The acyl-acceptor specificity of microsomal diacylglycerol acyltransferase as a possible determinant in regulating hepatic triacylglycerol synthesis in rats fed a polyunsaturated fat diet

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Rat liver microsomal diacylglycerol acyltransferase activity was determined using molecular species of 1,2-diacylglycerol substrates differing in the degree of unsaturation. When $C_{16:0}$-CoA was used as an acyl-donor, molecular species of diacylglycerol substrates dispersed in ethanol modified the V_{max} without influencing K_m values of the enzyme reaction. The enzyme activities measured with $diC_{18:2}$ and $diC_{18:3}$glycerols were lower than that obtained with $diC_{18:1}$glycerol. The latter value was the same as that obtained with 1-$C_{16:0}$, 2-$C_{18:1}$glycerol. On the other hand, 1-$C_{16:0}$, 2-$C_{18:2}$glycerol gave the lowest activity among the various diacylglycerol substrates examined. The similar results were obtained when $C_{18:2}$-CoA was served as an acyl-donor. To test the possibility that observed differences in dependencies on molecular species of diacylglycerols of diacylglycerol acyltransferase in vitro may be a factor in regulating hepatic triacylglycerol synthesis in vivo, rats were fed diets containing 20% fats with different degrees of unsaturation for 3 days after 2 days of fasting. Safflower oil compared with palm and olive oils increased the proportions of polyunsaturated fatty acids in liver microsomal diacylglycerol. Safflower oil compared with the other oils significantly decreased the microsomal diacylglycerol acyltransferase activity measured with endogenous diacylglycerols as the acyl-acceptors and $C_{16:0}$-CoA as an acyl-donor irrespective of the fact that microsomal concentration of diacylglycerol was the same among the rats fed these 20% fat diets. Thus, it is plausible that the acyl-acceptor specificity of microsomal diacylglycerol acyltransferase is a determinant in regulating triacylglycerol synthesis in rat liver.

Keywords: diacylglycerol acyltransferase; triacylglycerol synthesis; microsomal diacylglycerol; dietary fat unsaturation

Introduction

Diacylglycerol acyltransferase (EC 2.3.1.20) catalyzes the last step in the pathway of triacylglycerol synthesis in endoplasmic reticulum. This enzyme acylates 1,2-diacylglycerol with acyl-CoAs to form triacylglycerol. The 1,2-diacylglycerol is also utilized by diacylglycerol:choline phosphotransferase (EC 2.7.8.2) or diacylglycerol:ethanolamine phosphotransferase (EC 2.7.8.1) in the synthesis of phosphatidylcholine or phosphatidylethanolamine. Thus, the existence of control mechanism(s) at this branch point of triacylglycerol and phospholipid syntheses has been suspected.[1,2] There is some evidence[3-6] to indicate that the alteration in the activity of diacylglycerol acyltransferase plays a critical role in regulating triacylglycerol synthesis in rat liver. It is also possible that the substrate specificity of this enzyme may be a factor that determines triacylglycerol synthesis in the liver. However, only a few

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studies[7-9] evaluating the substrate specificity of this enzyme are available. In the present study, we measured the activity of rat liver microsomal diacylglycerol acyltransferase using various molecular species of 1,2-diacylglycerol. We also tested the possibility that the observed acyl-acceptor specificity of this enzyme may be a determinant in regulating the hepatic triacylglycerol synthesis in vivo in a feeding study using dietary fats differing in the degrees of unsaturation.

Materials and methods

Materials

Various molecular species of 1,2-diacylglycerol containing various fatty acids were prepared by phospholipase C treatment[10] from corresponding molecular species of 1,2-diacylglycerophosphorylcholines (1,2-di$C_{18:1}$, 1,2-di$C_{18:2}$, 1,2-di$C_{18:3}$, 1-$C_{16:0}$-2-$C_{18:1}$, 1-$C_{16:0}$-2-$C_{18:2}$), which were purchased from Avant Polar-Lipids Laboratories, Alabaster, AL USA, and purified by silicic acid column chromatography.[11] The effluents corresponding to diacylglycerols were analyzed by thin-layer chromatography using solvent systems of hexane/diethylether/acetic acid (82/18/1, vol/vol/vol) and hexane/diethylether (60/40, vol/vol). When the thin-layer chromatograms were visualized by iodine vapor, a lipid spot corresponding to 1,2-diacylglycerol was only detectable using these solvent systems in various samples from molecular species of 1,2-diacylglycerophosphorylcholines. Thus, the acyl migration to form 1,3-diacylglycerol from 1,2-diacylglycerol appeared to occur at a negligible rate under the present silicic column chromatographic system. When fatty acid contents of the various fractions on the thin-layer chromatograms were determined by gas-liquid chromatography using $C_{17:0}$ acid as an internal standard, more than 98% of fatty acids were found in the 1,2-diacylglycerol fractions in various samples. When 1,2-diacylglycerols were stored as the ethanolic solutions at −40° C, detectable amounts of 1,3-diacylglycerols were not found when analyzed by thin-layer chromatography even after 6 months of the storage. [9,10(n)-^{3}H]$C_{16:0}$ and [1-^{14}C]$C_{18:2}$ acids were obtained from Amersham International, Bucks and corresponding CoA esters were prepared according to the method of Kawaguchi et al.[12] [methyl-^{14}C]Choline chloride was purchased from Amersham International, Bucks and CDP-[methyl-^{14}C]choline was enzymatically prepared using a commercial preparation of choline kinase (EC 2.7.1.32, Sigma Chemical, St. Louis, MO USA) and partially purified rat liver CTP:phosphocholine cytidylyltransferase (EC 2.7.7.15) as described previously.[13-16]

Animals and diets

Male Sprague-Dawley rats (Charles River Japan, Kanagawa, Japan) were used throughout the study. The animals (8–9 weeks ages, body weights 250–300 g), fed a commercial diet (Type NMF, Oriental Yeast Co., Tokyo, Japan), were employed for the experiment to examine the acyl-acceptor specificity of diacylglycerol acyltransferase in liver microsomes. Rats were killed by withdrawing the blood from inferior vena cava under Nembutal anesthesia. Livers were quickly excised and a portion of each liver (approximately 3 g) was homogenized in 20 mL of 0.25 M sucrose. Approximately 10 mL of the homogenate was centrifuged at 8,000*g* for 10 min and the supernatant was processed for the preparation of microsomes for the enzyme assay.[15-17] In a feeding experiment, rats (6 weeks age) were fasted for 2 days and refed a fat-free diet or the diet containing 20% various fats differing in the degree of unsaturation (palm, olive, and safflower oils). The basal composition of the experimental diet was (in wt.%):casein 20; corn starch, 15; mineral mixture,[18] 3.5; vitamin mixture,[18] 1.0; DL-methionine, 0.3; choline bitartrate, 0.2; cellulose, 2; and sucrose, up to 100. Various dietary fats were added to the experimental diet at the expense of sucrose. Fatty acid compositions of these dietary fats are shown in *Table 1*. After 3 days of refeeding period, rats were killed by withdrawing blood from inferior vena cava under Nembutal anesthesia and liver microsomes were prepared as described above.

Enzyme assays

The diacylglycerol acyltransferase activity was determined according to the method of Rustan et al.[9] The assay mixture (0.5 mL) contained 8 mmol/L $MgCl_2$, 1 mg/mL bovine serum albumin (fatty acid-free), 30 μmol/L acyl-CoA and microsomes in 175 mmol/L Tris-HCl buffer (pH 7.8). Various molecular species of 1,2-diacylglycerol dispersed in ethanol (final concentration 10%, vol/vol) were employed as the acyl acceptors. Either [9,10(n)-^{3}H]$C_{16:0}$-CoA (7400 dpm/nmol) or [1-^{14}C]$C_{18:2}$-CoA (7800 dpm/nmol) served as a tracer. The amount of microsomes employed and the enzyme reaction periods were 15–20 μg protein and 10 min. In a feeding experiment in rats fed various types of dietary fats, the enzyme activity was determined in the presence and absence of 1,2-di$C_{18:1}$glycerol substrate (125 μmol/L) dispersed in ethanol. When an endogenous microsomal diacylglycerol was the substrate, ethanol was not included in the assay media and the amount of microsomes and the enzyme reaction periods were 200–300 μg protein and 3 minutes. The radiolabeled $C_{16:0}$-CoA was served as an acyl-donor in this feeding experiment. It has been reported that liver microsomes contain an ethanol acyltransferase activity[19,20] and the enzyme product, fatty acid ethyl ester, behaves like triacylglycerol during solvent extraction and various chromatographic procedures.[20] As exogenous diacylglycerol was added as an ethanol-dispersion in the present study, there is a concern that the ethanol acyltransferase reaction may confound the assay of diacylglycerol acyltransferase. However in the present study we included 1 mg/mL bovine serum albumin in the assay media to measure the diacylglycerol acyltransferase activity, which almost completely inhibits the ethanol acyltransferase.[19] In fact, when the microsomes were incubated in the assay media containing 10% ethanol but not diacylglycerols, the rates of the formation of "triacylglycerol" did not exceed 2% of those observed with 125 μmol/L of various molecular species of diacylglycerols dispersed in ethanol and these values were subtracted as the blank.

Table 1 Fatty acid composition of dietary fats (wt%)

Fatty acids	Dietary fats		
	Palm oil	Olive oil	Safflower oil
$C_{12:0}$	0.1	—	—
$C_{14:0}$	1.1	0.1	0.3
$C_{16:0}$	45.3	10.2	7.1
$C_{16:1}$	—	1.1	0.1
$C_{18:0}$	4.4	3.2	2.3
$C_{18:1}$	39.1	78.7	13.3
$C_{18:2}$	9.4	5.6	76.2
$C_{18:3}$	—	1.0	—

As we did not add ethanol using endogenous microsomal diacylglycerols as the substrates, the ethanol acyltransferase activity would not interfere with the assay of diacylglycerol acyltransferase. The value obtained without microsomes was subtracted as the blank for this assay. In the feeding experiment, the activity of diacylglycerol:choline phosphotransferase, which shares a common microsomal diacylglycerol substrate, was also determined. The enzyme activity was determined in the absence or presence of 1,2-di$C_{18:1}$glycerol substrate (3.2 mmol/L) solubilized with Tween 20 (0.3 mg/mL) using 0.1 mmol/L CDP-[methyl-^{14}C]choline (2,220 dpm/nmol) as a tracer.[15,16,21] The amounts of microsomes employed and the enzyme reaction periods were 30–40 and 200–300 μg protein and 6 and 2 min for the enzyme assays in the presence and absence of 1,2-di$C_{18:1}$glycerol substrate, respectively. When the endogenous microsomal diacylglycerol was the substrate, we did not add Tween 20 in the assay media.

Lipid analyses

Liver triacylglycerol[22] and the liver microsomal diacylglycerol[15,16,23] concentrations were determined as described elsewhere. Fatty acid compositions of microsomal diacylglycerol were determined in the pooled samples by gas-liquid chromatography as described previously.[16]

Statistical analysis

Data were analyzed by one-way analysis of variance and significant differences of means were inspected by Duncan's multiple range test at the level of $P < 0.05$.[24]

Results

Activities of rat liver microsomal diacylglycerol acyltransferase measured with various molecular species of diacylglycerol

Microsomal diacylglycerol acyltransferase activities in liver microsomes from rats fed a laboratory chow were determined with varying concentrations of molecular species of 1,2-diacylglycerol substrates differing in the degree of unsaturation as acyl-acceptors and [9,10(n)-^{3}H]$C_{16:0}$-CoA as an acyl-donor *(Figure 1)*. These diacylglycerol substrates were added to the assay media as an ethanol dispersion (final ethanol concentration was 10%). The highest activity was obtained with di$C_{18:1}$glycerol. The activities obtained with di$C_{18:2}$ and di$C_{18:3}$ species were 20–30% lower than that obtained with di$C_{18:1}$glycerol. The 1-$C_{16:0}$-2-$C_{18:1}$glycerol gave an activity similar to that obtained with di$C_{18:1}$glycerol. On the other hand, the activity observed using 1-$C_{16:0}$-2-$C_{18:2}$glycerol was the lowest among those obtained with various diacylglycerol substrates. A computer-assisted least-square analysis of the data according to the method of Lineweaver and Burk[25] revealed that there were considerable differences in V_{max} but not in the apparent K_m values for various diacylglycerol substrates of the enzyme reaction *(Table 2)*. The dependencies of diacylglycerol acyltransferase activity on

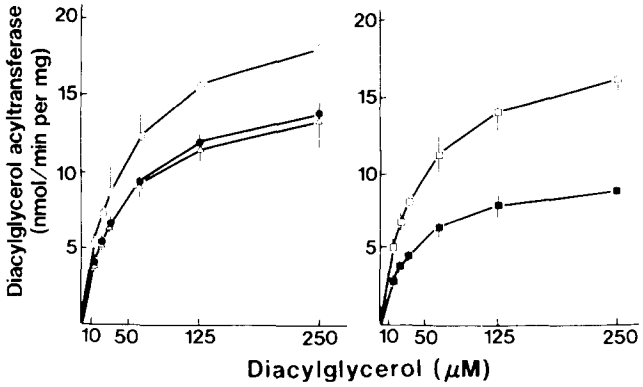

Figure 1 Liver microsomal diacylglycerol acyltransferase activity (mean ± SD of six rats fed laboratory chow) determined with varying concentrations of various molecular species of diacylglycerols as the acyl-acceptors (di$C_{18:1}$, ○; di$C_{18:2}$, ●; di$C_{18:3}$, △; 1-$C_{16:0}$-2-$C_{18:1}$, □; and 1-$C_{16:0}$-2-$C_{18:2}$glycerols, ■, respectively) and $C_{16:0}$-CoA as an acyl-donor.

Table 2 Rat liver microsomal diacylglycerol acyltransferase activities determined with 125 μmol/L of various diacylglycerol substrates (nmol/min per mg protein) and V_{max} (nmol/min per mg protein) and K_m values (μmol/L) for the diacylglycerol substrates of the enzyme reaction

	Diacylglycerol acyltransferase		
Diacylglycerol substrate	Activity obtained with 125 μmol/L diacylglycerol	V_{max}	Apparent K_m
$C_{16:0}$-CoA substrate			
di$C_{18:1}$	16.0 ± 1.2^a	21.6 ± 3.4^a	45.9 ± 9.3^a
di$C_{18:2}$	13.0 ± 1.5^{bc}	16.6 ± 1.5^b	48.0 ± 6.9^a
di$C_{18:3}$	11.7 ± 1.5^{cd}	15.7 ± 2.7^b	44.8 ± 13.5^a
$C_{16:0}$-$C_{18:1}$	14.3 ± 1.7^{ab}	19.0 ± 2.2^{ab}	42.5 ± 8.8^a
$C_{16:0}$-$C_{18:2}$	9.89 ± 1.10^d	10.4 ± 1.2^c	39.0 ± 5.4^a
$C_{18:2}$-CoA substrate			
di$C_{18:1}$	13.8 ± 1.2^a	—	—
di$C_{18:2}$	10.5 ± 1.5^{bc}	—	—
di$C_{18:3}$	10.8 ± 1.2^b	—	—
$C_{16:0}$-$C_{18:1}$	11.9 ± 1.2^{ab}	—	—
$C_{16:0}$-$C_{18:2}$	8.18 ± 0.64^c	—	—

Each value represents mean ± SD of different enzyme preparations from individual rats fed a laboratory chow ($n = 7$–9 for the activity obtained with 125 μmol/L diacylglycerols and $n = 6$ for V_{max} and K_m values, respectively). Values in a column not sharing a superscript letter are significantly different at $P < 0.05$.

various diacylglycerols added to assay media at the concentration of 125 μmol/L were confirmed when 30 μmol/L of [1-^{14}C]$C_{18:2}$-CoA was employed as an acyl-donor. The activities obtained with this substrate appeared to be somewhat less than the corresponding values obtained with $C_{16:0}$-CoA. However, these differences were statistically insignificant.

Activities of diacylglycerol acyltransferase and diacylglycerol:choline phosphotransferase in liver microsomes in rats fed various fats differing in degree of unsaturation for 3 days after 2 days fasting

To test the possibility that observed differences in the dependency on molecular species of diacylglycerol of diacylglycerol acyltransferase in vitro may be a factor in regulating hepatic triacylglycerol synthesis in vivo, rats were fed diets containing 20% fats with different degrees of unsaturation *(Table 1)* or no fat for 3 days after 2 days of fasting. There were no significant differences in average food intake during the 3 days of the feeding period (77–79 g/3 days). Also, the average body weights at time of killing were approximately the same among the groups (229–237 g).

As shown in *Table 3*, types of dietary fats characteristically modified fatty acid composition of liver microsomal diacylglycerol. In rats refed a fat-free diet, $C_{16:0}$ and $C_{18:1}$ acids were prominent in the lipid molecule and the sum of these acids comprised approximately 80% of total fatty acids. The decreases in the proportions of $C_{16:0}$ and $C_{16:1}$ acids and compensatory increases in those of $C_{18:0}$ acid and polyunsaturated fatty acids (sum of $C_{18:2}$, $C_{20:4}$, and $C_{22:6}$ acids) were the general consequences in rats fed 20% fat diets. However, $C_{16:0}$ and $C_{18:1}$ acids were still prominent (70–80% of total fatty acids) in rats refed palm and olive oils, and polyunsaturated fatty acids comprised less than 13.5% in these groups. In contrast, a profound decrease in the proportion of $C_{18:1}$ acid and a great increases in those of $C_{18:2}$ acid and a derivative ($C_{20:4}$ acid) were noted in rats refed safflower oil. As a consequence, these polyunsaturated fatty acids comprised approximately 30% of total fatty acids in this group.

As shown in *Table 4*, there were no significant differences in the activities of liver microsomal diacylglycerol acyltransferase determined using saturating concentrations of di$C_{18:1}$glycerol dispersed in ethanol as an acyl-acceptor and $C_{16:0}$-CoA as an acyl-donor among the groups. However, when the activities were measured with endogenous diacylglycerol substrate, diets containing various fats relative to a fat-free diet significantly decreased diacylglycerol acyltransferase activity. The most prominent reduction in the activity was obtained in rats fed safflower oil and the observed value was significantly lower than those in the animals fed palm and olive oils. The activities of diacylglycerol:choline phosphotransferase, which also utilizes microsomal diacylglycerol as a substrate, were also measured in rats fed various fats. A significant reduction in the enzyme activity measured in the presence of di$C_{18:1}$glycerol substrate solubilized with Tween 20 was obtained with the diet containing olive oil relative to a fat-free diet but not in the other groups. When the enzyme activity was assayed in the absence of the

Table 3 Effects of dietary fats differing in the degree of unsaturation on fatty acid compositions (wt%) of liver microsomal diacylglycerol in fasted-refed rats

	Dietary fats			
Fatty acids	Fat-free	Palm oil	Olive oil	Safflower oil
$C_{16:0}$	43.7	30.4	29.0	28.6
$C_{16:1}$	8.6	3.5	3.9	2.8
$C_{18:0}$	6.0	10.9	7.1	10.9
$C_{18:1}$	35.2	39.2	49.7	21.2
$C_{18:2}$	1.6	5.0	2.8	19.4
$C_{20:4}$	2.0	6.5	3.3	9.3
$C_{22:6}$	0.6	2.0	0.7	1.2

Fatty acids from diacylglycerol in each group of rats were pooled, methylated, and analyzed for the fatty acid composition. Figures for some minor fatty acids were excluded from the table.

Table 4 Effects of dietary fats differing in the degree of unsaturation on the activities of liver microsomal diacylglycerol acyltransferase (nmol/min per mg protein) and diacylglycerol choline phosphotransferase (nmol/min per mg protein) and concentrations of liver microsomal diacylglycerol (nmol/mg protein) and liver triacylglycerol (μmol/g) in fasted-refed rats

	Dietary fats			
	Fat-free	Palm oil	Olive oil	Safflower oil
Diacylglycerol acyltransferase				
with di$C_{18:1}$ glycerol substrate	15.2 ± 2.1^a	15.5 ± 1.1^a	13.4 ± 1.9^a	13.2 ± 1.3^a
without di$C_{18:1}$ glycerol substrate	1.21 ± 0.11^a	0.97 ± 0.08^b	0.84 ± 0.11^b	0.62 ± 0.08^c
Diacylglycerol:choline phosphotransferase				
with di$C_{18:1}$ glycerol substrate	23.9 ± 1.3^a	23.0 ± 1.3^{ab}	19.3 ± 1.3^b	21.0 ± 2.4^{ab}
without di$C_{18:1}$glycerol substrate	4.41 ± 0.40^a	3.00 ± 0.56^b	3.02 ± 0.53^b	2.85 ± 0.42^b
Microsomal diacylglycerol	35.3 ± 3.7^a	22.0 ± 3.7^b	20.4 ± 4.8^b	19.5 ± 3.4^b
Liver triacylglycerol	268 ± 26^a	220 ± 29^b	198 ± 24^b	110 ± 40^c

Each value represents mean ± SD of seven rats. Values in a line not sharing a common superscript letter are significantly different at $P < 0.05$.

$diC_{18:1}$glycerol substrate, the diets containing various fats differing in the degree of unsaturation relative to a fat-free diet profoundly decreased the value to a similar level.

The diets containing various fats relative to a fat-free diet significantly decreased the microsomal concentration of diacylglycerol *(Table 4)*. However, the modifications due to the types of dietary fats were not detected in this parameter. Various dietary fats significantly decreased the triacylglycerol concentration in the liver. Among the groups of rats fed 20% fat diets, safflower oil compared with palm and olive oils significantly decreased this parameter.

Discussion

Although diacylglycerol acyltransferase has been presumed to play a key role in regulating triacylglycerol synthesis in the tissue,[1-6] only a few studies[7-9] examined the substrate specificity of this enzyme. With regard to the acyl-donor specificity of diacylglycerol acyltransferase, Hosaka et al.[7] reported that the enzyme solubilized from rat microsomes efficiently utilized various acyl-CoAs (including $C_{14:0}$-, $C_{16:0}$-, $C_{18:1}$, $C_{18:2}$-, and $C_{20:4}$-CoAs) except for $C_{18:0}$-CoA as substrates. Rustan et al.[9] reported that $C_{20:5}$-CoA compared with $C_{18:1}$-CoA was a poor substrate for microsomal diacylglycerol acyltransferase in rat liver. They suggested that the acyl-donor specificity of the enzyme may be responsible for the reduction in lipoprotein synthesis in rat hepatocytes cultured in the medium fortified with $C_{20:5}$ acid. Coleman and Bell[8] showed that activities of microsomal diacylglycerol acyltransferase in fat cells are dependent on the chain length and degree of unsaturation of acyl-CoA substrates. They also examined the efficiency of various molecular species of diacylglycerol added to the assay media as an ethanol-dispersion for the acyl-acceptor of the enzyme reaction in fat cell microsomes and found that various diacylglycerols of 1,2-saturated fatty acid species (including $C_{6:0}$ to $C_{16:0}$ species) compared with $diC_{18:1}$glycerol are the poor substrates for diacylglycerol acyltransferase. Employing ethanol-dispersed substrates, we provided evidence, for the first time, that rat liver microsomal diacylglycerol acyltransferase also discriminates the molecular species of diacylglycerol substrate *(Figure 1 and Table 2)*. Our present study indicates that the modification in the degree of unsaturation of diacylglycerol substrate is a determinant in modulating the activity of diacylglycerol acyltransferase in rat liver microsomes. Moreover, the experiment in rats fed various type of dietary fats differing in degrees of unsaturation *(Table 4)* strongly suggested that the acyl-acceptor specificity of diacylglycerol acyltransferase plays a role in regulating hepatic triacylglycerol synthesis in vivo.

As the ethanol-dispersed diacylglycerol substrates were used to determine the acyl-acceptor specificity of diacylglycerol acyltransferase in the present study, we carefully employed the assay condition that excludes the interference by ethanol acyltransferase activity that is contained in rat liver microsomes (see Materials and methods). Diacylglycerol acyltransferase and ethanol acyltransferase are localized in the cytoplasmic[26] and the lumenal[19] surfaces of microsomal vesicles, respectively. As fatty acyl-CoA substrate can not be transported across the microsomal membrane,[19] the ethanol acyltransferase activity may not be expressed so far as the microsomal vesicles remain to be intact. However, the possibility that the ethanol acyltransferase activity is expressed at a significant degree under the particular assay condition owing to the disruption of microsomal vesicles can not be excluded. Thus, it remains necessary to confirm our results with regard to the acyl-acceptor specificity of diacylglycerol acyltransferase employing the choices other than ethanol-dispersion for the addition of diacylglycerol substrates in the assay media. However, the other reported methods for the addition of diacylglycerol in the assay media using detergents (Tween 20,[7] Tween 80,[3] or taurocholate[26]) appear to give the extremely lower diacylglycerol acyltransferase activity compared with those obtained using ethanol-dispersed substrates.[8,9] In fact, Coleman and Bell[8] reported that Tween 20 severely inhibited the diacylglycerol acyltransferase activity in fat cell microsomes. Thus, the development of a novel method for the addition of diacylglycerol substrate in the assay media to measure the diacylglycerol acyltransferase activity is necessary before the confirmation of the present finding is made.

Caloric densities of a fat-free diet and the diets containing 20% of various fats employed in the present study were considerably different from each other. As dietary fats were added to the experimental diets at the expense of sucrose, rats fed a fat-free diet consumed more of the sucrose compared with those fed fat-supplemented diets. Thus, there is the possibility that the differences in the caloric intake and the amounts of sucrose consumed may be the factors that cause the alterations in the lipid metabolism seen in the rats fed a fat-free diet and those fed 20% fat diets. However, these parameters are considered to be the same among the various groups of rats fed 20% fat diets. Thus, the differences in caloric intake and the amounts of sucrose consumed do not account for the observed changes in various parameters for lipid biosynthesis among the three groups of rats fed various fats differing in the degree of unsaturation.

Diacylglycerol acyltransferase and diacylglycerol: choline phosphotransferase share a common diacylglycerol substrate.[1,2] When saturating concentrations of $diC_{18:1}$glycerol were present in assay media, neither the acyltransferase nor the phosphotransferase activities were affected by types of dietary fats. Also, the activities of these enzymes in rats fed various 20% fat diets were comparable with those in the animals fed a fat-free diet except for one instance in the phosphotransferase. Although we employed artificial forms of $diC_{18:1}$glycerol substrate (ethanol-dispersed and Tween-solubilized for acyltransferase and phosphotransferase, respectively) for the enzyme assays, the results suggested that various dietary fats were irrelevant in mod-

ulating the enzyme amounts or catalytic activities of these enzymes. Reductions in the activities of these enzymes measured in the absence of $diC_{18:1}$glycerol substrate in rats fed various types of dietary fats relative to those fed a fat-free diet are apparently due to the reductions in the availability of endogenous microsomal diacylglycerol substrate[15,16] *(Table 4)*. However, the responses of these enzymes to types of dietary fats were considerably different from each other. Our present finding that V_{max} but not K_m values of diacylglycerol acyltransferase for ethanol-dispersed diacylglycerol substrates were affected by the degree of unsaturation of constituent fatty acids *(Table 2)* may be extrapolated to the responses of the acyltransferase to endogenous microsomal substrates, and thus may account for the reduction of the enzyme activity in rats fed safflower oil relative to the animals fed palm and olive oils.

Previous studies showed that choline phosphotransferase does not show any selectivity toward both microsome-bound[27] and Tween-solubilized[21] diacylglycerol molecular species differing in the degree of unsaturation. This supports our present observation that types of dietary fats alter the fatty acid composition of microsomal diacylglycerol but did not affect the transferase activity measured with the endogenous substrate. There is the concern that various dietary fats may modify the hepatic concentration of CDP-choline. The consequence may alter the specific activity of radiolabeled CDP-choline employed as a tracer to measure the activity of choline phosphotransferase and thus make the accurate determination of the enzyme activity difficult. The concentration of CDP-choline in rat liver has been reported to be 28–50 nmol/g.[28] The amount of liver microsomes employed in the enzyme assays in the present study approximated to merely 13 and 2.6 mg liver in the absence and presence of exogenous diacylglycerol substrate, respectively. Thus, the amounts of endogenous CDP-choline in the assay mixture available for choline phosphotransferase are considered to be extremely low compared with those added exogenously (0.1 mmol/L, 20 nmol in 0.2 mL of the incubation mixture). Although subcellular localization of CDP-choline has not been studied yet, it is also plausible that the water-soluble CDP-choline mainly localizes in cytoplasmic fraction after cell fractionation and may not be contained in isolated microsomal fraction used as the enzyme source in the present study.

Microsomal enzyme reactions transfer phosphocholine moiety of CDP-choline not only to diacylglycerols to form phosphatidylcholine, but also to 1-alkyl-2-acylglycerols and ceramides to form plasmalogen and sphingomyeline, respectively.[29] Our present method for the assay of choline phosphotransferase[15,16,21] does not discriminate the phosphatidylcholine, plasmalogen, and sphingomyeline syntheses. Also, our assay for microsomal diacylglycerol[15,16,23] may also detect 1-alkyl-2-acylglycerols and ceramides. It has been reported that rat liver contained a significant amount of ceramides.[30] Thus, it is also possible that differences in the microsomal concentrations of these lipid molecules among the groups may at least in part be responsible for the different responses to types of dietary fats in diacylglycerol acyltransferase and choline phosphotransferase. Thus, exact determinations of these lipid molecules in liver microsomes and careful clarification of the enzyme products of cholinephosphotransferase may be required to draw a definite conclusion.

In conclusion, the examination of the acyl-acceptor specificity of diacylglycerol acyltransferase in rat liver microsomes employing ethanol-dispersed diacylglycerol substrates and the result of a feeding study indicate that modifications in the fatty acid composition of microsomal diacylglycerol is a determinant in regulating triacylglycerol synthesis in the liver. A detailed study with regard to the acyl-acceptor specificity of diacylglycerol acyltransferase using a physiological form of membrane-bound diacylglycerol substrate should be made to draw a definite conclusion.

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