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Dietary diacylglycerol suppresses high fat diet-induced hepatic fat accumulation and microsomal triacylglycerol transfer protein activity in rats

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Abstract

We have recently shown that the long-term ingestion of dietary diacylglycerol (DAG) mainly containing 1,3-isoform reduces body fat accumulation in humans as compared to triacylglycerol (TAG) with the same fatty acid composition. The fat reduction in this human experiment was most pronounced in visceral fat and hepatic fat. Recent animal studies have also indicated that dietary DAG induces alteration of lipid metabolism in the rat liver. In the present study, the dietary effects of DAG on high fat diet-induced hepatic fat accumulation and hepatic microsomal triglyceride transfer protein (MTP) activity were examined in comparison with those of TAG diet in rats. When the TAG oil content was increased from 10 to 30 g/100 g diet, hepatic TAG concentration, hepatic MTP activity and MTP large subunit mRNA levels were significantly increased after 21 days. However, when the dietary TAG oil (30 g/100 g diet) was replaced with the same concentration of DAG oil with the same fatty acid composition, the increase of the TAG concentration and the MTP activity in the liver were significantly less and the mRNA levels remained unchanged. The MTP activity levels correlated significantly with hepatic TAG concentration.

These results showed that dietary DAG may suppress high fat diet-induced MTP activity in the liver, and indicated the possibility that hepatic TAG concentration may regulate hepatic MTP activity.

Keywords: Diacylglycerol; Triacylglycerol; MTP; Liver; Rat

1. Introduction

Diacylglycerol (DAG) is found naturally as a minor component in various vegetable oils and fats [1,2]. Although human adults ingest 1 to 5g of DAG every day, little attention has been paid to its nutritional characteristics because DAG has been recognized only as an intermediate in the process of triacylglycerol (TAG) digestion [3]. We recently reported that the long-term ingestion of dietary DAG mainly containing 1,3-DAG, in contrast to TAG ingestion, reduces body fat accumulation in humans [4]. The fat reduction was most pronounced in visceral fat and hepatic fat in human study. We have also shown that postprandial response after ingestion of DAG emulsion was significantly less than that after ingestion of TAG emulsion in healthy human subjects [5,6]. These studies indicated that structural differences between DAG and TAG, not fatty acid composition, markedly affect nutritional behavior of lipids.

In animal studies, the long-term ingestion of dietary DAG, in contrast to TAG, prevented the accumulation of visceral fat and body weight gain in obesity-prone mice [7]. It was also reported that dietary DAG decreases the activity of enzymes for fatty acid synthesis but increases those of the fatty acid oxidation pathway resulting in a reduced fat content in the rat liver [8]. Since it has been shown that the absorption efficiency of DAG in rats was not different from that of TAG [9], alteration of the fatty acid metabolism in the liver may be one of the factors responsible for the long-term effect of dietary diacylglycerol.

[1] *Abbreviations:* DAG, diacylglycerol; TAG, triacylglycerol; MTP, microsomal triacylglycerol transfer protein; HNF-4, hepatic nuclear factor-4; FFA, free fatty acid.

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Many studies have shown that microsomal triacylglyceride transfer protein (MTP) plays a crucial role in lipid metabolisms in the liver [10,11]. MTP is a protein that mediates transport of TAG from its site of synthesis on the smooth endoplasmic reticulum to the site of apo B containing lipoprotein assembly in the liver and intestine. MTP also catalyzes the transfer of cholesterol ester and phosphatidylcholine between phospholipid surfaces. It has been shown that MTP activity increases in the rat fed the high fat diet [12]. In the present study, we investigated the effects of dietary DAG on the high fat diet-induced TAG accumulation and MTP activity in the rat liver as compared to TAG.

2. Materials and methods

2.1. Materials

The material oils for the diet were obtained from The Nisshin Oil Mills (Tokyo, Japan). Rats were obtained from CLEA Japan (Tokyo, Japan). Kits for ketone body analysis were obtained from Sanwa Kagaku Kenkyusho (Nagoya, Japan). Kits for lipid analysis were obtained from Wako (Osaka, Japan). Protein assay kit was obtained from Pierce (Rockford, IL). MTP assay kit was obtained from Roar Biomedical (New York, NY). Rat liver total RNA was obtained from Clontech Laboratories (Palo Alto, CA). Oligo d(T)18 primer was obtained from New England BioLabs (Beverly, MA). Cloning vector pCRII-TOPO was obtained from Invitrogen (Carlsbad, CA). Protease inhibitor cocktail, DNA labeling kit, nylon membrane filter were obtained from Boehringer Mannheim (Mannheim, Germany). ISOGEN was obtained from Nippon Gene (Tokyo, Japan). X-ray films were obtained from Eastman Kodak (Rochester, NY).

2.2. Dietary fats

DAG rich oil was prepared by esterifying glycerol with fatty acids from soybean oil using a reverse reaction of 1,3-specific lipase [13]. TAG oil was prepared by mixing soybean oil, rapeseed oil, and safflower oil so that the fatty acid composition was similar to that of the DAG rich oil. The DAG rich oil contained 83g/100g of DAG and 17g/100g of TAG. The ratio of 1 [3],2-DAG to 1,3-DAG was 32:68. Acylglycerol and fatty acid composition of the DAG rich oil and the blended TAG oil are shown in Table 1.

2.3. Animals and diets

Male Sprague-Dawley rats at the age of 7 weeks were housed in metal cages and maintained in a room with controlled temperature (22 ± 1°C) and lighting (light on from 7:00 am to 7:00 pm). They were allowed free access to drinking water, but food was given with in a manner such that feeding time was fixed from 8:30 am to 11:30 am in order to control the length of the post absorptive period. The composition of experimental diets containing TAG oil or DAG rich oil is shown in Table 2.

Twenty-four rats were fed 10% (w/w) TAG oil diet for 6 days prior to transfer to the experimental diet. After this acclimatization period of 6 days, they were divided into three groups of 8 animals so that the body weights were distributed evenly among the groups. Rats in group 1 (30% DAG group) were fed the diet containing 30% (w/w) DAG rich oil and the rats in group 2 (30% TAG group) were fed the diet containing 30% (w/w) TAG oil for 3 weeks. The rest of the rats were fed 10% TAG diet for 3 weeks. Food intake of all rats was recorded everyday and body weight was recorded every week. On day 21 of the experimental period, at 8 hr after the last feeding, the rats were anesthetized with diethyl ether and approximately 8 mL of blood was collected from the abdominal aorta. The liver was perfused with ice-cold saline prior to removal, and approximately 100 mg of the median lobe of the liver was excised for RNA isolation as described below. The rest of the liver was immediately frozen in liquid N_2. The frozen samples

Table 1
Fatty acid and acylglycerol compositions of diacylglycerol and triacylglycerol oils

Acylglycerols and fatty acids	DAG rich oil	TAG oil
	g/100 g oil	
Triacylglycerol	10.0	98.8
1,2 (2,3)-diacylglycerol	28.8	0.4
1,3-diacylglycerol	61.2	0.8
Monoacylglycerol	ND[a]	ND
Free fatty acid	ND	ND
Fatty acids	g/100 g fatty acid	
16:0	3.18	6.04
18:0	3.67	2.38
18:1	43.87	46.45
18:2	39.99	30.61
18:3	3.07	8.35
20:0	0.80	0.73
20:1	0.71	0.90
22:0	0.26	0.23
22:1	0.10	0.22

[a] Not detected.

Table 2
Composition of the test diets

Ingredients	10% TAG diet	30% DAG diet	30% TAG diet
		g/100 g diet	
DAG rich oil	0	30	0
TAG oil	10	0	30
Casein	20	20	20
Cellulose	4	4	4
Mineral mixture (AIN-76[a])	3.5	3.5	3.5
Vitamin mixture (AIN-76)	1	1	1
Potato starch	61.5	41.5	41.5

[a] AIN-76 prescription (27).

were then stored at −80°C until they were used for MTP activity measurement and for lipid analysis. The study was approved by the Ethical Committee for the Experimental Animals of Kao Corporation.

2.4. Lipid analysis

Serum triacylglycerol, total and free cholesterol, phospholipid and non-esterified fatty acid concentrations were measured by enzymatic methods using a Super Z 818 autoanalyzer (MC Medical, Tokyo, Japan). Serum total ketone body was measured by enzymatic method. Serum immunoreactive insulin was determined by the rat insulin enzyme immunoassay system. Liver lipids were extracted and purified as previously reported [14]. Triacylglycerol, cholesterol and phospholipid contents in the lipid extract were determined by enzymatic methods.

2.5. Measurement of MTP activity

Approximately 1 g of frozen liver tissue was minced and homogenized in a Potter-Elvehjem homogenizer in 10 mL of ice-cold homogenization buffer (10 mM Tris, pH 7.4/150 mM NaCl/1 mM EDTA/complete EDTA-free protease inhibitor cocktail). The homogenate was then centrifuged at 700 g at 4°C for 10 min. The supernatant was assayed for MTP activity. Protein concentration of the supernatant was measured by protein assay kit using bovine serum albumin as a standard protein.

Lipid transfer activity was determined using the MTP assay kit according to the manufacturer's instructions. Briefly, 10 μL of donor vesicle containing fluorescent neutral lipid and 10 μL of acceptor vesicle were incubated with 100 μg of liver homogenate protein in 0.5 mL of assay buffer (10 mM Tris, pH 7.4/150 mM NaCl/2 mM EDTA) at 37°C for 12 h. Fluorescence intensity of the assay buffer was measured by a Hitachi F-2000 fluorescence spectrophotometer (Hitachi, Tokyo, Japan) at excitation wavelength of 465 nm and emission wavelength of 535 nm to determine the amount of MTP mediated lipid transfer.

2.6. Preparation of complementary DNA probe for rat liver MTP large subunit

cDNA encoding a portion of the rat MTP large subunit was cloned by reverse transcription PCR amplification. The first cDNA strand was reverse transcribed from rat liver total RNA using an oligo d(T)18 primer. PCR Primers 5′-AAGGTCACACAACTGGTCTCTCAT-3′ and 5′-GAGCCTGGTAGGTCACTTTACAAT-3′ were designed from regions of 100% homology between the human [15] and hamster [16] sequences. PCR was carried out for 27 cycles by a program consisting of 94°C for 30 sec, 60°C for 30 sec, 72°C for 90 sec. PCR product was cloned into the TA cloning site of pCRII-TOPO. The clone was sequenced to confirm its identity and orientation. The sequence of the amplified fragment of the rat MTP large subunit (483 bp) is as follows:

AAGGTCACACAACTGGTCTCTCATTAAATAAT
GAGCGGCTATACAAGCTCACGTACTCCAC
CGAAGTGTTTCTCGATGGGGGCAAAGGAA
AACTGCGGACAGCGTGGGCTACCGGATCTCA
TCGGACGTGGACGTTGTGTTACTGTGGAGGA
ATCCCGACGGTGACGATGATCAACTGATCC
AAGTCACGATAACGGCTGTCAATGTTGAAAAT
GCGGGTCAGCAGAGAGGAGAGAAGAGCATCT
TCAAGGGCAAAAGTACACCTAAGATCGTAG
GAAAGGACAACTTGGAGGCTCTACGGAGAC
CCATGCTTCTTCATCTGGTCCGGGGGAAGGT
CAAAGAGTTCTACTCCTATGAAAATGAGC
CCGTGGGCATAGAAAATCTCAAGAGAGGCTTG
GCTAGCTTATTCCAGATGCAGTTAACCTCTG
GAACCACCAATGAGGTAGATATCTCTGGGGAT
TGTAAAGTGACCTACCAGGCTC.

The nucleotide sequence had 85%, 92%, 83% and 93% identity with the reported human [15], hamster [16], bovine [17], and mouse [18] sequences, respectively. A [^{32}P]-labeled cDNA probe was prepared by a random priming method using a high prime DNA labeling kit.

2.7. Isolation of total RNA from rat liver and determination of MTP mRNA concentration

For total RNA isolation, approximately 100 mg of the median lobe of the liver was excised after removal, and immediately homogenized in 1 mL of ISOGEN. The total RNA was extracted according to the manufacturer's instructions.

Fifteen μg of total RNA was denatured, applied to 1% agarose gel, and electrophoresis was carried out. The RNA was transferred onto a nylon membrane filter. After UV crosslinking, the filter was pre-hybridized in 50% formamide, 5 × SSC, 5 × Denhardt's, 0.5% SDS, 200 μg/mL of denatured salmon sperm DNA at 42°C for 2 hr, and then hybridized to the [^{32}P]-labeled cDNA probe (1×10^6 cpm/mL) in the same buffer at 42°C for 16 hr. After washing, the filter was exposed to Kodak Bio Max MS X-ray film.

The amount of mRNA was determined by quantitative slot blotting. Five μg of total RNA was denatured and applied to a nylon membrane using a Bio-Dot SF slot blot apparatus (Bio Rad, Hercules, CA). The conditions of prehybridization and hybridization were the same as those for Northern blot analysis. The cDNA probe was tested for specific hybridization before slot blot analysis and showed a single band of appropriate size by Northern blot analysis under the same hybridization conditions. Autoradiograms of the slot blot hybridization were quantified by scanning densitometry. The signal from the experimental probe was normalized to that of G3PDH.

Table 3
Effect of high fat diets on liver weight and hepatic lipid concentrations

	10% TAG diet	30% TAG diet	30% DAG diet
Liver weight (g)	13.4 ± 0.4[a]	13.2 ± 0.3[a]	13.4 ± 0.4[a]
Hepatic triacylglycerol (mmol/g liver)	0.185 ± 0.018[a]	0.373 ± 0.032[b]	0.272 ± 0.041[a]
Hepatic total-cholesterol (mmol/g liver)	0.056 ± 0.004[a]	0.108 ± 0.010[b]	0.099 ± 0.013[b]
Hepatic phospholipid (mmol/g liver)	0.163 ± 0.007[a]	0.171 ± 0.007[a]	0.167 ± 0.005[a]

Data are expressed as means ± SEM (n = 8 per group). Same alphabets denote no significant differences between diet groups as determined by student's t test (significant level: $p < 0.05$).

2.8. Statistical analysis

Data were expressed as means ± SEM. Statistical significance of the difference between the groups were determined by Student's t-test (two tailed). A simple linear regression equation was calculated by the least-squares method. Correlation was tested for equality using the Fisher's Z transformation [19]. The significance levels were set at $p < 0.05$. The statistical calculations were performed with Stat View for windows version 4.58 (Abacus Concepts. Inc., Berkley, CA).

3. Results

3.1. Effect of dietary fat on hepatic lipid concentrations

There was no significant difference between the groups in food consumption throughout the experiment (data not shown). Although the body weight was slightly increased in high fat diet groups (353.3 ± 5.5 g for high TAG diet group, and 357.4 ± 3.5 g for high DAG diet group) as compared to the control low TAG diet group (346.4 ± 6.2 g), the differences among groups were not significant. We compared the effects of high fat diets containing TAG or DAG oils. The hepatic lipid concentration and liver weight in the rat fed 10% (w/w) TAG diet, 30% (w/w) TAG diet and 30% (w/w) DAG diet are shown in Table 3. Hepatic TAG concentration was significantly increased as the dietary fat content was increased. Although 30% (w/w) DAG diet increased hepatic lipid concentrations, the increment was significantly lower than that of the rat fed 30% (w/w) TAG diet. The hepatic total cholesterol content also increased by high fat diets, but it did not show any significant difference between TAG and DAG diet. The hepatic phospholipid content, on the other hand, was not affected by the high fat diets.

The serum lipids did not show any significant differences between the 30% (w/w) TAG diet group and the 30% (w/w) DAG diet group (Table 4). As shown in Table 4, there was a trend that ketone body and glucose concentrations increase in the DAG group as compared to the 10% (w/w) and the 30% (w/w) TAG groups. The difference, however, was not significant ($p = 0.271$) for the ketone body.

3.2. Effect of dietary fats on hepatic MTP activity

Liver homogenates were prepared and assayed for MTP activity. Increasing the concentration of dietary TAG from 10% (w/w) to 30% (w/w) caused a significant rise in hepatic MTP activity (Table 5). The increment of the MTP activity by increasing the concentration of dietary fat from 10% TAG to 30% (w/w) DAG was significantly less than that obtained by increasing from 10% TAG to 30% (w/w) TAG.

3.3. Effect of dietary fats on hepatic MTP large subunit mRNA levels

Hepatic mRNA levels for the MTP large subunit were then determined by slot blot assay. Increasing the concentration of dietary TAG from 10 to 30% (w/w) caused a significant rise in hepatic large subunit mRNA levels (Table 5). However, increasing the concentration of dietary fat from 10% TAG to 30% (w/w) DAG did not cause any difference in the MTP mRNA levels. These results clearly demonstrated that the increased MTP activity by the high fat diet was associated with the increased mRNA levels and that dietary DAG does not lead to this increase by preventing the elevation of mRNA levels.

3.4. Correlation of hepatic MTP activity with serum and hepatic lipid levels

The data of all animals fed either 10% TAG diet, 30% TAG diet or 30% (w/w) DAG diet were combined and the

Table 4
Effect of high fat diets on serum lipids and other composition concentrations

	10% TAG diet	30% TAG diet	30% DAG diet
Triacylglycerol (mmol/L)	2.95 ± 0.42[a]	2.33 ± 0.23[a]	3.49 ± 0.63[a]
Total-cholesterol (mmol/L)	1.95 ± 0.10[a]	1.87 ± 0.09[a]	1.77 ± 0.04[a]
Phospholipid (mmol/L)	2.15 ± 0.09[a]	1.91 ± 0.07[a,c]	1.86 ± 0.07[b,c]
Glucose (mmol/L)	9.42 ± 0.22[a]	9.69 ± 0.18[a]	10.29 ± 0.22[b]
Total ketone body (μmol/L)	194 ± 36[a]	189 ± 26[a]	234 ± 30[a]

Data are expressed as means ± SEM (n = 8 per group). Same alphabets denote no significant differences between diet groups as determined by student's t test (significant level: $p < 0.05$).

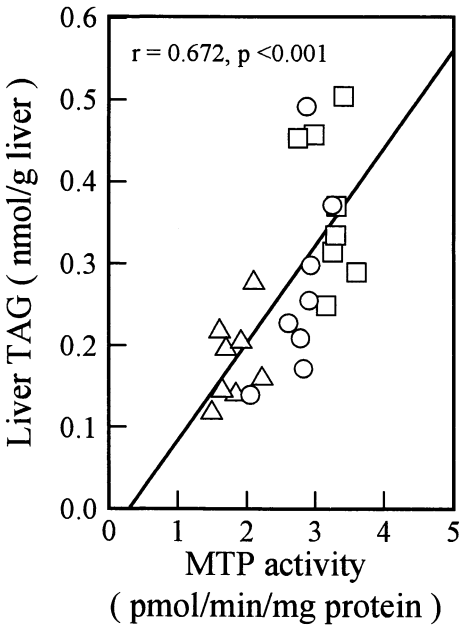

Fig. 1. Correlation between hepatic MTP activity and hepatic triacylglycerol concentrations. Scatter plot of the hepatic MTP activities versus hepatic triacylglycerol concentration is presented. Data for 24 animals fed either the control TAG diet (triangle) containing 10% (w/w) TAG oil or the high TAG diet (square) containing 30% (w/w) TAG oil or the high DAG diet (circle) containing 30% (w/w) DAG rich oil for 21 days were used for the analysis. The correlation coefficients and *p* values are shown in the panel.

correlation between the MTP activity and hepatic lipids were examined by linear correlation coefficient analysis. Strong positive correlations were observed between hepatic MTP activity and hepatic TAG concentration ($r = 0.672$ $p < 0.001$, Fig. 1).

4. Discussion

The effect of dietary DAG on hepatic fat accumulation [8] was confirmed in the present study, where lipid content was increased from 10 to 30g/100g diet, as compared to TAG. Moreover, the present study provided evidence that dietary DAG reduced the MTP activity in the liver, and that its modulation was mediated by the mRNA level. However, the results of the present study, in contrast to previous reports [8,20], did not show the lowering effect on the serum TAG concentration. This discrepancy may be, at least in part, due to the high lipid contents in the diet.

Murata et al. [8] have reported that dietary DAG, compared with TAG, decreased the activities of the enzymes of fatty acid synthesis but increased those of the enzymes involved in the fatty acid oxidation pathway in the rat liver. Recently, Murase et al. [7] have also shown that high DAG feeding up-regulated hepatic acyl-coenzyme oxidase and acyl-coenzyme synthetase mRNA expression in obesity-prone mice. It is therefore probable that dietary DAG, compared with TAG, modifies the rates of fatty acid synthesis and oxidation in the liver and these alterations lead to reduced hepatic TAG concentration.

Although there are a number of reports on the in vitro regulation of MTP large subunit mRNA concentration, the studies on the dietary regulation of MTP are limited. Bennett et al. [12] reported that increasing the dietary fat contents increased hepatic MTP large subunit mRNA in a dose dependent manner. They showed that increasing the dietary fat contents from 11.7 energy % to 46.8 energy % caused a 60% increase in hepatic MTP large subunit mRNA levels in the hamster. In the present study with rats, the hepatic MTP activity and mRNA levels were significantly increased (78% in activity and 46% in mRNA levels) by increasing the fat contents in the diet from 10% to 30% (w/w) corresponding to the increase from 21.6 to 52.3 energy %. These results were comparable to those reported by Bennett et al. [21]. In contrast, increase of dietary fat concentration to 30% (w/w) DAG oil showed no increase in MTP large subunit mRNA levels and less increase in MTP activity in the present study.

Correlation between hepatic TAG concentration and MTP activity (Fig. 1) indicated that dietary lipids regulate MTP activity on the mRNA level through modifying hepatic TAG accumulation. Hagan et al. [22] showed that the 5′-promotor region of the human MTP gene contained the consensus recognition sequences for liver cell-specific factors, including HNF-4. HNF-4 is a transcription factor of the steroid hormone receptor superfamily. HNF-4 consensus sequences in the MTP promoter region has been reported to regulate a direct response to fatty acids [23]. Recently, the ligand for HNF-4 was shown to be fatty acylCoA [24]. As described above, long term ingestion of dietary DAG has been reported to enhance β-oxidation of fatty acids in the liver [8,25]. Tendency towards an increase in serum ketone body concentration in the present study was consistent with these previous results. This enhanced β-oxidation in the liver may be related to the limited availability of the fatty acid for MTP expression in the liver. It has been suggested that the regulation of hepatic fatty acid binding protein mRNA is mediated by fatty acid metabolites resulting from an impaired or overloaded mitochondrial β-oxidation pathway [26]. Perhaps a similar intracellular signaling pathway

Table 5
Effect of high fat diets on hepatic MTP activity and its large subunit mRNA levels

	10% TAG diet	30% TAG diet	30% DAG diet
Activity (pmol/min/mg protein)	1.82 ± 0.09^a	3.21 ± 0.09^b	2.78 ± 0.34^c
MTP/G3PDH (%)	100.0 ± 13.3^a	146.3 ± 13.2^b	104.4 ± 19.5^a

Data are expressed as means ± SEM (n = 8 per group). Same alphabets denote no significant differences between diet groups as determined by student's t test (significant level: $p < 0.05$).

responsive to increased intracellular lipid levels also regulates MTP mRNA levels.

These results showed that dietary DAG may suppress high fat diet-induced MTP activity in the liver, and indicated the possibility that hepatic TAG concentration may regulate hepatic MTP activity on the mRNA level. Although precise mechanisms of the effect of dietary DAG on the regulation of hepatic MTP activity should be addressed in future studies, the present results provide a useful model for studying the regulation of hepatic MTP by dietary lipids.

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