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

Yasutoshi Ando, Shinichiro Saito*, Sachiko Oishi, Nami Yamanaka, Masanobu Hibi, Noriko Osaki and Yoshihisa Katsuragi

Healthcare Food Research Laboratories, Kao Corporation (2-1-3 Bunka Sumida-ku Tokyo 131-8501, JAPAN)

Abstract: Alpha linolenic acid-enriched diacylglycerol (ALA-DAG) reduces visceral fat area and body fat in rodents and humans compared to conventional triacylglycerol (TAG). Although ALA-DAG increases dietary fat utilization as energy in rodents, its effects in humans are not known. The present study was a randomized, placebo-controlled, double-blind, crossover intervention trial performed to clarify the effect of ALA-DAG on postprandial energy metabolism in humans. Nineteen healthy subjects participated in this study, and postprandial energy metabolism was evaluated using indirect calorimetry followed by 14-d repeated pre-consumption of TAG (rapeseed oil) as a control or ALA-DAG. As a primary outcome, ALA-DAG induced significantly higher postprandial fat oxidation than TAG. As a secondary outcome, carbohydrate oxidation tended to be decreased. In addition, postprandial energy expenditure was significantly increased by ALA-DAG compared to TAG. These findings suggest that daily ALA-DAG consumption stimulates dietary fat utilization as energy after a meal, as well as greater diet induced thermogenesis in healthy humans. In conclusion, repeated consumption of ALA-DAG enhanced postprandial fat metabolism after a meal, which may partially explain its visceral fat area-reducing effect.

Key words: energy expenditure, fat oxidation, indirect calorimetry, human, ALA-DAG

1 INTRODUCTION

Disruption of the balance between energy intake and expenditure induces obesity. Severe obesity is a risk factor for type 2 diabetes, stroke, and cardiovascular disease¹⁻³. Managing body weight is important to decrease these risk factors. Energy expenditure, fat oxidation, and fat storage capacity also affect body fat and visceral fat area (VFA)⁴⁻⁶. Increasing fat for use as energy is an evolving concept as a way to maintain fat and energy balance. Therefore, food ingredients affecting energy metabolism may help people to successfully control or manage their weight.

Diacylglycerol (DAG), which comprises mainly 1,3-DAG, is a minor component of various edible oils^{7,8}. The nutritional characteristics and physiologic effects of DAG are thought to be due to differences in the chemical structure from that of TAG. Long-term ingestion of DAG consisting mainly of linoleic and oleic acids, compared to TAG with a similar fatty acid composition, is effective for reducing both body weight and VFA in rodents^{9,10} and humans¹¹⁻¹³. In addition, DAG activates the enzymes involved in

β -oxidation in the liver⁹ and small intestine¹⁰, and increases fat utilization¹⁴. On the other hand, α -linolenic acid (ALA), which is abundant in linseed and perilla oil, is also reported to have nutritional properties distinct from those of other fatty acids. For example, ALA is easier to burn in the body than palmitic, stearic, oleic, or linoleic acid in rodents^{15,16} and humans¹⁷. Based on these reports, alpha linolenic acid-enriched diacylglycerol (ALA-DAG) is a potentially useful fat ingredient to improve postprandial energy metabolism. Indeed, previous studies suggested that ALA-DAG consumption activates β -oxidation and up-regulates gene expression of thermogenesis-related enzymes in the small intestine and the liver in animal models^{18,19}. The relationship between daily ALA-DAG ingestion and postprandial energy metabolism in humans, however, remains unclear. The purpose of the present study was to investigate the effect of ALA-DAG consumption on postprandial energy metabolism, particularly fat oxidation as a primary outcome, in humans.

*Correspondence to: Shinichiro Saito, Healthcare Food Research Laboratories, Kao Corporation, 2-1-3 Bunka Sumida-ku Tokyo 131-8501, JAPAN

E-mail: saito.shinichiro@kao.co.jp

Accepted April 6, 2016 (received for review March 11, 2016)

Journal of Oleo Science ISSN 1345-8957 print / ISSN 1347-3352 online

<http://www.jstage.jst.go.jp/browse/jos/> <http://mc.manuscriptcentral.com/jjoc>

2 EXPERIMENTAL

2.1 Ethics and registration

This study was performed in accordance with the Declaration of Helsinki (2013) and approved by the Ethics Review Board of Kao Corporation. After receiving a full explanation of the study, all participants provided written informed consent. The study was conducted under the supervision of a physician and managed by Kao Corporation (Tokyo, Japan). The study protocol was registered at the University Hospital Medical Information Network (UMIN-CTR, <http://www.umin.ac.jp/ctr/index-j.htm>) prior to enrolling the first subject (UMIN ID: UMIN000018843).

2.2 Subjects

Nineteen healthy Japanese subjects were recruited based on the inclusion and exclusion criteria. Inclusion criteria were as follows: 1) age 25 to 59 years, 2) ability to approve medical record access by the supervising physician, 3) ability and willingness to comply with the study protocol, 4) ability to provide informed consent, and 5) a male employee of Kao Corporation. Exclusion criteria were as follows: 1) presence of liver, kidney, and heart disease; respiratory, endocrine, metabolic, nervous system, or cognitive disorders; or diabetes or other diseases, 2) surgery within 2 months before the trial, 3) unpleasant feeling during drawing blood or energy expenditure analysis, 4) donated 200 mL or more blood within 1 month before the trial, 5) taking medications for hyperglycemia, lipidemia, or hypertension, 6) VFA under 25 cm², 7) unstable energy expenditure, 8) inability or unwillingness to conform to the alcohol limitation, 9) taking supplements or foods that affect body weight and serum lipid levels, 10) changes in body weight of ≥ 2 kg within 1 month before the trial, 11) planned business trip lasting 5 consecutive days or more, 12) allergies to ingredients in the test food or equipment used for indirect calorimetry, 13) not accustomed to the test diet, 14) smoker, 15) inability or unwillingness to record daily diet, 16) inability or unwillingness to consume the test diet during the study period, 17) participating or planning to participate in other clinical studies, 18) determined to be unqualified by the supervising physician based on the medical record, 19) determined to be unqualified by the supervising physician for other reasons.

The sample size was estimated based on a previous study performed by Hibi *et al.*²⁰⁾. In their study, a significant difference between TAG and DAG was observed in 8 subjects when postprandial fat oxidation was measured using indirect calorimetry. The VFA-reducing effect is estimated to be similar between 10.0 g DAG²¹⁾ and 2.5 g ALA-DAG²²⁾. Moreover, the mechanisms of the VFA-reducing effect are also similar (activate β -oxidation in the liver and the small intestine in animal models^{9, 10, 18, 19)}). Thus, we estimated that a similar sample size was needed in this study. Therefore, we planned to recruit more than 8 but a

maximum of 30 subjects from volunteers to obtain significance and remain within our capacity to manage the study.

2.3 Design and protocol

This was a randomized, double-blind, controlled crossover study with two 14-d intervention periods, separated by washout periods of more than 14-d. The subjects consumed either ALA-DAG or control TAG daily, and postprandial energy metabolism was measured by indirect calorimetry at the end of each 14-d intervention period. The participants were randomly assigned to each treatment using computer-generated random numbers. The primary outcome was postprandial fat oxidation and secondary outcomes were postprandial energy expenditure, carbohydrate oxidation, and respiratory quotient.

During each intervention period, all subjects were instructed to maintain their habitual lifestyle, including usual physical activity and dietary intake. The subjects were asked to record their meals for 3 days before the measurements, and nationally registered dietitians analyzed the meals by referring to the fifth edition of the Food Composition Table (Kagawa Nutrition University Publishing Division). Alcohol intake and hard exercise were not allowed for the 3 days before the measurements. On the day before the measurements, the subjects consumed identical designed meals for breakfast, lunch, and dinner (total energy: 2253 kcal/day; protein: fat: carbohydrate = 14: 32: 53 as energy value). All measurements were performed after fasting for at least 12 h.

2.4 Test diet

The ALA-DAG was prepared using the method reported by Watanabe *et al.*²³⁾ 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). DAG-bound ALA comprised 0.9 g as fatty acid weight per 2.5 g of ALA-DAG. The control TAG was rapeseed oil (The Nisshin OilliO Group, Ltd, Tokyo, Japan). The composition of TAG and ALA-DAG is shown in the **Table 1**. The prepared ALA-DAG was mixed with rapeseed oil, anti-oxidants, and emulsifying agents to produce a cooking oil. A test shortbread was prepared for accurate and certain ingestion of ALA-DAG or TAG, and contained hard flour, soft flour, superfine sugar, salt, egg, pullulan, and water in addition to the cooking oil. The test shortbread had 291 kcal (protein: fat: carbohydrate = 8: 39: 53 as energy value) per serving (2.5 g ALA-DAG or TAG in 60 g of shortbread). The test shortbread was individually packaged and whether it contained TAG or ALA-DAG could not be determined based on its appearance, taste, or odor.

2.5 Indirect calorimetry

On the day of the measurement, the subjects were

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

Constituent	TAG	ALA-DAG
Acylglycerols (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 acids (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

adapted to a room maintained 25°C for 15 min at 8:00 AM. Breath analysis was performed between 8:30 and 9:30 for the 0-min time-point. After breath analysis at 0-min, the subjects ingested a 583 kcal (protein: fat: carbohydrate = 15: 34: 51 as energy value) test meal containing 5.0 g TAG or ALA-DAG and then a breath analysis was performed intermittently for up to 300 min using an ARCO-2000 open-circuit breath-by-breath gas-exchange measurement system (Arco Systems Inc., Chiba, Japan). The subjects were asked to remain sedentary throughout the study period, except for getting up to go to the bathroom.

Breath analysis was performed to obtain values of oxygen consumption (VO_2) and carbon dioxide production (VCO_2) for 17 min at each time-point, which comprised 2 min for equilibrating and 15 min for measurement. The energy expenditure and substrate utilizations were calculated from the measured values of VO_2 and VCO_2 according to the following equations^{24, 25}. Protein oxidation (P) was estimated from test meal.

$$\text{Energy expenditure (kcal)} = 3.9 \times \text{VO}_2 (\text{L}) + 1.1 \times \text{VCO}_2 (\text{L})$$

$$\text{Fat oxidation (g)} = 1.718 \times \text{VO}_2 (\text{L}) - 1.718 \times \text{VCO}_2 (\text{L}) - 0.315 \times \text{P (g)}$$

$$\text{Carbohydrate oxidation (g)} = 4.170 \times \text{VCO}_2 (\text{L}) - 2.965 \times \text{VO}_2 (\text{L}) - 0.390 \times \text{P (g)}$$

2.6 Other parameters

Body composition (height, weight, body fat ratio, and VFA) was measured in a fasting state before the breath analysis. VFA was measured using EW-FA90 (Panasonic Corporation, Osaka, Japan) based on the bio-impedance method, which highly correlates with the computed tomography method²⁶.

2.7 Statistical analysis

Analyses were performed with SPSS version 19 software (IBM Inc. Tokyo, Japan). Significant differences between treatments were assessed using a paired *t* test when the data were normally distributed. When the data were not normally distributed, the Wilcoxon signed-rank test was applied. Changes in postprandial fat oxidation were analyzed using a linear mixed effects model with fixed effects for treatment, time-point, and their interaction. A *P* value of less than 0.05 was considered statistically significant.

3 RESULTS

3.1 Subjects and characteristics

All subjects (40 ± 8 y of age, mean $\pm$ SD) completed the study and were included in the analysis. The dietary records indicated high compliance with the test diet during the 14-d intervention periods, and the consumption rate of the test shortbread was 100% for both treatments. Body composition did not significantly differ between the TAG and ALA-DAG treatments after the 14-d intervention

Table 2 Anthropometric parameter.

Parameter	TAG	ALA-DAG
Body Weight (kg)	68.2 $\pm$ 8.4	68.3 $\pm$ 8.5
Body mass index (kg/m ²)	23.0 $\pm$ 2.6	23.0 $\pm$ 2.6
Body fat ratio (%)	18.4 $\pm$ 3.1	18.2 $\pm$ 2.8
Visceral fat area (cm ²)	71 $\pm$ 41	70 $\pm$ 35
Waist (cm)	83.2 $\pm$ 7.9	82.8 $\pm$ 7.5

Mean $\pm$ SD; *n* = 19. Differences between treatments were assessed using paired *t*-tests. No significant differences were observed between treatments.

Table 3 Dietary records.

	TAG	ALA-DAG
Energy intake (kcal/day)	2217 $\pm$ 257	2176 $\pm$ 227
Protein intake (g/day)	76.4 $\pm$ 10.4	77.3 $\pm$ 10.6
Fat intake (g/day)	81.0 $\pm$ 12.2	80.3 $\pm$ 11.9
Carbohydrate intake (g/day)	285.1 $\pm$ 43.9	277.0 $\pm$ 33.8

Mean $\pm$ SD; *n* = 19. Differences between treatments were assessed using paired *t*-tests. No significant differences were observed between treatments.

(Table 2). Energy, protein, fat, and carbohydrate intake did not significantly differ between the two treatments for the 3 days before the measurement (Table 3).

3.2 Outcomes

As a primary outcome, changes in postprandial fat oxidation were significantly greater following ALA-DAG treatment compared with TAG treatment when assessed by a linear mixed effects model with fixed effects for treatment, but not significant for time and treatment interaction (Fig.

1). The incremental area under the curve (iAUC) of fat oxidation was also significantly higher following ALA-DAG treatment, but the AUC of fat oxidation did not differ significantly between the treatments (Table 4). On the other hand, the iAUC of postprandial carbohydrate oxidation was slightly decreased following ALA-DAG treatment, but were not significantly different between the two treatments. The postprandial total energy expenditure and respiratory quotient are shown in Table 5. Mean postprandial energy expenditure was significantly increased by ALA-DAG treat-

Fig. 1 Changes in postprandial fat oxidation measured using the indirect calorimeter for up to 300 min after ingesting either TAG or ALA-DAG for 14 d. The dashed line connecting open circles indicates the TAG treatment, and the solid line connecting the solid circles indicates the ALA-DAG treatment. Data are delta values from baseline and expressed as means $\pm$ SE. P value assessed by a linear mixed effects model with fixed effects for treatment is expressed.

Table 4 Area under the curve (AUC) and incremental area under the curve (iAUC) of substrate oxidation.

	AUC		iAUC	
	TAG	ALA-DAG	TAG	ALA-DAG
Fat oxidation (g/300min)	9.67 $\pm$ 3.77	9.96 $\pm$ 6.89	3.34 $\pm$ 2.92	2.12 $\pm$ 2.62*
CHO oxidation (g/300min)	33.80 $\pm$ 6.13	34.96 $\pm$ 15.93	15.51 $\pm$ 8.04	13.45 $\pm$ 5.95

Mean $\pm$ SD; n = 19. Differences between treatments were assessed using paired *t*-tests. *, significantly greater than TAG treatment; *p* < 0.05. CHO, carbohydrate.

Table 5 Average of energy expenditure and respiratory quotient.

	TAG	ALA-DAG
Energy expenditure (kcal/min for 300 min)	1.096 $\pm$ 0.095	1.124 $\pm$ 0.109*
Respiratory quotient (L/L for 300 min)	0.854 $\pm$ 0.025	0.854 $\pm$ 0.051

Mean $\pm$ SD; n = 19. Differences between treatments were assessed using paired *t*-tests. *, significantly greater than TAG treatment; *p* < 0.05.

ment compared with TAG treatment.

4 DISCUSSION

In the present study, we observed enhanced postprandial fat oxidation and total energy expenditure after 14-d of repeated consumption of ALA-DAG compared to TAG. Postprandial carbohydrate utilization was slightly, but not significantly, decreased by ALA-DAG. These findings suggested that ALA-DAG induced higher diet-induced thermogenesis mainly by enhancing postprandial fat utilization. In previous studies, subjects who consumed ALA-DAG for ~12 to 16-wk exhibited reduced VFA compared to subjects who consumed TAG^{22, 27)}. Because diet-induced thermogenesis accounts for approximately 10% of daily energy expenditure²⁸⁾, our observation could partially explain the VFA-reducing effect induced by ALA-DAG consumption. Additionally, the mean difference in postprandial energy expenditure between the TAG and ALA-DAG treatments was 8.4 kcal per 300 min, correlating with 0.63 kg of body fat over a 16-wk period. Thus, our observation is an appropriate predictor of the VFA-reduction effect. Of interest, Katsuragi *et al.*²⁷⁾ reported that fasting energy expenditure was significantly increased after 6-wk consumption of ALA-DAG. Although their study was performed as a single-arm trial with no comparison with a control group, the findings suggested that ALA-DAG enhances not only diet-induced thermogenesis, but also resting metabolic rate. Taken together, both factors are considered to be involved in the VFA reduction by ALA-DAG. We observed no significant increase in fasting energy expenditure after 14-d consumption of ALA-DAG, therefore further studies with longer treatment durations are needed to clarify the impact of ALA-DAG on resting metabolic rate.

Previous studies in rodents suggested that ALA-DAG enhances fat metabolism via the up-regulation of enzymes and gene expression involved in β -oxidation and thermogenesis in the small intestine¹⁸⁾ and liver¹⁹⁾. ¹³C-Labeled TAG was used to determine if ALA-DAG consumption alters dietary induced fat oxidation. Watanabe *et al.*²⁹⁾ demonstrated a significantly increased recovery rate of ¹³C in rats fed ALA-DAG compared with rats fed TAG. This finding suggests that ALA-DAG enhances the utilization of dietary fat as energy, possibly in association with enhanced fat metabolism in the small intestine and the liver. Thus, these animal reports could provide a reasonable explanation for the mechanism underlying the increase in postprandial fat oxidation and energy expenditure after ALA-DAG treatment in the present human study.

Previously, the effect of ALA-DAG, ALA-TAG and TAG were compared in rodents. In these report, not only ALA-DAG treatment but also ALA-TAG treatment up-regulated enzymes and gene expression involved in β -oxidation

compared to TAG treatment^{18, 19)}. The magnitudes of the up-regulated level, however, were higher after ALA-DAG treatment than those after ALA-TAG treatment. Additionally, the mRNA expression of uncoupling protein-2 which is associated with thermogenesis and energy expenditure was up-regulated after ALA-DAG treatment but not after ALA-TAG. These evidences suggest that ALA alone does not have sufficient effect, but the effect of DAG structure was enforced by ALA. Indeed, Hibi *et al.*²⁰⁾ reported that postprandial fat oxidation was significantly increased followed by 10 g/d of 14-d repeated consumption of DAG which consisted mainly of oleic acid and linoleic acid. In this study, however, significant increase in postprandial fat oxidation was observed followed by 2.5 g/d of 14-d repeated consumption of ALA-DAG, suggesting that ALA-DAG showed the similar effect to oleic and linoleic acid-rich DAG only for a quarter of the dosage.

In summary, compared with TAG, consumption of ALA-DAG oil for 14-d enhanced postprandial fat oxidation and energy expenditure. Our results could partially explain the mechanism underlying the reduction in VFA and body fat by ALA-DAG consumption.

5 CONCLUSION

Repeated consumption of ALA-DAG enhanced postprandial fat metabolism, which may partially explain the VFA-reducing effect of ALA-DAG.

ACKNOWLEDGMENTS

We thank Dr. Shigeru Kobayashi (Tokyo Rinkai Hospital) for instruction and advice regarding this study. We thank Dr. Haruo Nakamura (Mitsukoshi Health and Welfare Foundation) for instruction and advice regarding the study design, protocol, and discussion. We thank Mr. Masao Shimizu, Mr. Akihiro Ise, Mr. Hiroshi Aoyama, Mr. Katsuyoshi Saito, Mr. Sho Aida, Ms. Yuumi Matsuda, Ms. Aya Sasaki, Ms. Mizue Ouchi, Ms. Yui Ishikawa, Mr. Yusuke Sugiyura, Mr. Yuki Matsui, Mr. Iyori Shirakura, Mr. Shinpei Fukuhara, Mr. Tsukasa Miyatani, and Mr. Minoru Kase for preparing the test diets.

References

- 1) Vazquez, G.; Duval, S.; Jacobs, Jr. D. R.; Silventoinen, K. Comparison of body mass index, waist circumference, and waist/hip ratio in predicting incident diabetes: a metaanalysis. *Epidemiol. Rev.* **29**, 115-128 (2007).
- 2) Song, Y. M.; Sung, J.; Davey Smith, G.; Ebrahim S. Body mass index and ischemic and hemorrhagic

- stroke: a prospective study in Korean men. *Stroke* **35**, 831-836 (2004).
- 3) Wormser, D.; Kaptoge, S.; Di Angelantonio, E.; Wood, A.M.; Pennells, L.; Thompson, A.; Sarwar, N.; Kizer, J. R.; Lawlor, D. A.; Nordestgaard, B. G.; Ridker, P.; Salomaa, V.; Stevens, J.; Woodward, M.; Sattar, N.; Collins, R.; Thompson, S. G.; Whitlock, G.; Danesh, J. Separate and combined associations of body-mass index and abdominal adiposity with cardiovascular disease: collaborative analysis of 58 prospective studies. *Lancet* **377**, 1085-1095 (2011).
- 4) Jackman, M. R.; Kramer, R. E.; MacLean, P. S.; Besse- sen, D. H. Trafficking of dietary fat in obesity-prone and obesity-resistant rats. *Am. J. Physiol. Endocrinol. Metab.* **291**, E1083-E1091 (2006).
- 5) Seidell, J. C.; Muller, D. C.; Sorkin, J. D.; Andres, R. Fasting respiratory exchange ratio and resting metabolic rate as predictors of weight gain: the Baltimore Longitudinal Study on Aging. *Int. J. Obes. Relat. Metab. Disord.* **16**, 667-674 (1992).
- 6) Laville, M.; Cornu, C.; Normand, S.; Mithieux, G.; Bey- lot, M.; Riou, J. P. Decreased glucose-induced thermo- genesis at the onset of obesity. *Am. J. Clin. Nutr.* **57**, 851-856 (1993).
- 7) Abdel Nabey, A. A.; Shehata, A. A. Y.; Ragab, M. H.; Rossell, J. B. Glycerides of cottonseed oils from Egyptian and other varieties. *Riv. Ital. Stostanze Grasse* **69**, 443-447 (1992).
- 8) D'Alonzo, R. P.; Kozarek, W. J.; Wade, R. L. Glyceride composition of processed fats and oils as determined by glass capillary gas chromatography. *J. Am. Oil Chem. Soc.* **59**, 292-295 (1982).
- 9) Murase, T.; Mizuno, T.; Omachi, T.; Onizawa, K.; Ko- mine, Y.; Kondo, H.; Hase, T.; Tokimitsu, I. Dietary diacylglycerol suppresses high fat and high sucrose diet-induced body fat accumulation in C57BL/6J mice. *J. Lipid Res.* **42**, 372-378 (2001).
- 10) Murase, T.; Aoki, M.; Wakisaka, T.; Hase, T.; Tokimitsu, I. Anti-obesity effect of dietary diacylglycerol in C57BL/6J mice: dietary diacylglycerol stimulates intestinal lipid metabolism. *J. Lipid Res.* **43**, 1312-1319 (2002).
- 11) Nagao, T.; Watanabe, H.; Goto, N.; Onizawa, K.; Tagu- chi, H.; Matsuo, N.; Yasukawa, T.; Tsushima, R.; Shima- saki, H.; Itakura, H. Dietary diacylglycerol suppresses accumulation of body fat compared to triacylglycerol in men in a doubleblind controlled trial. *J. Nutr.* **130**, 792-797 (2000).
- 12) Maki, K. C.; Davidson, M. H.; Tsushima, R.; Matsuo, N.; Tokimitsu, I.; Umporowicz, D. M.; Dicklin, M. R.; Fos- ter, G. S.; Ingram, K. A.; Anderson, B. D.; Frost, S. D.; Bell, M. Consumption of diacylglycerol oil as part of a reduced-energy diet enhances loss of body weight and fat in comparison with consumption of a triacylglycer- ol control oil. *Am. J. Clin. Nutr.* **76**, 1230-1236 (2002).
- 13) Kawashima, H.; Takase, H.; Yasunaga, K.; Wakaki, Y.; Katsuragi, Y.; Mori, K.; Yamaguchi, T.; Hase, T.; Matsuo, N.; Yasukawa, T.; Tokimitsu, I.; Koyama, W. One-year ad libitum consumption of diacylglycerol oil as part of a regular diet results in modest weight loss in compari- son with consumption of a triacylglycerol control oil in overweight Japanese subjects. *J. Am. Diet Assoc.* **108**, 57-66 (2008).
- 14) Osaki, N.; Meguro, S.; Onizawa, K.; Mizuno, T.; Shimo- toyodome, A.; Hase, T.; Tokimitsu, I. Effects of a single and short-term ingestion of diacylglycerol on fat oxi- dation in rats. *Lipids* **43**, 409-417 (2008).
- 15) Leyton, J.; Drury, P. J.; Crawford, M. A. Differential ox- idation of saturated and unsaturated fatty acids in vivo in the rat. *Br. J. Nutr.* **57**, 383-393 (1987).
- 16) Bessesen, D. H.; Vensor, S. H.; Jackman, M. R. Traf- ficking of dietary oleic, linolenic, and stearic acids in fasted or fed lean rats. *Am. J. Physiol. Endocrinol. Metab.* **278**, E1124-1132 (2000).
- 17) DeLany, J. P.; Windhauser, M. M. Champagne, C. M.; Bray, G. A. Differential oxidation of individual dietary fatty acids in humans. *Am. J. Clin. Nutr.* **72**, 905-911 (2000).
- 18) Murase, T.; Nagasawa, A.; Suzuki, J.; Wakisaka, T.; Hase, T.; Tokimitsu, I. Dietary alpha-linolenic acid-rich diac- ylglycerols reduce body weight gain accompanying the stimulation of intestinal beta-oxidation and related gene expressions in C57BL/KsJ-db/db mice. *J. Nutr.* **132**, 3018-3022 (2002).
- 19) Murase, T.; Aoki, M.; Tokimitsu, I. Supplementation with alpha-linolenic acid-rich diacylglycerol suppress- es fatty liver formation accompanied by an up-regula- tion of beta-oxidation in Zucker fatty rats. *Biochim. Biophys. Acta* **1733**, 224-231 (2005).
- 20) Hibi, M.; Takase, H.; Yasunaga, K.; Yamaguchi, T.; Shiiba, D.; Saito, S.; Yokoyama, R.; Kudo, N.; Katsuragi, Y.; Megu- ro, S.; Shimizu, A.; Tokimitsu, I. Greater fat oxidation with diacylglycerol oil consumption for 14 days com- pared with triacylglycerol oil consumption in over- weight men and women. *Int. J. Obes. (Lond.)* **32**, 1841-1847 (2008).
- 21) Shoji, K.; Takase, H.; Tomonobu, K.; Hibi, M.; Kudo, N.; Katsuragi, Y.; Meguro, S.; Tokimitsu, I. Effects of diac- ylglycerol oil on body weight and fat in obese people. *Prog. Med.* **28**, 203-210 (2008).
- 22) Takei, A.; Katsuragi, Y.; Abe, C.; Mori, K.; Takeda, Y.; Seo, Y.; Takase, H.; Takahashi, H.; Tohata, M.; Chika- ma, A.; Fumoto, S.; Meguro, S.; Komine, Y.; Nagao, T.; Hase, T.; Tokimitsu, I.; Shimasaki, H.; Itakura, H. Ef- fects of dietary alpha-linolenic acid-rich diacylglycerol on body fat in man (1): Lowering effect on body fat. *J. Oleo Sci.* **50**, 735-746 (2001).
- 23) Watanabe, T.; Shimizu, M.; Sugiura, M.; Sato, M.; Koh-

- ori, J.; Yamada, N.; Nakanishi, K. Optimization of reaction conditions for the production of DAG using immobilized 1,3-regiospecific lipase lipozyme RM IM. *J. Am. Oil Chem. Soc.* **80**, 1201-1207 (2003).
- 24) Weir, J. B. New methods for calculating metabolic rate with special reference to protein metabolism. *J. Physiol.* **109**, 1-9 (1949).
- 25) Brouwer, E. On simple formulae for calculating the heat expenditure and the quantities of carbohydrate and fat oxidized in metabolism of men and animals, from gaseous exchange (Oxygen intake and carbonic acid output) and urine-N. *Acta Physiol. Pharmacol. Neerl.* **6**, 795-802 (1957).
- 26) Ryo, M.; Maeda, K.; Onda, T.; Katashima, M.; Okumiya, A.; Nishida, M.; Yamaguchi, T.; Funahashi, T.; Matsuza-wa, Y.; Nakamura, T.; Shimomura, I. A new simple method for the measurement of visceral fat accumulation by bioelectrical impedance. *Diabet. Care* **28**, 451-453 (2005).
- 27) Katsuragi, Y.; Takeda, Y.; Abe, C.; Mori, K.; Toi, T.; Takei, A.; Shimasaki, H.; Itakura, H. Effects of dietary alpha-linolenic acid rich diacylglycerol on body fat in man (2): Effects on resting metabolism and fat metabolism. *J. Oleo Sci.* **50**, 747-752 (2001).
- 28) Van Zant R. Influence of diet and exercise on energy expenditure-a review. *Int. J. Sport Nutr.* **2**, 1-19 (1992).
- 29) Watanabe, H.; Yamaguchi, T.; Onizawa, K.; Osaki, N.; Harada, U.; Matsuo, N.; Tokimitsu, I.; Shimasaki, H.; Itakura, H. Effects of alpha-linolenic acid-rich diacylglycerol intake on the oxidation of dietary fats in rats. *J. Oleo Sci.* **50**, 839-842 (2001).