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Effects of Three Different Highly Purified n-3 Series Highly Unsaturated Fatty Acids on Lipid Metabolism in C57BL/KsJ-*db*/*db* Mice

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Triglycerides (TG) consisting of highly purified (>97%) n-3 series highly unsaturated fatty acids, eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA), and docosahexaenoic acid (DHA), were administered to C57BL/KsJ-*db*/*db* mice for 4 weeks by pair-feeding to compare their effects on lipid metabolism and to evaluate the effects of DPA on lipid metabolism. The hepatic TG level and total amount was decreased by treatment with DHA and DPA compared to the control. The efficacy of DPA was greater than that of EPA, but less than that of DHA. In contrast, EPA had the greatest serum TG reducing effect. The hepatic cytosol fraction of the DHA-treated group contained the lowest fatty acid synthase (FAS) and malic enzyme (ME) activity levels. Furthermore, the DHA-treated group contained the highest serum adiponectin concentrations. These findings indicate that the strong hepatic TG-lowering effect of DHA is due to the suppression of TG synthesis. The same tendencies were observed in DPA-treated mice, and the effect was stronger than that observed in EPA-treated mice, but equivalent to that observed in DHA-treated mice. Based on these results, DPA possesses lipid metabolism-improving effects. The beneficial effects of DPA for lipid metabolism were not superior to those of EPA and DHA, and the effect was always intermediate between those of EPA and DHA.

KEYWORDS: C57BL/KsJ-*db*/*db* mice; docosahexaenoic acid; docosapentaenoic acid; eicosapentaenoic acid; lipid metabolism; n-3 series highly unsaturated fatty acids; triglyceride

INTRODUCTION

Fish oil has attracted widespread attention as a functional fat and oil. The effects of fish oil are thought to originate from n-3 series highly unsaturated fatty acids (n-3HUFA), such as eicosapentaenoic acid (20:5n-3, EPA) and docosahexaenoic acid (22:6n-3, DHA), which are contained in fish oil. Though several studies have reported their efficacy for lowering triglycerides (TG) and cholesterol levels in the plasma and liver (*1*−*3*), increasing β-oxidation activity in the mitochondria and peroxisomes in hepatic cells (*4*), and suppressing TG synthesis in the liver (*5*), some of the results and proposed mechanisms are still conflicting or are unclear (*6*, *7*). Many kinds of foods and medicines containing these functional fatty acids are already on the market and applied practically. For example, EPA decreases plasma TG levels, and EPA ethyl ester is used as a medicine to treat hyperlipidemia (*3*). DHA also improves lipid metabolism. In Japan, some foods enriched with DHA have been approved as a Food for Specified Health Use by the Ministry of Health, Labor, and Welfare, and packaging of the foods is permitted to display health claims regarding the improvement of blood TG levels (*8*).

EPA and DHA are n-3HUFA and cannot be de novo synthesized in a mammalian body because mammals lack the essential Δ-12 and Δ-15 desaturase (*9*). Therefore, they must be obtained from other living organisms. The conversion of an n-3 series polyunsaturated fatty acid taken into the mammalian body to other kinds of n-3 series polyunsaturated fatty acids is possible, however, through chain elongation, desaturation, and β-oxidation reactions (*9*). For example, EPA taken into the body can be converted to DHA by two elongation reactions, a desaturation reaction, and subsequent β-oxidation. The first elongation reaction for EPA forms docosapentaenoic acid (22:5n-3, DPA). Although DPA is not a major fatty acid, it is contained in fairly high amounts in seal oil (*10*, *11*). The n-6 series DPA is increased in the mammalian brain when there are limited amounts of n-3 series (*12*−*14*). This n-6 series DPA (22:5n-6, n-6DPA) is produced from the shorter chain length of n-6 series polyunsaturated fatty acids such as linoleic and arachidonic acids.

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In fact, several effects of n-3 series DPA itself have been reported over the last several years. Kanayasu-Toyoda et al. reported that DPA possesses a 10-fold greater endothelial cell migration ability than EPA, and that DPA might act as a potent preventive nutrient against arteriosclerosis (*15*). Akiba et al. compared the inhibition effects among n-3 series EPA, DPA and DHA on platelet aggregation stimulated by collagen or arachidonic acid (*16*). DPA was the most potent inhibitor and is thought to act as a potent antithrombosis factor in the arteries. Therefore, n-3 series DPA is now attracting attention as a functional fatty acid.

Seal oil is a marine oil that contains relatively high amounts of n-3 series DPA compared to other marine oils such as tuna and sardine oils (*10*, *11*). The reducing effect of DPA in seal oil on plasma TG levels was confirmed in rats and found to be even stronger than the effect of a mixture of tuna orbital oil and sardine oil (*17*). The plasma TG reducing ability is thought to be due to the existence position of n-3HUFA on a glycerol backbone of TG, but relatively high amounts of DPA might also induce this effect. One study compared the lipid metabolism-improving effects of EPA and DHA using highly purified EPA ethyl ester (> 95%) and DHA ethyl ester (> 95%) in rats (*18*); however, the effects have not been compared among EPA, DPA, and DHA using TGs consisting of highly purified fatty acids in animal experiments. The metabolism pathways of fatty acids in fatty acid ethyl ester and TG are different (*19*). The fatty acids hydrolyzed from fatty acid ethyl ester in the small intestine are absorbed into small-intestine epithelial cells to be synthesized to TG or phospholipids in the phosphatidic acid pathway. In contrast, the fatty acids hydrolyzed from TG are mainly synthesized to TG in the monoacylglycerol pathway in the small-intestine epithelial cells because the 2-monoacylglycerol is also formed in small intestine when TG is hydrolyzed in small intestine. The fatty acid ethyl ester is an artificially synthesized form. Therefore, in using the TG form of n-3HUFA, the main naturally existing form of fatty acid, it is important to understand the effect of n-3HUFA on the body. C57BL/KsJ-*db*/*db* mice carry a mutation in the leptin receptor gene that results in impaired function of leptin receptor b and eventually exhibit hyperlipidemic, diabetic, and obese symptoms due to a lack of appetite control (*20*−*25*). Therefore, this model mouse is commonly used for studies of lipid and glucose metabolism. The C57BL/KsJ-*db*/*db* mouse is also thought to be a suitable model for evaluating the effects of n-3HUFA on lipid metabolism. In this study, TGs consisting of highly purified fatty acids were administered to C57BL/KsJ-*db*/*db* mice to compare their effects on lipid metabolism, aimed toward understanding the effects of DPA on lipid metabolism in comparison with those of EPA and DHA.

MATERIALS AND METHODS

Chemicals and Materials. AAA-type TGs such as tripalmitate (tri18:0), trioleate (tri18:1n-9), trilinoleate (tri18:2n-6), trilinolenate (tri18:3n-3), triEPA, triDPA, and triDHA were obtained from Tsukishima Foods Industry Co., Ltd. (Tokyo, Japan). The purity of the fatty acids consisting of AAA-type TG, with the exception of α-linolenic acid, was greater than 97%. The purity of α-linolenic acid was greater than 90%. Dithiothreitol, Triton X-100, 6-phosphogluconatedehydrogenase, casein, DL-methionine, and choline bitartrate were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Beta-corn starch, cellulose, nicotinamide adenine dinucleotide, reduced nicotinamide adenine dinucleotide, and reduced nicotinamide adenine dinucleotide phosphate were obtained from Oriental Yeast Co., Ltd. (Tokyo, Japan). Mineral mixture (AIN-76) and vitamin mixture (AIN-76) were purchased from Nosan Corporation (Tokyo, Japan). 5,5′-Dithiobis(2-nitro-benzoic acid) was purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). Sucrose was obtained from Mitsui Sugar Co., Ltd. (Tokyo, Japan). Silica gel (silicic acid 100 mesh) was purchased from Mallinckrodt Baker, Inc. (Phillipsburg, NJ). Palmitoyl CoA was obtained from Funakoshi Co., Ltd. (Tokyo, Japan). Glucose 6-phosphate was purchased from CALZYME LABORATORIES, Inc. (San Luis Obispo, CA). Acetyl CoA, L-malic acid, malonyl CoA, L-carnitine, flavin adenine dinucleotide (FAD), phosphatidylcholines, and phosphatidic acid were bought from SIGMA-Aldrich Japan K.K. (Tokyo, Japan). Other reagents were obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan).

Table 1. Composition of Experimental Diet (g/100 g)

	C57BL/6J	C57BL/KsJ-*db*/*db*			
ingredient	normal control group	control group	EPA-TG group	DPA-TG group	DHA-TG group
casein	20.00	20.00	20.00	20.00	20.00
corn starch	15.00	15.00	15.00	15.00	15.00
cellulose	5.00	5.00	5.00	5.00	5.00
mineral mixture (AIN-76)	3.50	3.50	3.50	3.50	3.50
vitamin mixture (AIN-76)	1.00	1.00	1.00	1.00	1.00
DL-methionine	0.30	0.30	0.30	0.30	0.30
choline bitartrate	0.20	0.20	0.20	0.20	0.20
tripalmitate (tri16:0)	3.00	3.00	3.00	3.00	3.00
trioleate (tri18:1n-9)	3.00	3.00	3.00	3.00	3.00
trilinoleate (tri18:2n-6)	2.90	2.90	1.90	1.90	1.90
trilinolenate (tri18:3n-3)	0.10	0.10	0.10	0.10	0.10
triEPA (tri20:5n-3)			1.00		
triDPA (tri22:5n-3)				1.00	
triDHA (tri22:6n-3)					1.00
sucrose	46.00	46.00	46.00	46.00	46.00

Animal Tests. All experiments were performed in accordance with the Saga University guidelines for animal use and care provided by the ethics committee. Five-week-old male C57BL/KsJ-*db*/*db* mice and C57BL/6J mice were supplied by Nippon Clea Co. (Tokyo, Japan). The mice were housed individually in plastic cages lined with sawdust in a temperature (23 ± 2 °C) and light (12-h cycle) controlled environment. The mice were initially fed a commercial diet CE-2 (Nippon Clea Co.) for 1 week and then divided into four groups: control group (control diet; $n = 5$), EPA-TG group (1% [w/w] of trilinoleate in the control diet was replaced with triEPA; $n = 5$), DPA-TG group (1% of trilinoleate in control diet was replaced with triDPA; $n = 5$), and DHA-TG group (1% of trilinoleate in control diet was replaced with triDHA; $n = 5$). TG comprised 9% of the control diet and consisted of tripalmitate (3%), trioleate (3%), trilenoleate (2.9%), and trilinolenate (0.1%). All the diets were prepared based on an AIN-76 composition, and the detailed diet composition is shown in **Table 1**. Each group of C57BL/KsJ-*db*/*db* mice was given their diet for 4 weeks by pair-feeding. Furthermore, as the positive control group, a group of C57BL/6J mice was also fed a control diet for 4 weeks (normal control). All the mice were allowed free access to water. The weight and consumption amount of the feed for each mouse were measured every day. At the end of the feeding period, the mice were killed by withdrawing blood from the heart under nembutal anesthesia after a 9 h starvation period. Liver, epididymal, perirenal, and omental fat, and testis, kidney, spleen, heart, muscle, and brain were harvested and their weights, with the exception of that of muscle, were obtained. All the organs and tissues were preserved in liquid nitrogen. The withdrawn blood samples were centrifuged at 1750*g* for 15 min at 4 °C to collect the serum.

Serum Analysis. Serum TG and cholesterol levels were analyzed using commercial enzyme assay kits (Wako Pure Chemical Industries, Ltd.). Insulin and adiponectin levels were measured using an Insulin ELISA kit (Shibayagi Co. Ltd., Gunma, Japan) and Mouse/Rat Adiponectin ELISA kit (Otsuka Pharmaceutical Co., Ltd., Tokyo, Japan), respectively. Alanine aminotransferase (ALT) activity was analyzed using a Transaminase CII-test Wako kit (Wako Pure Chemical Industries, Ltd.).

Liver Lipid Analysis. Hepatic lipid was extracted from liver by the method of Folch et al. (*26*). Hepatic concentrations of TG and phospholipid were measured according to Fletcher et al. (*27*) and Rouser et al. (*28*),

Table 2. Effect of Experimental Diet on Growth Parameters in C57BL/6J and C57BL/KsJ-*db*/*db* Mice[a]

	C57BL/6J	C57BL/KsJ-*db*/*db*			
	normal control group	control group	EPA-TG group	DPA-TG group	DHA-TG group
initial body wt (g)	21.8 ± 0.3	27.4 ± 0.1*	27.4 ± 0.3	27.4 ± 0.2	27.4 ± 0.4
final body wt (g)	24.0 ± 0.3	33.6 ± 1.0 ab*	35.0 ± 0.5 a	32.7 ± 1.1 ab	30.5 ± 0.9 b
body wt gain (g)	2.20 ± 0.14	6.28 ± 1.07 ab*	7.64 ± 0.72 a	5.34 ± 1.01 ab	3.18 ± 1.18 b
food intake (g)	70.1 ± 0.6	115 ± 4*	115 ± 5	115 ± 2	114 ± 2
liver wt (g/100 g BW)	3.93 ± 0.08	6.37 ± 0.68*	6.13 ± 0.40	5.29 ± 0.17	5.18 ± 0.21
testis (g/100 g BW)	0.850 ± 0.019	0.412 ± 0.016*	0.407 ± 0.020	0.335 ± 0.009	0.351 ± 0.031
kidney (g/100 g BW)	1.20 ± 0.04	1.06 ± 0.07	1.00 ± 0.03	1.10 ± 0.05	1.18 ± 0.03
spleen (g/100 g BW)	0.230 ± 0.014	0.0912 ± 0.0084*	0.105 ± 0.007	0.0924 ± 0.0047	0.103 ± 0.006
heart (g/100 g BW)	0.425 ± 0.003	0.276 ± 0.011*	0.273 ± 0.008	0.278 ± 0.005	0.291 ± 0.013
accumulated fat (white adipose tissue) wt (g/100 g BW)					
brain (g/100 g BW)	1.73 ± 0.03	1.04 ± 0.03 ab*	1.00 ± 0.02 a	1.06 ± 0.02 ab	1.14 ± 0.04 b
total	3.52 ± 0.39	11.5 ± 0.3 ab*	11.4 ± 0.1 ab	12.0 ± 0.4 a	10.8 ± 0.2 b
epididymal	1.74 ± 0.17	5.02 ± 0.19*	4.77 ± 0.12	5.05 ± 0.35	4.68 ± 0.04
perirenal	0.807 ± 0.081	2.89 ± 0.14*	3.00 ± 0.18	3.21 ± 0.13	2.75 ± 0.17
omental	0.974 ± 0.159	3.59 ± 0.10 ab*	3.66 ± 0.10 ab	3.73 ± 0.03 a	3.34 ± 0.07 b

[a] Each value represents mean ± SE. Different letters indicate significant difference at $P < 0.05$ among C57BL/KsJ-*db*/*db* groups. (*) $P < 0.05$ (normal control vs control group).

respectively. Hepatic cholesterol concentration was analyzed using the Cholesterol E-Test Wako (Wako Pure Chemical Industries, Ltd.).

Hepatic Enzyme Activity Assay. Liver mitochondrial and cytosol fractions were prepared by homogenization and centrifugation. The protein concentration of each fraction was analyzed by the method of Lowry (*29*). The enzyme activity of fatty acid synthase (FAS) (*30*) and glucose 6-phosphate dehydrogenase (G6PDH) (*31*) in each cellular fraction was measured by the method of Kelly. Malic enzyme (ME), carnitine palmitoyl transferase (CPT), peroxisomal β-oxidation, and phosphatidate phosphohydrolase (PAP) in each cellular fraction were assayed by the method of Ochoa et al. (*32*), Markwell et al. (*33*), Lazarow et al. (*34*), and Walton et al. (*35*), respectively.

Hepatic mRNA Level Assay. Total RNA was extracted from liver using TRIZOL Reagent (Invitrogen, Tokyo, Japan). TaqMan Universal PCR Master Mix (Applied Biosystems, Tokyo, Japan); Assay-on-Demand, Gene Expression Products (Mm01204659_m1 for acetyl-CoA carboxylase 2 (ACC2), Mm000662319_m1 for FAS, Hs99999901_s1 for 18S RNA, Applied Biosystems), and TaqMan MGB Gene Expression Kit for sterol regulatory element binding protein-1 (SREBP-1) were used for quantitative real-time RT-PCR analysis of ACC, FAS, 18S RNA, and SREBP-1 expression in the liver, respectively. The details of the TaqMan MGB Gene Expression assay were as follows: SREBP-1 (forward primer, 5′-GCCCACAATGCCATTGAGA-3′; reverse primer, 5′-GCAAGACAGCAGATTTATTCAGCTT-3′; and TaqMan MGB probe, 5′-FAM-TATCAATGACAAGATTGTG-MGB-3′). The amplification was performed with a real-time PCR system (ABI Prism 7000 Sequence Detection System; Applied Biosystems). Results were quantified with a comparative method and expressed as a relative value after normalization to 18S RNA expression.

Analysis of Fatty Acid Composition in Organs and Tissues. Lipids contained in the organs and tissues were also extracted by the method of Folch et al. (*26*). The extracted lipids were dried and weighed. Methyl esterification of the extracted lipids was performed using a boron trifluoride methanol solution. The methyl-esterified fatty acid was subjected to a gas chromatography−flame ionization detector (GC−FID) system (GC14B, Shimadzu, Tokyo, Japan) equipped with a capillary column (Omegawax320, 30 m × 0.25 mm ID, SIGMA-Aldrich Japan K. K., Tokyo, Japan) and a Chromatopac integrator (C-R6A; Shimadzu) to analyze the fatty acid composition and relative ratio. The temperature of the injection port and detector was 250 and 260 °C, respectively. The initial column temperature of 175 °C was increased to 225 °C at a rate of 1 °C/min. Helium was used as the carrier gas at a flow rate of 32 cm/s. The fatty acid species was identified using the retention time of a fatty acid methyl ester standard solution (Supelco 37 Component FAME Mix, SIGMA-Aldrich Japan K.K.). The relative content of the respective fatty acids was calculated using a GC−FID chromatogram.

Statistical Analyses. Each value is presented as mean ± SE. Differences between C57BL/KsJ-*db*/*db* groups were analyzed by one-way ANOVA, and all detected differences were further analyzed using a Tukey−Kramer posthoc test. Differences between normal control and the control group were evaluated by Student's *t* test. The difference was considered significant when P was less than 0.05.

RESULTS AND DISCUSSION

C57BL/KsJ-*db*/*db* mice carry a G-to-T point mutation at the leptin receptor b coding gene and therefore lack the ability to bind leptin (*20*, *21*). Other mutations in the C57BL/KsJ-*db*/*db* mouse have not been reported. This one mutation leads to an excessive appetite, thereby causing obesity, hyperlipidemia, hyperglycemia, etc. (*22*−*25*). In the present study, pair-feeding was employed to compare the relative effects of n-3HUFA on lipid metabolism in C57BL/KsJ-*db*/*db* mice. Growth parameters, such as body weight, food intake amount, organ weight, and tissue weight for each experimental diet group of C57BL/KsJ-*db*/*db* and the C57BL/6J mice, are shown in **Table 2**. The body weight gain of the control group (C57BL/KsJ-*db*/*db* mice) was almost 3-fold higher than that of the normal control (C57BL/6J mice), even though the food intake amount of the control group was less than double that of the normal control. Leptin is involved in the control of metabolic rate, thermogenesis, inflammation, etc. (*20*−*25*). The big difference in weight gain between the control group and the normal control group might be also due to these other roles of leptin. Therefore, the defective leptin receptor function affects not only appetite control but also other homeostatic mechanisms, and therefore observations of lipid metabolism in C57BL/KsJ-*db*/*db* mice is thought to be the best way to understand the effects of functional food components on lipid metabolism. Differences in the these accumulated TG amounts in the liver, particularly between the normal control and the control group (**Table 3**), indicate that the metabolism rate of hepatic TG is inherently lower in C57BL/KsJ-*db*/*db* mouse liver than that in the C57BL/6J mouse liver and/or that the TG synthesis rate in C57BL/KsJ-*db*/*db* mouse liver is inherently higher than that in C57BL/6J mouse, because the food intake amount of the control group was less than double that of the normal control. There was a clear effect of n-3HUFA ingestion on hepatic TG levels (**Table 3**). Hepatic TG levels in the DHA-TG were approximately 3-fold lower than that in the control group, and that in the DPA-TG group was about half that in the control group. Ingestion of EPA-TG, however, did not significantly decrease hepatic TG levels. Incidentally, an improvement in hepatic TG levels was not observed in the EPA-TG group. The hepatic TG lowering effect was DHA-TG > DPA-TG > EPA-TG.

Table 3. Effect of Experimental Diet on Hepatic Lipids in C57BL/6J and C57BL/KsJ-*db*/*db* Mice (mg/g liver)[a]

	C57BL/6J	C57BL/KsJ-*db*/*db*			
	normal control group	control group	EPA-TG group	DPA-TG group	DHA-TG group
triglyceride (mg/g liver)	25.1 ± 3.9	173 ± 21 a*	148 ± 20 ab	101 ± 13 bc	67.3 ± 11.0 c
triglyceride (mg/liver)	24.0 ± 4.4	398 ± 98 a*	327 ± 64 ab	180 ± 30 ab	109 ± 22 b
total cholesterol (mg/g liver)	2.57 ± 0.06	3.15 ± 0.14*	3.81 ± 0.29	3.42 ± 0.27	3.84 ± 0.28
total cholesterol (mg/liver)	2.42 ± 0.07	6.83 ± 1.00*	8.12 ± 0.70	5.84 ± 0.30	3.07 ± 0.53
phospholipid (mg/g liver)	31.3 ± 0.6	23.1 ± 1.0*	24.4 ± 1.0	25.4 ± 1.3	25.8 ± 0.9
phospholipid (mg/liver)	29.5 ± 0.7	49.6 ± 6.6*	52.0 ± 2.7	43.7 ± 2.1	40.5 ± 1.3

[a] Each value represents mean ± SE. Different letters indicate significant difference at $P < 0.05$ among C57BL/KsJ-*db*/*db* groups. (*) $P < 0.05$ (normal control vs control group).

This finding might be related to hepatic enzyme activity. The activities of several kinds of hepatic TG metabolism-related enzymes were measured (Table **5**), and peroxisomal β-oxidation and CPT activity in the cytosolic and mitochondrial fractions, PAP in the microsomal fractions, and G6PDH in cytosolic fraction did not significantly differ among the four C57BL/KsJ-*db*/*db* groups. In contrast, the enzymatic activities of FAS and ME, relating to TG synthesis, in the cytosolic fraction were affected by the n-3HUFA species. Also the mRNA levels of FAS and SREBP-1 in liver were also decreased by supplementation with n-3HUFA, but the reduction was not significant (**Table 6**). The FAS activity in the DHA-TG group was lower than that in the other groups. Though a significant difference was not detected, the mean FAS activity in the DPA-TG group was lower than that in the control and EPA-TG groups. The tendency was similar for ME activity. These results might indicate that n-3HUFA comprising 22 carbons like DPA and DHA is important for lowering the lipogenic enzyme activities of FAS and ME to decrease TG levels in the liver. Willumsen et al. also observed acute alterations in lipid metabolism after the administration of EPA and DHA in Mol:WIST rats (*7*). In their study, both lipogenic and lipolytic enzyme activities in the liver were examined and the results were similar to ours. For example, the activity of the lipogenic enzyme PAP was significantly decreased compared to that of the control group. In our study, however, the reduction in PAP activity was not significant. Also, FAS and ME were reduced in the DPA-TG and DHA-TG groups. Therefore, DHA and DPA act to suppress the synthesis of TGs in the liver. Willumsen et al. also observed a significant increase in the activity of lipolytic enzyme such as fatty acyl-CoA oxidase and 2,4-dienoyl-CoA reductase activity in an EPA-treated group (*7*). The increases in these enzymes might also reduce hepatic TG levels, and other studies reported that EPA reduces hepatic TG levels (*5*). In the present study, we observed no significant increase in lipolytic enzyme activity, e.g., CPT and peroxisomal β-oxidation, following EPA treatment. As a result, hepatic TG levels (mg/g liver) and total amounts (mg/liver) in the EPA-TG group were not improved compared to the control group. This difference might be due to differences in leptin receptor function between animals. Further discussion about these differences is not possible at present because the same enzymatic activities were not evaluated in both experiments. In contrast, serum TG levels were lowest in the EPA-TG group among the four C57BL/KsJ-*db*/*db* groups. This might be due to lower hepatic VLDL levels due to enhanced intercellular degradation of apolipoprotein (Apo) B100 by EPA (*36*). Interestingly, the effect of lowering serum TG levels by EPA-TG was the reverse of that for hepatic TG levels. Therefore, it might be that the number of double bonds in the structure is an important factor for the enhancement of intercellular degradation of Apo B100. The serum TG level of the DHA-TG group was not significantly different from that of the control group, which is not consistent with other reports that DHA decreases blood TG levels (*18*, *37*). A possible reason for this discrepancy is that the blood TG lowering effects of DHA might be due to a reduction of the hepatic TG level. As indicated above, DHA strongly decreases hepatic TG levels. In the present study, the test feed administration period was 4 weeks, and this might be not sufficient for complete lowering of the hepatic TG levels, which is reflected by the serum TG levels. The lowering efficacy of both serum and hepatic TG levels of DPA was always intermediate between that of EPA and DHA. These results might indicate that the ability of DPA to reduce serum and hepatic TG levels is not superior to that of the other n-3HUFAs.

The effects of n-3HUFAs on cholesterol levels were different from those on serum and hepatic TG levels. For example, mean hepatic cholesterol level (mg/g liver) and amount (mg/liver) were higher in the groups administered n-3HUFA than in the control group (**Table 3**). In contrast, mean serum cholesterol level in the n-3HUFA groups was lower than that in the control group (**Table 4**). Plasma cholesterol levels are reported to be decreased by n-3HUFA (*38*). Our results are consistent with these previous findings, even though the difference we observed was not significant. There was no difference in the serum and hepatic TG levels among the groups receiving n-3HUFAs, however, which might indicate that n-3HUFA suppressed the secretion of cholesterol from the liver because the cholesterol levels in the liver of the three groups receiving n-3HUFAs were higher than those of the control group, but the levels in the serum were lower.

Adiponectin is a protein hormone related to glucose and lipid metabolism, and the blood concentration is inversely correlated with body fat percentage (*39*, *40*). Low levels of plasma adiponectin increase the risk for cardiovascular diseases (*41*, *42*). The concentration of adiponectin in the control group (C57BL/KsJ-*db*/*db* mice) was significantly lower than that of the normal control (C57BL/6J mice) (**Table 4**), indicating that all the C57BL/KsJ-*db*/*db* mice exhibited abnormal lipid or glucose metabolism. The level was improved, however, by the administration of n-3HUFA. The beneficial effect of DHA was the strongest, followed by DPA, among the three groups administered n-3HUFA. EPA also increased adiponectin, and significantly higher adiponectin levels were observed in the EPA-TG group than in the control group. The increase in adiponectin by n-3HUFA has been reported in both animals and humans by several groups (*43*, *44*). Therefore, the adiponectin findings in the present study are consistent with previous results. DHA-TG decreased the enzymatic activity of FAS and ME in the hepatic cytosol fraction (**Table 5**) as well as the hepatic TG level (mg/g liver) and amount (mg/liver) (**Table 3**). Adiponectin suppresses hepatic enzymatic activity related to fatty acid synthesis (*45*, *46*). Thus, there is a possibility that the decrease in FAS and ME activity induced by the administration of DHA-TG might be due to the increase in the adiponectin levels. Furthermore, a tendency toward an improved serum insulin concentration was observed in the DHA-TG and DPA-TG groups (**Table 4**), but the improvement was not significant. As mentioned above, the C57BL/KsJ-*db*/*db* mouse is also commonly used in glucose metabolism

Table 4. Effect of Experimental Diet on Serum Parameters in C57BL/6J and C57BL/KsJ-*db*/*db* Mice[a]

	C57BL/6J	C57BL/KsJ-*db*/*db*			
	normal control group	control group	EPA-TG group	DPA-TG group	DHA-TG group
triglyceride (mg/dL)	46.4 ± 3.8	85.1 ± 7.0*	66.9 ± 5.2	76.8 ± 10.9	92.7 ± 7.4
total cholesterol (mg/dL)	93.3 ± 2.9	177 ± 12*	152 ± 13	137 ± 5	153 ± 14
ALT (GPT) (IU/L)	6.02 ± 0.8	45.6 ± 20.1	37.0 ± 7.2	27.7 ± 2.7	32.0 ± 3.3
adiponectin (μg/mL)	19.1 ± 0.8	10.6 ± 0.3 a*	13.7 ± 0.6 b	14.6 ± 0.6 b	15.6 ± 0.4 b
insulin (ng/mL)	0.986 ± 0.270	17.0 ± 3.8*	25.3 ± 9.1	11.4 ± 0.7	11.5 ± 2.2

[a] Each value represents mean ± SE. Different letters indicate significant difference at $P < 0.05$ among C57BL/KsJ-*db*/*db* groups. (*) $P < 0.05$ (normal control vs control group). ALT, alanine aminotransferase.

Table 5. Activities of Hepatic Triglyceride Metabolism Related Enzymes in C57BL/6J and C57BL/KsJ-*db*/*db* Mice (nmol/min/mg protein)[a]

	C57BL/6J	C57BL/KsJ-*db*/*db*			
	normal control group	control group	EPA-TG group	DPA-TG group	DHA-TG group
		Cytosol Fraction			
FAS	16.3 ± 1.5	18.4 ± 2.7 ab	22.3 ± 2.5 a	17.8 ± 1.6 ab	11.0 ± 1.8 b
ME	148 ± 11	146 ± 24 ab	181 ± 11 a	127 ± 15 ab	99.1 ± 5.4 b
G6PDH	16.6 ± 2.2	5.65 ± 0.98*	5.94 ± 0.48	5.03 ± 0.45	5.04 ± 0.27
		Mitochondrial Fraction			
CPT	9.88 ± 0.42	13.4 ± 0.7*	14.1 ± 0.4	12.8 ± 0.6	14.0 ± 0.8
peroxisomal β-oxidation	11.2 ± 0.4	23.6 ± 1.6*	23.2 ± 1.8	21.9 ± 1.0	24.0 ± 0.9
		Homogenate			
CPT	10.0 ± 0.5	11.6 ± 0.0*	12.0 ± 0.8	11.0 ± 0.5	12.2 ± 0.4
peroxisomal β-oxidation	5.12 ± 0.55	9.45 ± 0.96*	11.8 ± 0.5	8.86 ± 1.44	9.83 ± 0.91
		Microsome Fraction			
PAP	16.9 ± 2.1	10.6 ± 0.6*	10.6 ± 0.5	11.2 ± 0.6	9.09 ± 0.34

[a] Each value represents mean ± SE. Different letters indicate significant difference at $P < 0.05$ among C57BL/KsJ-*db*/*db* groups. (*) $P < 0.05$ (normal control vs control group). FAS, fatty acid synthase; ME, malic enzyme; G6PDH, glucose 6-phosphate dehydrogenase; CPT, carnitine palmitoyl transferase; PAP, phosphatidate phosphohydrolase.

Table 6. Relative Hepatic mRNA Levels in C57BL/6J and C57BL/KsJ-*db*/*db* Mice[a]

	C57BL/6J	C57BL/KsJ-*db*/*db*			
	normal control group	control group	EPA-TG group	DPA-TG group	DHA-TG group
FAS	17.1 ± 4.2	100 ± 33*	58.7 ± 18.4	48.0 ± 10.4	52.4 ± 14.1
ACC2	152.6 ± 4.7	100 ± 13 a*	81.3 ± 12.8 ab	59.5 ± 8.6 ab	42.6 ± 7.2 b
SREBP-1	20.5 ± 1.6	100 ± 20*	86.1 ± 14.9	73.9 ± 9.3	66.7 ± 19.5

[a] Each value represents mean ± SE. Different letters indicate significant difference at $P < 0.05$ among C57BL/KsJ-*db*/*db* groups. (*) $P < 0.05$ (normal control vs control group). FAS, fatty acid synthase; ACC2, acetyl-CoA carboxylase 2; SREBP-1, sterol regulatory element binding protein-1.

experiments because the mouse exhibits hyperglycemia. Adiponectin is also strongly related to glucose metabolism, and increases in adiponectin mitigate the effects of insulin in the liver, which improves fatty liver (*47*). An increase in adiponectin might also improve insulin levels in the DPA-TG and DHA-TG groups. The effect of DPA to increase adiponectin levels was between those of DHA and EPA. Thus, the chain length and unsaturation number in n-3HUFAs might affect hepatic lipid metabolism through increasing adiponectin. According to this perspective, our results are rational because the chain length of DPA is longer than that of EPA and the unsaturation number of DPA is lower than that of DHA.

Except for DHA in the liver, kidney, testis, and brain in the control group, there was very little accumulation of n-3HUFAs in the other organs and tissues (**Figure 1**). The ratio of accumulated hepatic DHA in the DHA-TG group or hepatic DPA in the DPA-TG group against total lipids was significantly higher than that in the other groups. In contrast, the hepatic EPA ratio in the EPA-TG group was not significantly higher than that in the DHA-TG or DPA-TG groups. This finding might be due to an increase in EPA in the DHA-TG or DPA-TG groups by a retroconversion of DHA or DPA to form EPA. The β-oxidation mechanism of polyunsaturated fatty acids is different from that of saturated fatty acids, and DHA is retroconverted to EPA without DPA (*48*). Therefore, the finding that little DPA accumulated in the kidney and liver in the DHA-TG groups is reasonable. In fact, the EPA content among the three groups administered n-3HUFA was almost the same. This result might be explained by defective hepatic lipid metabolism in the EPA-TG group compared to the other groups. In contrast, the ratio of arachidonic acid in liver lipids of the three groups administered n-3HUFA was significantly lower in the EPA-TG group than that of the control group. Several studies have reported that n-3HUFA suppresses the elongation reaction of n-6 series polyunsaturated fatty acids to generate arachidonic acid (*49*). Moreover, the presence of DPA, but not EPA, in the brain was observed in the EPA-TG and DPA-TG groups. It is well-known that both n-3 and n-6 series DPA permeate the blood−brain barrier (*50*, *51*). Therefore, the distribution, conversion, and retroconversion of n-3HUFA observed in C57BL/KsJ-*db*/*db*

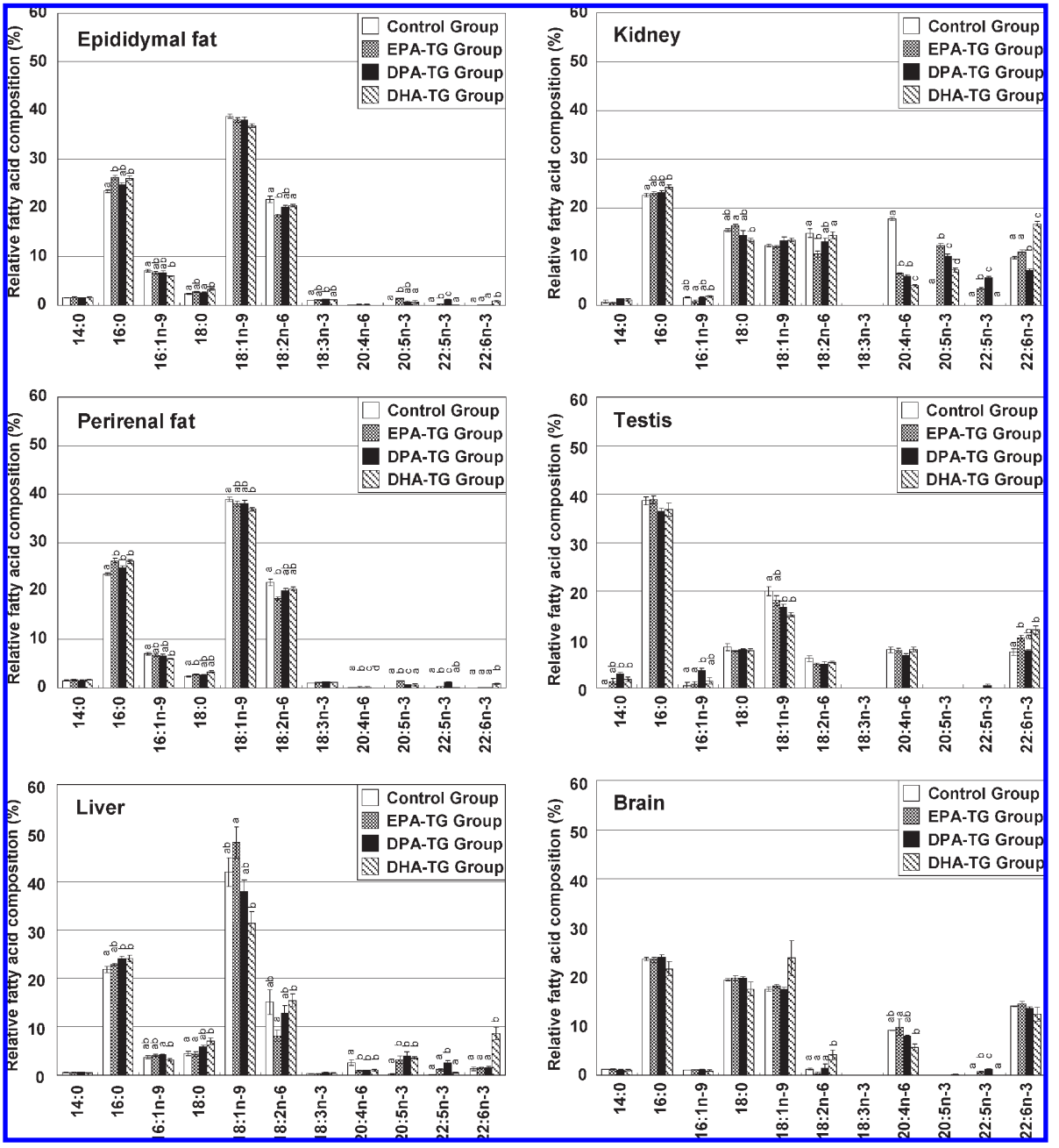

Figure 1. Comparison of the distribution of main fatty acids in the epididymal fat, perirenal fat, liver, kidney, testis, and brain. Values represent mean ± SE. Different letters indicate significant difference at $P < 0.05$. 14:0, myristic acid; 16:0, palmitic acid; 16:1n-9, palmitoleic acid; 18:0, stearic acid; 18:1n-9, oleic acid; 18:2n-6, linoleic acid; 18:3n-3, α-linolenic acid; 20:4n-6, arachidonic acid; 20:5n-3, eicosapentaenoic acid (EPA); 22:5n-3, docosapentaenoic acid (DPA); 22:6n-3, docosahexaenoic acid (DHA).

mice are consistent with the results obtained in other animals and are apparently not affected by impaired leptin receptor function.

The main purpose of the present study was to understand the effects of DPA on lipid metabolism by comparing them with the effects of EPA and DHA. The results indicated that DPA also improves lipid metabolism. The beneficial effects of DPA, however, were not superior to those of the other n-3HUFAs. Moreover, the effects of DPA were always between those of EPA and DHA. In this study, the C57BL/KsJ-*db*/*db* mouse was used because it was thought that the effects of n-3HUFA on lipid metabolism would be amplified so that the results could be easily evaluated. The same comparison using wild-type animals is needed to better characterize the effects of DPA on lipid metabolism.

ABBREVIATIONS USED

ACC2, acetyl-CoA carboxylase 2; ALT, alanine aminotransferase; CPT, carnitine palmitoyl transferase; DHA, docosahexaenoic acid (22:6n-3); DPA, docosapentaenoic acid (22:5n-3); EPA, eicosapentaenoic acid (20:5n-3); FAS, fatty acid synthase; G6PDH, glucose 6-phosphate dehydrogenase; ME, malic enzyme; n-3HUFA, n-3 series highly unsaturated fatty acid; PAP, phosphatidate phosphohydrolase; SREBP-1, sterol regulatory element binding protein-1; tri18:0, tripalmitate; tri18:1n-9, trioleate; tri18:2n-6, trilinoleate; tri18:3n-3, trilinolenate.

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