NOTE**Effects of α -Linolenic Acid-rich Diacylglycerol Intake on the Oxidation of Dietary Fats in Rats**

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Abstract : The effects of ingestion of α -linolenic acid-rich diacylglycerol (ALA-DAG) on the oxidation of dietary fat were investigated. Rats were fed ALA-DAG for 3 wk, deprived of food for 6 h, and then administered lipids containing $[1-^{13}\text{C}]$ -tripalmitin. All of the gas expired by the rats was collected at 2-h intervals for 6 h after administering the $[1-^{13}\text{C}]$ -tripalmitin. Rats fed triacylglycerol (TAG) for 3 wk were used as controls. The ^{13}C content in the carbon dioxide expired from the ALA-DAG-fed rats was significantly greater ($p < 0.05$) compared to that from the TAG-fed rats until 4-h after $[1-^{13}\text{C}]$ -tripalmitin administration. This result indicates that the long-term ingestion of ALA-DAG, as compared to TAG, induces an accelerated oxidation rate of dietary fat in rats.

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Key words : diacylglycerol, β -oxidation, dietary fats, rat, stable isotope

1 Introduction

Animal studies indicate that diacylglycerol (DAG) ingestion limits the increase of triacylglycerols secreted into the lymph system (1) and decreases the accumulation of body fat during long-term ingestion (2,3) when compared with triacylglycerol (TAG), which has the same fatty acid composition as DAG. Human studies also indicate that DAG limits the increase of TAG concentration in the serum, especially the increase in chylomicron TAG content after a single oral dose of DAG (4,5). Long-term ingestion of DAG also suppresses the accumulation of abdominal fat, particularly visceral fat (6). The production of 1-monoacylglycerol (1

-MAG) as a digestion product in the small intestine (2) and the activation of β -oxidation in the liver (7) are the major metabolic characteristics of DAG distinct from TAG. These results were obtained using DAG, which contains approximately 1 to 4% n-3 fatty acids (2,6).

The n-3 fatty acids have been studied with respect to their function in lipid metabolism, such as activation of lipoprotein lipase (LPL) (8) and β -oxidation (9,10,11), stimulation of brown adipose tissue thermogenic activity (12), up-regulation of uncoupling proteins (UCPs) (13,14,15), and down-regulation of enzymes for fatty acid synthesis (15).

Therefore, the present study focused on the effects of long-term ingestion of α -linolenic acid (ALA)-diacylglycerol (ALA-DAG), which is a combination of the structure of 1,3-DAG and n-3 fatty acids, on the oxidation rate of orally administered $[1-^{13}\text{C}]$ -

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tripalmitin by measuring carbon dioxide expired by the rats.

2 Experiment

2.1 Reagents

ALA-DAG was prepared from perilla oil in the presence of immobilized lipase following the method described by Huge-Jensen *et al.* (16). The fatty acid compositions of ALA-DAG and TAG are listed in Table 1 (analyzed by the Japan Food Analysis Center, Tokyo). ALA-DAG contained 14.1% TAG, and the ratio of 1,3-DAG to 1,2-DAG was 7:3 for the remaining 85.2% of the diacylglycerol structures.

[1-¹³C]-hexadecanoic acid (palmitic acid, 99.7% purity by gas chromatography, isotope enrichment is 99.0%) was purchased from Nippon Sanso Co. We added glycerol (Wako Pure Chemical Industries, Ltd.) to palmitic acid in lipozyme IM (Novo Nordisk Co.) and synthesized glyceryl tri-([1-¹³C]-hexadecanoate) ([1-¹³C]-tripalmitin). The tripalmitin obtained was 99.0% pure, as determined using gas chromatography.

Mixed lipids were prepared by adding 6.1% of the [1-¹³C]-tripalmitin to TAG, the fatty acid composition of which is listed in Table 1. To administer stable isotope to the rats, 5% mixed lipid emulsion was prepared by mixing with 0.2% lecithin (Wako Pure Chemical Industries, Ltd.), 2% bovine serum albumin (Sigma Chemical Co.), and 92.8% water using an ultrasonic treatment apparatus. The [1-¹³C]-tripalmitin was administered to the rats at a rate of 20 mg/kg.

2.2 Animals

Male Sprague Dawley rats (4 wk old) with a mean body weight of 92±3 g, (Charles River Japan, Inc.) were used for the experiments. Rats were allowed free access to food and water, and maintained in a temperature (23±3°C) and humidity (55±10%) controlled room, lights on from 7:00 am to 7:00 pm. The materials used for the diet preparation were purchased from Oriental Yeast Co., Ltd.

2.3 Administration of Stable Isotope

Rats were divided into two groups (ALA-DAG ingestion group: n=8, TAG ingestion group: n=8), each group received the corresponding diet for 3 wk (Table 2).

Food was withheld for 6 h before transferring the rats to a glass metabolic chamber (Metabolica MC-ST, Sugiyama Gen Iriki Co., Ltd.). All of the gas exhausted by the rats for 1 h was collected into 100 ml of 1 N sodium hydroxide solution in water. The gas was collected at a rate of 350 ml/min. After collecting the gas for 1 h, the 5% lipid emulsion containing [1-¹³C]-tripalmitin was fed to the rats by oral administration using a feeding tube (SAFEED feeding tube Fr. 3.5, Terumo Co.). Expired gas was collected every 2 h until 6 h after the administration of the [1-¹³C]-tripalmitin. During collection of the gas, all feeding was stopped except for water. To separate the carbon dioxide from the remainder of the gas sampled from the metabolic cage, the 1 N aqueous sodium hydroxide solution was neutralized with ammonium chloride, and the carbon dioxide was precipitated in the form of calcium carbonate by the

Table 1 Fatty Acid Composition of Experimental Lipids (%).

Fatty acid	ALA-DAG ^a	TAG ^b
C16:0	5.33	5.17
C16:1	n.d.	0.19
C18:0	3.29	1.94
C18:1	18.21	36.35
C18:2	15.09	46.90
C18:3	50.77	7.70
C20:0	n.d.	0.43
C20:1	n.d.	0.78
C22:0	n.d.	0.27
C22:1	n.d.	0.10

^aALA-DAG: α-linoleic acid rich diacylglycerol

^bTAG: triacylglycerol

n.d.: not determined

Table 2 Nutrient Content of Experimental Diets (%).

Nutrient	ALA-DAG ^a group	TAG ^b group
ALA-DAG	20	—
TAG	—	20
Casein	20	20
Cellulose	4	4
Mineral mixture*	3.5	3.5
Vitamin mixture**	1	1
α-Potato starch	61.5	60.5

*Mineral mixture AIN76

**Vitamin mixture AIN76

^aALA-DAG: α-linoleic acid rich diacylglycerol

^bTAG: triacylglycerol

addition of calcium chloride. The resulting calcium carbonate was then analyzed to determine its ^{13}C content.

2.4 Measurement of ^{13}C

The ^{13}C -carbon dioxide analysis in the calcium carbonate was performed by the Nippon Sanso Co., using a mass spectrometer (ANCA-SL, PDZ Europa). For quantitative analysis, the δ -value of each sample was calculated using Craig's method (17) with Pee Dee Belemnite Limestone as a standard. The transition values of ^{13}C in the carbon dioxide were expressed as the differences (Δ) between the initial σ -value obtained from the gas breathed for 1 h by the rats before $[1-^{13}\text{C}]$ -tripalmitin administration, and the σ -values obtained from the gas sampled at 2 h intervals after $[1-^{13}\text{C}]$ -tripalmitin administration.

2.5 Statistical Analysis

The results were expressed as mean $\pm$ standard deviation. Statistical analysis was performed using Student's *t* test for the comparison of the two groups at 2-h intervals. Repeated measures ANOVA was performed to determine any significant differences between the two groups.

3 Results and Discussion

Although it has been reported that fats containing *n*-3 fatty acids enhance the activity of β -oxidation (9, 10, 11), the effect of the long-term intake of the dietary fat has not been evaluated by measuring the amount of CO_2 in the expiration directly from the ingested fat. The present study employed the stable isotope method using energy substrates that are used in the field of energy metabolism to analyze various metabolic activities and metabolic quantities (18). Figure 1 shows the changes in the ^{13}C content in the carbon dioxide exhaled into the atmosphere in the metabolic chamber after $[1-^{13}\text{C}]$ -tripalmitin administration.

The ^{13}C content in the gas expired by both the ALA-DAG and TAG ingestion groups increased after $[1-^{13}\text{C}]$ -tripalmitin administration. There was a significant increase in the ^{13}C -carbon dioxide discharge at 0 to 2 h and 2 to 4 h after $[1-^{13}\text{C}]$ -tripalmitin administration in the ALA-DAG ingestion group when compared to the corresponding time points of the TAG ingestion group ($p < 0.05$). Moreover, repeated measures ANOVA indicated that there was a significant treatment effect between the two groups ($p = 0.036$).

The ingestion of DAG for 2 to 3 wk enhances activity of the β -oxidation enzymes in the liver in comparison with TAG, which has a similar fatty acid composition of DAG (7). It is in agreement with the observations of the hepatic enzymes in rats fed ALA-DAG diet for 3 wk in the present study. This might have caused the accelerated dietary fat oxidation in the rats fed ALA-DAG. The metabolic characteristics of DAG that are distinct from TAG are that DAG generates more 1-MG in the small intestine (2) and DAG decreases TAG discharge into the lymph and circulation (1). The mechanism underlying these phenomena might be that, unlike 2-MG, 1-MG is not easily utilized for TAG re-synthesis in the small intestinal epithelial cells (19). As a consequence, more 1-MG and free fatty acids remain in the epithelial cells after DAG ingestion as compared to TAG ingestion. These substances that remain after DAG ingestion might activate β -oxidation enzymes in the liver by acting as a type of signal. We are currently investigating the mechanism by which DAG activates β -oxidation enzymes in the liver.

Other than in the liver, where enzyme activation occurs, the small intestine might have a role in the regulation of β -oxidation and the digestion of lipids. That is, after ingestion of DAG, the remaining 1-MG

Fig. 1 Changes in ^{13}C in Carbon Dioxide Exhaled by Rats after Single Dose of Lipid.

Data represent mean $\pm$ standard deviation.

●; α -Linolenic acid rich-diacylglycerol diet group ($n = 8$), $\triangle$; Triacylglycerol diet group ($n = 8$).

*Significant difference between two groups by Student's *t*-test ($P < 0.05$).

Significant difference between two groups by repeated ANOVA ($P_{\text{treatment}} = 0.036$, $P_{\text{time}} < 0.001$).

and free fatty acids that are not used for TAG synthesis might actually accelerate β -oxidation in the small intestinal epithelial cells. Free fatty acid is a ligand for the peroxisome proliferator-activated receptor (PPAR) α , which belongs to the nuclear hormone receptor superfamily (20). PPAR α activates the generation of the uncoupling protein-2 (UCP-2) (14). Murase *et al.* examined the small intestine as the largest internal organ where lipids and free fatty acids generated from lipid digestion are abundant, and reported that UCP-2 expression is up-regulated in the small intestinal cell line IEC-6 by free fatty acids and PPAR α agonists (21). They suggested that the small intestine functions as an anti-obesity internal organ because the free fatty acids generated through lipid digestion can control the energy consumption of humans and animals by regulating the expression of UCP-2 in the small intestinal epithelial cells (21). The behavior of the free fatty acids generated from DAG in the small intestine suggests that the acceleration of dietary fat combustion observed in the present study might be related to the acceleration of β -oxidation in the small intestine via a mechanism involving PPAR α .

In the present study, the DAG diet had a higher ALA content because the fatty acid compositions of ALA-DAG and TAG were not adjusted to be equivalent. Various functions have been reported for n-3 fatty acids, such as LPL activation (8), β -oxidation activation (9), stimulation of brown adipose tissue thermogenic activity (12), up-regulation of UCP-1 (13), UCP-2 (14), and UCP-3 (15), and inhibition of the enzymes for fatty acid synthesis (15). Therefore, we can not identify whether the major factor contributing to the ALA-DAG effect is the DAG structure or the altered fatty acid composition. Our findings, together with those of Murase *et al.* (21), suggest that both the DAG structure and altered fatty acid composition act in combination to accelerate β -oxidation of ingested fat. To investigate the acceleration mechanism of fatty acid oxidation by ALA-DAG, the actions of TAG and DAG must be compared using the same fatty acid composition.

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