a pedometer. Compliance was assessed from the records provided by the subjects and also checked by collected containers after consumption.

TABLE 1 Composition of the test oils

Constituents	ALA-TAG	ALA-DAG
Acylglycerols, g/100 g		
DAG	2.8	78.1
ALA esterified to DAG	1.6	37.0
Monoacylglycerol	0.0	0.5
Free fatty acid	0.1	0.1
TAG and others	97.2	21.3
Fatty acids, g/100 g		
C16:0	5.1	3.5
C18:0	3.2	2.1
C18:1	18.0	23.2
C18:2	14.9	16.5
C18:3	57.6	53.4
C20:0	0.0	0.0
C20:1	0.2	0.2
C22:0	0.2	0.2
Others	0.8	0.9

Subjects

Potential subjects were recruited from among volunteers who lived in the Tokyo area in Japan. The subjects were screened based on the inclusion and exclusion criteria. Inclusion criteria were as follows: (1) BMI 25 to < 30 kg/m^2; (2) VFA ≥ 120 cm^2; (3) men and postmenopausal women age 35 to < 65 years; and (4) ability to provide informed consent. Exclusion criteria were as follows: (1) presence of liver, kidney, and cardiovascular disease; respiratory, endocrine, metabolic, nervous system, or cognitive disorders; and diabetes mellitus or other diseases; (2) surgery within 2 months before the trial; (3) taking medications for hyperglycemia, lipidemia, or hypertension; (4) taking supplements or foods authorized by the government as specific health treatments; (5) unpleasant feeling during blood drawing; (6) donated 200 mL or more blood within 1 month before the trial; (7) changes in weight of 2 kg or more within 3 months before the trial; (8) shift worker; (9) business trip planned for 10 consecutive days or more during the trial; (10) allergies to any constituents in the test diet; (11) planned participation in another clinical trial; (12) unable to provide informed consent; and (13) determined to be unqualified by the physician in charge.

The power calculation was based on the results of a related study showing reduced VFA by ALA-DAG treatment (20). The power calculation indicated that 44 subjects per group were required (power 0.8 and type I error 0.05) to detect a significant difference in VFA. Assuming a 20% dropout rate, target enrollment was set at 60 per group. Potential subjects were allocated to each group by stratified randomization with sex, age, VFA, and BMI as stratification factors using computer-generated random numbers under blinded conditions.

Study period

Recruitment of participants was conducted in April 2016. Screening visits were performed from May 14 to May 29, 2016. The treatment period was for 12 weeks from June 18 to September 13, 2016. Visits for the measurements, including posttreatment observations, were completed by December 17, 2016.

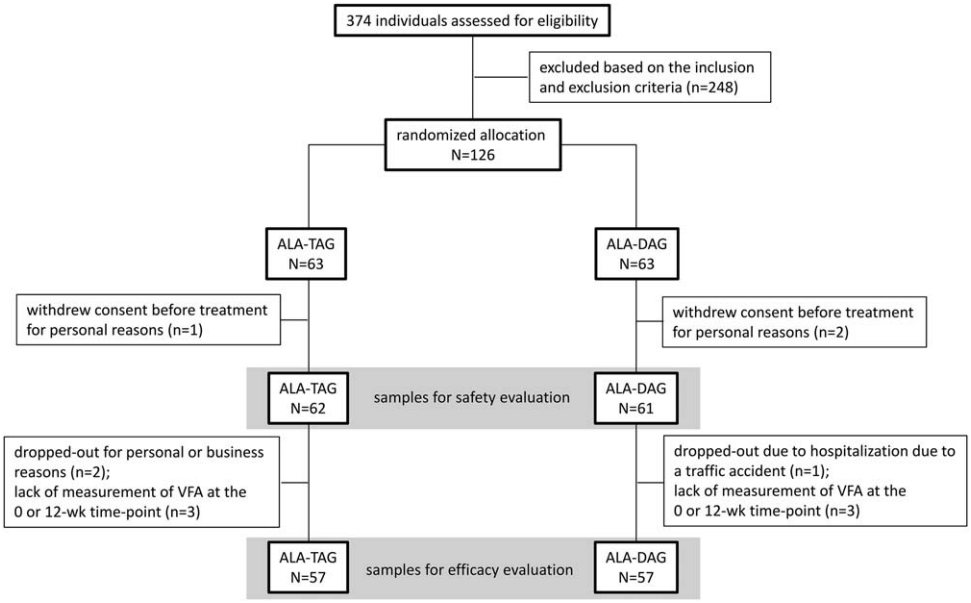

Figure 1 Participant flow through the study. Values are expressed as the number of participants.

Test oil

Crude ALA-DAG was manufactured from linseed oil (Summit Oil Corporation, Chiba, Japan) by the Kao Corporation (Tokyo, Japan) according to the method reported by Watanabe et al. (22). Crude ALA-TAG comprised linseed oil (Summit Oil Corporation, Chiba, Japan). To prepare the test oils for the trial, the crude oils were mixed with antioxidants and emulsifying agents to confer higher stability for storage. The test oils consisted of more than 99% pure oil and less than 1% stabilizers and were packaged in single doses to provide a daily dose of 2.5 g (22.5 kcal). ALA esterified to DAG constituted 0.9 g as fatty acid weight per 2.5 g of the ALA-DAG. ALA-TAG and ALA-DAG could not be distinguished by appearance, taste, or odor and were provided to the CRO after concealment. The CRO then reconcealed the test oils and provided them to the subjects. The subjects were instructed to store the test oils in a refrigerated state, and prior to consumption, they were to leave the test oil unrefrigerated until it reached room temperature. The compositions of the test oils are shown in Table 1.

Measurements

VFA was measured at weeks −4 (screening visit and start of pre-nontreatment period), 0 (baseline), 4, 8, 12 (end of treatment period), and +4 (end of post-nontreatment period) using an impedance instrument (EW-FA90; Panasonic Corporation, Osaka, Japan), which has results highly correlated with the computed tomography method (23) and is authorized as a medical device in Japan (No. 22500BZX00522000). The urinary measurements included glucose, protein, bilirubin, urobilinogen, ketone bodies, occult blood reaction, pH, and gravity. Overnight fasting blood samples were obtained to measure hematologic parameters such as white blood cells, red blood cells, hemoglobin, hematocrit, mean corpuscular volume, mean cell hemoglobin, mean corpuscular hemoglobin concentration, and platelets. Blood samples for hemoglobin A_{1c} measurement were collected using EDTA-2Na-containing tubes. Plasma samples for glucose measurement were obtained by centrifugation (4°C, 3000 rpm, 15 min) of the blood samples collected using EDTA-2Na-containing tubes. The other blood biochemistries or blood electrolytes (sodium, chlorine, calcium, phosphorus, and iron) were measured using serum samples obtained by centrifugation (4°C, 3000 rpm, 15 min) of the blood samples after standing at room temperature for 15 minutes. The urinary and blood measurements were analyzed by Health Sciences Research Institute, Inc. (Kanagawa, Japan). All samples were collected after the subjects fasted for more than 12 hours.

Statistics

Analyses were performed with SPSS Statistics version 19 software (IBM Inc., Armonk, New York). The samples for efficacy evaluation included data from all subjects who completed at least week 0 and 12 of the measurements. To determine the primary and secondary outcomes, significant differences were assessed using a linear mixed effects model with fixed effects for treatment and time interaction. As exploratory data analyses and safety evaluations, significant differences were assessed between groups using Student's *t* test when the data were normally distributed. When the data were not normally distributed, the data were log-transformed prior to analysis. When the data could not be log-transformed, the Mann-Whitney U test was performed. The incidence of adverse events was assessed with Fisher's exact test (two-tailed) in the samples, including data from all subjects who had at least one clinic visit after consuming at least one test oil. As additional analyses, correlation between the changes in VFA and all of the measured parameters was assessed in the ALA-DAG group for Pearson's coefficient of correlation when the data from both data sets were normally distributed and for Spearman's coefficient of correlation when the data from either data set were not normally distributed. A P value of less than 0.05 was considered statistically significant. Data are shown as mean ± SD.

TABLE 2 Dietary and step records during the study

	Week −4	Week 0	Week 4	Week 8	Week 12	Week +4
Energy, kJ/d						
ALA-TAG	6,990 ± 2,831	7,274 ± 2,971	7,354 ± 2,931	7,164 ± 2,812	7,329 ± 3,032	7,201 ± 2,835
ALA-DAG	6,825 ± 2,883	7,233 ± 2,930	6,967 ± 2,848	7,311 ± 3,373	7,167 ± 2,937	7,275 ± 3,067
Protein, g/d						
ALA-TAG	65.9 ± 14.9	68.4 ± 15.3	70.7 ± 18.2	66.3 ± 22.4	68.3 ± 17.9	67.7 ± 16.4
ALA-DAG	65.6 ± 15.6	71.7 ± 16.1	68.2 ± 15.3	67.9 ± 18.7	69.9 ± 18.6	70.5 ± 19.3
Fat, g/d						
ALA-TAG	65.6 ± 23.0	66.9 ± 22.9	67.2 ± 23.1	65.5 ± 21.2	67.7 ± 23.9	69.7 ± 21.7
ALA-DAG	62.9 ± 21.2	66.8 ± 19.0	61.0 ± 17.0	66.3 ± 25.2	68.3 ± 19.2	67.0 ± 21.9
CHO g/d						
ALA-TAG	232.6 ± 50.6	245.3 ± 64.2	248.6 ± 54.4	244.5 ± 43.4	248.1 ± 56.9	236.5 ± 48.3
ALA-DAG	223.6 ± 63.8	237.9 ± 64.1	236.7 ± 69.6	247.7 ± 85.4	233.5 ± 64.6	241.0 ± 73.0
Fiber, g/d						
ALA-TAG	11.0 ± 3.8	10.5 ± 3.7	11.5 ± 4.3	10.9 ± 3.4	11.6 ± 4.1	10.6 ± 2.8
ALA-DAG	9.9 ± 3.6	11.2 ± 3.4	10.5 ± 3.1	11.3 ± 3.5	11.0 ± 3.7	10.6 ± 3.3
		Week −4 ∼ −1	Week 0 ∼ 4	Week 5 ∼ 8	Week 9 ∼ 12	Week +1 ∼ +4
Step, steps/d						
ALA-TAG		7,408 ± 3,139	7,626 ± 3,128	7,502 ± 2,976	7,361 ± 2,594	7,545 ± 2,514
ALA-DAG		8,588 ± 3,142*	8,609 ± 3,304	8,539 ± 3,378	8,173 ± 3,132	8,617 ± 3,666

Data are mean ± SD.
*$P < 0.05$ between groups.
CHO, carbohydrate.

Results

Subjects and characteristics

As shown in Figure 1, of the 374 individuals who underwent screening, 126 were randomly assigned to one of the two treatment groups (63 subjects per group). In the ALA-TAG group, three subjects dropped out of the study (one subject withdrew before the treatment for personal reasons, one subject withdrew at week 4 for personal reasons, one subject dropped out at week 8 for business reasons), and three subjects did not complete the measurement of the primary outcome at the 0- or 12-week time points because of problems related to the measuring instruments or measuring conditions. In the ALA-DAG group, three subjects dropped out from the study (two subjects withdrew before the treatment for personal reasons and one subject withdrew at week 12 because of hospitalization following a traffic accident), and three subjects did not complete the measurement of the primary outcome at the 0- or 12-week time points because of problems related to the measuring instruments or measuring conditions. The sample for efficacy evaluation comprised 57 subjects (male/female, 45/12; age, 52 ± 7 years) in the ALA-TAG group and 57 (male/female, 45/12; age, 51 ± 7 years) in the ALA-DAG group. None of the parameters measured at baseline differed significantly between groups.

Daily habit

The consumption rates of the test oils were 99.6% ± 1.9% in the ALA-TAG group and 99.2% ± 4.0% in the ALA-DAG group, with no significant difference between groups. The subjects recorded their

TABLE 3 Changes in VFA

	Week −4	Week 0	Week 4	Week 8	Week 12	Week +4
Absolute value, cm^2						
ALA-TAG	152 ± 24	148 ± 28	152 ± 27	149 ± 28	150 ± 25	151 ± 26
ALA-DAG	150 ± 21	148 ± 27	144 ± 27	142 ± 24	143 ± 28†	145 ± 26
Change from week 0, Δcm^2						
ALA-TAG		0 ± 0	+4 ± 14	+1 ± 18	+2 ± 15	+3 ± 14
ALA-DAG		0 ± 0	−5 ± 21*	−7 ± 17*	−6 ± 19*	−4 ± 24

Data are mean ± SD.
Treatment-by-time interaction from week 0 to week 12 significantly different between groups, as assessed by linear mixed model; †$P < 0.05$.
As exploratory data analyses, significant differences in changes from baseline (delta value) between groups assessed; *$P < 0.05$.
VFA, visceral fat area.

TABLE 4 Changes in BMI, body weight, and WC

	Week −4	Week 0	Week 4	Week 8	Week 12	Week +4	Δ at week 12
BMI, kg/m^2							
ALA-TAG	27.0 ± 1.3	26.9 ± 1.3	26.9 ± 1.4	27.0 ± 1.4	27.0 ± 1.3	27.0 ± 1.3	+0.1 ± 0.6
ALA-DAG	26.9 ± 1.2	26.9 ± 1.3	26.8 ± 1.4	26.8 ± 1.4	26.7 ± 1.4†	26.7 ± 1.5	−0.2 ± 0.7*
Body weight, kg							
ALA-TAG	76.2 ± 6.7	76.1 ± 6.7	76.2 ± 6.8	76.3 ± 7.0	76.3 ± 7.2	76.3 ± 6.9	+0.3 ± 1.6
ALA-DAG	76.3 ± 6.9	76.1 ± 6.9	76.1 ± 7.2	76.1 ± 7.2	75.7 ± 7.3†	75.6 ± 7.3	−0.4 ± 1.8*
WC, cm							
ALA-TAG	98.7 ± 4.5	97.7 ± 4.6	98.7 ± 4.7	98.8 ± 5.1	98.8 ± 4.4	98.6 ± 4.3	+1.0 ± 2.9
ALA-DAG	98.7 ± 4.5	97.8 ± 5.1	98.5 ± 5.0	97.9 ± 4.7	98.1 ± 5.1	98.4 ± 5.1	+0.3 ± 2.8

Data are mean ± SD.
Treatment-by-time interaction from week 0 to week 12 significantly different between groups, as assessed by linear mixed model; †$P < 0.05$.
As exploratory data analyses, significant differences in changes from baseline (delta value) between groups assessed; *$P < 0.05$.
WC, waist circumference.

diets for 3 days before each clinic visit, and a registered dietician analyzed the dietary records based on the Food Composition Table (Kagawa Nutrition University Publishing Division, Saitama, Japan). The energy, protein, fat, carbohydrate, and fiber intake did not differ significantly between groups throughout the study. Although the mean number of steps differed significantly between groups from week −4 to week −1, no significant differences in number of steps were detected throughout the treatment period. The dietary and step records in the samples for efficacy evaluation are shown in Table 2.

Body composition variables as primary and secondary outcomes

The VFA (Table 3) and BMI (Table 4) in the ALA-DAG group were significantly decreased with a treatment-by-time interaction compared with those in the ALA-TAG group. Exploratory analyses indicated that the changes in VFA from baseline were significantly reduced in the ALA-DAG group compared with the ALA-TAG group at weeks 4, 8, and 12 (Table 3). Reductions of VFA were similar between men and women (delta values were −6 ± 20 cm^2 and −5 ± 18 cm^2, respectively), but no significant interactions were found between the groups in either sex presumably because of lack of power ($n = 45$ and 12, $P = 0.096$ and 0.125, respectively). The changes in BMI from baseline were also significantly reduced at 12 weeks (Table 4) in the ALA-DAG group but not at the other time points. Changes from baseline in VFA at week 12 in the ALA-DAG group correlated significantly with baseline VFA but not with baseline BMI (data not shown). Additionally, changes from baseline in VFA at week 12 in the ALA-DAG group correlated significantly with changes from baseline in BMI at week 12 (Figure 2A). WC did not differ significantly between groups (Table 4).

Blood biochemistry

There was a significant decrease in the serum TAG concentration in the ALA-DAG group compared with the ALA-TAG group even though there were small changes in the normal range (Table 5). One subject with normal serum TAG concentration at baseline (1.23

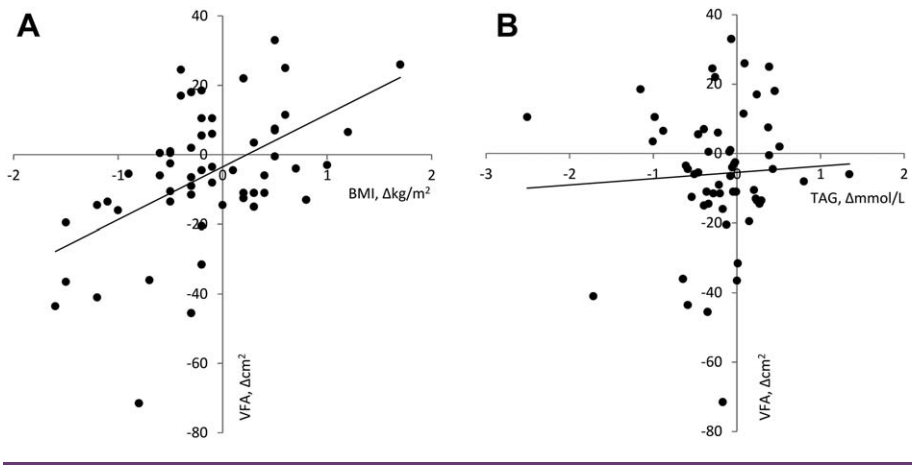

Figure 2 Correlation between (**A**) changes in VFA and BMI ($r = 0.522$, $P = 0.000$) and (**B**) VFA and serum TAG concentration ($r = 0.043$, $P = 0.750$) at week 12 from baseline in the ALA-DAG group.

TABLE 5 Changes in blood pressure and biochemistry

	Week −4	Week 0	Week 4	Week 8	Week 12	Week +4	Δ at week 12
SBP, mmHg							
ALA-TAG	116 ± 13	117 ± 14	119 ± 13	118 ± 14	119 ± 14	118 ± 15	+2 ± 10
ALA-DAG	117 ± 12	116 ± 11	115 ± 14	116 ± 13	117 ± 13	117 ± 13	+1 ± 9
DBP, mmHg							
ALA-TAG	75 ± 11	76 ± 12	75 ± 12	75 ± 12	75 ± 12	77 ± 12	−1 ± 8
ALA-DAG	76 ± 9	76 ± 9	75 ± 10	74 ± 11	77 ± 11	78 ± 10	+1 ± 8
TAG, mmol/L							
ALA-TAG	1.52 ± 0.73	1.43 ± 0.75	1.59 ± 1.74	1.71 ± 1.07	1.46 ± 0.78	1.48 ± 0.70	0.03 ± 0.47
ALA-DAG	1.58 ± 0.87	1.53 ± 0.89	1.57 ± 0.86	1.52 ± 0.73	1.35 ± 0.61	1.51 ± 0.91	−0.19 ± 0.58*
TAG in HN, mmol/L							
ALA-TAG	1.84 ± 0.56	1.53 ± 0.11	1.49 ± 0.46	1.80 ± 0.34	1.48 ± 0.35	1.69 ± 0.34	−0.03 ± 0.24
ALA-DAG	1.22 ± 0.37	1.52 ± 0.12	1.46 ± 0.73	1.28 ± 0.13*	1.05 ± 0.18*	1.82 ± 0.76	−0.48 ± 0.06**
TAG in MH, mmol/L							
ALA-TAG	2.03 ± 0.56	2.01 ± 0.12	1.80 ± 0.46	2.26 ± 0.76	1.97 ± 0.67	1.87 ± 0.35	−0.02 ± 0.48
ALA-DAG	1.75 ± 0.78	1.94 ± 0.17	1.89 ± 0.70	1.98 ± 0.91	1.48 ± 0.43	1.90 ± 0.64	−0.45 ± 0.42*
TAG in HT, mmol/L							
ALA-TAG	2.31 ± 0.67	2.49 ± 0.57	3.01 ± 2.96	2.62 ± 0.98	2.35 ± 0.85	2.16 ± 0.53	−0.10 ± 0.68
ALA-DAG	2.29 ± 0.91	2.50 ± 0.82	2.16 ± 0.77	2.05 ± 0.80	1.89 ± 0.58	2.10 ± 0.92	−0.60 ± 0.66*
TKB, μmol/L							
ALA-TAG		103 ± 104	82 ± 100	79 ± 104	77 ± 80	78 ± 74	−26 ± 86
ALA-DAG		127 ± 216	80 ± 121	101 ± 229	115 ± 209	118 ± 187	−11 ± 77
TC, mmol/L							
ALA-TAG	5.76 ± 0.84	5.65 ± 0.80	5.78 ± 0.86	5.62 ± 0.82	5.58 ± 0.88	5.69 ± 0.80	−0.06 ± 0.51
ALA-DAG	5.84 ± 0.70	5.71 ± 0.83	5.89 ± 0.81	5.71 ± 0.88	5.68 ± 0.87	5.80 ± 0.86	−0.03 ± 0.41
LDL-C, mmol/L							
ALA-TAG	3.67 ± 0.71	3.63 ± 0.69	3.65 ± 0.77	3.47 ± 0.67	3.52 ± 0.71	3.59 ± 0.68	−0.11 ± 0.45
ALA-DAG	3.77 ± 0.65	3.72 ± 0.76	3.76 ± 0.69	3.63 ± 0.80	3.66 ± 0.80	3.71 ± 0.80	−0.05 ± 0.38
HDL-C, mmol/L							
ALA-TAG	1.48 ± 0.27	1.47 ± 0.26	1.44 ± 0.29	1.40 ± 0.27	1.41 ± 0.27	1.45 ± 0.28	−0.06 ± 0.12
ALA-DAG	1.44 ± 0.35	1.41 ± 0.35	1.41 ± 0.33	1.37 ± 0.32	1.38 ± 0.33	1.42 ± 0.35	−0.03 ± 0.14
Glucose, mmol/L							
ALA-TAG	5.23 ± 0.39	5.36 ± 0.40	5.32 ± 0.36	5.22 ± 0.37	5.30 ± 0.52	5.35 ± 0.51	−0.06 ± 0.42
ALA-DAG	5.36 ± 0.43	5.50 ± 0.56	5.48 ± 0.55	5.41 ± 0.57*	5.41 ± 0.54	5.48 ± 0.65	−0.08 ± 0.46
HbA_{1c}, %							
ALA-TAG	5.5 ± 0.2	5.5 ± 0.2	5.4 ± 0.2	5.5 ± 0.2	5.4 ± 0.2	5.5 ± 0.3	0.0 ± 0.1
ALA-DAG	5.4 ± 0.4	5.4 ± 0.3	5.4 ± 0.3	5.4 ± 0.4	5.4 ± 0.4	5.5 ± 0.4	0.0 ± 0.1
Insulin, pmol/L							
ALA-TAG		56.5 ± 21.3	54.1 ± 21.3	63.0 ± 47.3	62.5 ± 67.9	67.8 ± 54.6	8.4 ± 58.8
ALA-DAG		54.4 ± 27.0	53.2 ± 34.3	57.4 ± 27.5	51.0 ± 26.1	56.4 ± 35.0	−2.2 ± 25.8
AST, U/L							
ALA-TAG	21 ± 5	23 ± 6	22 ± 6	21 ± 7	22 ± 8	23 ± 7	−1 ± 6
ALA-DAG	22 ± 5	23 ± 6	21 ± 5	21 ± 6	20 ± 4	21 ± 5	−3 ± 5
ALT, U/L							
ALA-TAG	27 ± 10	29 ± 15	27 ± 15	26 ± 13	28 ± 15	29 ± 16	−1 ± 8
ALA-DAG	26 ± 10	26 ± 11	23 ± 8	22 ± 9	23 ± 9*	23 ± 10*	−3 ± 7
γ-GT, U/L							
ALA-TAG	36 ± 19	37 ± 21	36 ± 21	37 ± 25	37 ± 20	37 ± 22	0 ± 8
ALA-DAG	34 ± 20	33 ± 20	33 ± 26	33 ± 22	33 ± 20	32 ± 23	0 ± 5
ALP, U/L							
ALA-TAG		216.6 ± 43.9	214.4 ± 53.9	212.1 ± 49.2	211.3 ± 51.5	210.7 ± 47.5	−5.3 ± 20.7
ALA-DAG		208.2 ± 54.1	209.7 ± 54.3	204.8 ± 52.3	210.1 ± 55.6	209.0 ± 55.3	1.9 ± 26.2

TABLE 5 (continued).

	Week −4	Week 0	Week 4	Week 8	Week 12	Week +4	Δ at week 12
LDH, U/L							
ALA-TAG		174.2 ± 21.8	171.6 ± 23.2	177.2 ± 25.3	172.0 ± 22.5	169.3 ± 22.2	−2.2 ± 12.3
ALA-DAG		176.6 ± 24.3	176.5 ± 25.3	179.5 ± 25.6	175.7 ± 23.0	172.5 ± 24.6	−0.9 ± 13.4
TP, g/L							
ALA-TAG		74.0 ± 3.3	73.3 ± 3.1	73.4 ± 3.3	72.6 ± 3.2	73.0 ± 3.2	−1.4 ± 2.5
ALA-DAG		73.3 ± 4.0	73.5 ± 4.1	73.0 ± 3.0	73.2 ± 3.9	73.3 ± 3.6	−0.1 ± 2.1**
Albumin, g/L							
ALA-TAG		46.1 ± 2.2	45.5 ± 2.7	43.7 ± 2.7	44.0 ± 2.3	43.7 ± 2.6	−2 ± 2
ALA-DAG		46.1 ± 2.5	45.6 ± 2.5	43.6 ± 2.3	44.7 ± 2.2	44.2 ± 2.3	−1 ± 2*
Uric acid, μmol/L							
ALA-TAG	376 ± 65	387 ± 71	380 ± 65	365 ± 67	375 ± 70	382 ± 72	−12 ± 35
ALA-DAG	363 ± 69	368 ± 61	375 ± 65	364 ± 72	370 ± 72	367 ± 70	2 ± 43
Creatinine, μmol/L							
ALA-TAG	78 ± 13	79 ± 14	76 ± 14	78 ± 14	75 ± 12	74 ± 14	−4 ± 5
ALA-DAG	77 ± 13	78 ± 13	77 ± 13	78 ± 13	76 ± 12	74 ± 13	−1 ± 5*
UN, mmol/L							
ALA-TAG	4.8 ± 1.2	5.2 ± 1.4	4.9 ± 1.3	4.8 ± 1.2	4.7 ± 1.3	4.7 ± 1.2	−0.5 ± 1.0
ALA-DAG	4.6 ± 1.2	5.1 ± 1.4	4.6 ± 1.1	4.7 ± 1.5	4.8 ± 1.3	4.7 ± 1.2	−0.3 ± 1.0

Data are mean ± SD.
Significant differences between groups, *$P < 0.05$, **$P < 0.01$.
TAG in high normal (HN), modest hypertriglyceridemia (MH), and hypertriglyceridemia (HT); stratified analyses performed in subgroup with high normal ($1.36 \leq$ TAG ≤ 1.69 mmol/L) and modest hypertriglyceridemia ($1.70 \leq$ TAG ≤ 2.25 mmol/L) and with hypertriglyceridemia (TAG ≥ 1.70 mmol/L).
TKB, insulin, ALP, LDH, TP, and albumin were not measured at week -4.
SBP, systolic blood pressure; DBP, diastolic blood pressure; TKB, total ketone bodies; TC, total cholesterol; LDL-C, low-density lipoprotein-cholesterol; HDL-C, high-density lipoprotein-cholesterol; HbA_{1c}, hemoglobin A_{1c}; AST, aspartate aminotransferase; ALT, alanine aminotransferase; γ-GT, γ-glutamyltransferase; ALP, alkaline phosphatase; LDH, lactase dehydrogenase; TP, total protein; UN, urea nitrogen.

mmol/L) showed a great change at week 8 (+5.13 mmol/L from baseline), and this may have contributed to the drastic change in serum TAG concentrations between weeks 8 and 12 in the ALA-TAG group. The large change did not persist up to week 12, and no other subjects had such a large change in either group at weeks 8 and 12. The changes from baseline in the serum TAG concentration at week 12 in the ALA-DAG group did not significantly correlate with the changes from baseline in VFA at week 12 (Figure 2B). The stratified analyses showed that the delta value at week 12 in the serum TAG concentration in the subgroup with high normal ($1.36 \leq$ TAG ≤ 1.69 mmol/L; $n = 7$ in the ALA-TAG and $n = 4$ in the ALA-DAG) and modest hypertriglyceridemia ($1.70 \leq$ TAG ≤ 2.25 mmol/L; $n = 7$ in the ALA-TAG and $n = 10$ in the ALA-DAG) (Table 5), and with hypertriglyceridemia (TAG ≥ 1.70 mmol/L;

TABLE 6 Incidence and list of adverse events

	ALA-TAG	ALA-DAG
Total number of subjects for safety evaluation	62	61
Number of subjects reporting adverse events	26	23
List of adverse events reported (alphabetical order)	anathema; backache; eye trouble (dry eye or eye fatigue); fatigue; gastrointestinal complaint; headache; high AST, ALT, or γ-GT level; high glucose, insulin, ketone bodies, TAG, or uric acid levels; incisura; joint ache; labial herpes; mouth inflammation; muscle ache; toothache; upper respiratory infection; urinary lithiasis	anathema; backache; bladder inflammation; broken bone; bruise; eye trouble (swelling); fatigue; gastrointestinal complaint; genital pain; headache; high AST, ALT, or γ-GT level; high ketone bodies or TAG levels; incisura; joint ache; labial herpes; mouth inflammation; sleeplessness; upper respiratory infection; urinary lithiasis

Number of subjects who reported an adverse event was not significantly different between groups, as assessed by Fisher's exact test.
AST, aspartate aminotransferase; ALT, alanine aminotransferase; γ-GT, γ-glutamyltransferase.

$n = 15$ in the ALA-TAG and $n = 20$ in the ALA-DAG) in the ALA-DAG group compared with the ALA-TAG group. The changes from baseline in the serum total protein and albumin concentrations at week 12 and the change from baseline in the serum glucose concentration at week 8 were significantly smaller in the ALA-TAG group than in the ALA-DAG group (Table 5). The serum alanine aminotransferase concentration as a liver function parameter was significantly improved in the ALA-DAG group compared with the ALA-TAG group at weeks 12 and +4. The serum creatinine concentration as a measure of kidney function was slightly decreased in both groups, and the change from baseline in the serum creatinine concentration at week 12 in the ALA-TAG group was significantly smaller than that in the ALA-DAG group.

Safety

As shown in Table 6, of the 62 subjects in the ALA-TAG group and 61 subjects in the ALA-DAG group included in the safety evaluation, adverse events were reported by 26 and 23 subjects, respectively. The incidence of adverse events did not differ significantly between groups as assessed by Fisher's exact test. The most common adverse events were upper respiratory infection, gastrointestinal complaint, and headache. Other adverse events were considered to be mild or moderate. Because these symptoms were not persistent or likely to occur on a daily basis, adverse effects induced directly by the ALA-DAG consumption were not considered to have occurred in this trial.

One serious adverse event was reported in one subject in the ALA-DAG group. The subject was hospitalized with broken bones from a traffic accident and dropped out of the study. The physician in charge deemed no cause-and-effect relationship between ALA-DAG and this serious adverse event.

In general, the incidence of abnormal blood values and urinary measurements was similar between groups. Throughout the study, there was no significant difference between groups in the absolute values of heart rate, body temperature, hematologic parameters, or blood electrolytes. Only the potassium values at weeks 4 and 12 and the magnesium values at week 12 were significantly higher in the ALA-DAG group than in the ALA-TAG group, but the means were within the normal range. Comprehensively, abnormal changes beyond physiologic variation in all measured safety parameters among the individual subjects throughout the study were not considered to have occurred in this trial.

Discussion

ALA-rich linseed and perilla oils are conventionally consumed globally (21) and are reported to improve fat metabolism and reduce body fat in rodents (7-9) but not in humans (10-12). In the present study, ALA-TAG did not reduce VFA and BMI from baseline (Tables 3 and 4). These results indicate that the effects of consuming ALA alone on VFA and BMI are not adequate in the amounts consumed by humans. Previous studies in rodents reported that ALA-DAG stimulates beta-oxidation-related enzymes and gene expression in the small intestine (24) and liver (25) compared with ALA-TAG. Therefore, we hypothesized that the DAG structure enhances the effect of ALA even in humans, and the results of this study support our hypothesis. The effect of ALA-DAG on VFA was moderate but clear and was detected in a relatively short period (4-week time points, Table 3). Additionally, the reduction of VFA correlated significantly with the reduction in BMI (Figure 2A), suggesting that weight loss was accompanied by a reduction in body fat. Weight loss was maintained and no rebound was observed during the follow-up period (Table 4). These findings led us to conclude that the DAG structure enhanced the effect of ALA beyond the improvement of visceral obesity in humans, leading to healthy weight loss with no rebound within a month.

In the present study, serum TAG concentrations were improved in the ALA-DAG group compared with the ALA-TAG group (Table 5). A possible mechanism is that the availability of fatty acids as a substrate for TAG synthesis was reduced because of induced beta-oxidation in the small intestine and liver by the ALA-DAG treatment (24,25), leading to reduced TAG accumulation in the liver (25) and serum TAG concentration. Although increasing VFA is strongly associated with the clustering of metabolic risk factors (4), reduction of the serum TAG concentrations did not correlate with the reduction of VFA (Figure 2B). This finding suggests that the reduction of serum TAG is independent from the reduction of VFA.

Although WC was reported to correlate with visceral fat in both diabetic and nondiabetic subjects (26), it was also reported that the correlation between VFA and WC varied considerably among individuals; for example, men with WC between 85.0 and 86.0 cm had VFA in the range of 67 to 137 cm^2 in a Japanese population (23). In the present study, these individual variations or other factors, such as seasonal variation of subcutaneous fat, could potentially affect the result showing no reduction of WC in the ALA-DAG group, despite the significant reduction of VFA in the present study. Because subcutaneous fat was not measured in this study, we cannot discuss the relationship among VFA, subcutaneous fat, and WC, and this is a limitation of the study.

Other limitations and potential biases of this trial are the imbalanced number of male and female subjects (45 men and 12 women in both groups, respectively); the recruitment of men and postmenopausal women but not premenopausal women; study performed in a single race (Japanese); some of the included authors being employees of the manufacturer of the test oil; and exploratory analyses being performed statistically without adjusting the P values for multiple comparisons. Although a slightly lower energy intake and a slightly greater number of steps during the treatment period were observed in the ALA-DAG group compared with the ALA-TAG group, which might affect primary and secondary outcomes, these differences were not statistically significant and were not considered to have enough of an impact to overturn the conclusion.

Conclusion

This study was performed under sufficiently powered and adequately blinded conditions. These findings suggest that incorporation of ALA-DAG in a regular diet for 12 weeks reduces VFA, BMI, and serum TAG in men and women with overweight.

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