Effect of Dietary Oil Rich in Docosahexaenoic Acid-Bound Lysophosphatidylcholine Prepared from Fishery By-Products on Lipid and Fatty Acid Composition in Rat Liver and Brain

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Abstract: The possibility of improving brain function coupled with its preferential uptake in the brain has garnered attention for docosahexaenoic acid-bound lysophosphatidylcholine (DHA-LPC). However, studies focusing on the health benefits of dietary DHA-LPC are lacking. We prepared a dietary oil rich in DHA-LPC (DHA-LPC rich oil) via enzymatic modification of phospholipids (PL) extracted from squid (Todarodes pacificus) meal and purification of active carbon, ion exchange resin, and silica gel. We then examined the effects of dietary DHA-LPC rich oil on male Wistar rats by evaluating serum and liver lipid profiles, fatty acid (FA) metabolizing enzyme activity, and the FA composition of serum and brain. The rats were fed a basal diet containing either soybean oil alone (7%) or soybean oil (4.5%) with DHA-LPC rich oil (2.5%) for 28 days, and then evaluated. The rats fed the diet containing DHA-LPC rich oil showed reduced triacylglycerol concentration due, in part, to the enhancement of carnitine palmitoyltransferase 2 and acyl-CoA oxidase activities and suppression of acetyl-CoA carboxylase and glucose-6-phosphate dehydrogenase activities in the liver. Moreover, the dietary DHA-LPC rich oil moderately increased DHA in the FA composition of the rat hippocampus, which may be due to elevated DHA composition in serum LPC. These results suggest that DHA-LPC rich oil has hypolipidemic effect and moderate increase in hippocampal DHA amount in normal rats.

Key words: docosahexaenoic acid, lysophosphatidylcholine, fatty acid, lipid metabolism, fishery by-products

1 Introduction

Docosahexaenoic acid (DHA) - and eicosapentaenoic acid (EPA) -bound triacylglycerol (TAG) and ethyl-ester (EE) forms of purified DHA and EPA are used as supplements and pharmaceutical products. Protection against hyperlipidemia\textsuperscript{1}, cardiovascular diseases\textsuperscript{2}, hypertension\textsuperscript{3}, and Alzheimer’s disease\textsuperscript{4} is provided by n-3 polyunsaturated fatty acids (PUFA), namely DHA and EPA. Fishery by-products are rich in DHA and EPA and are a viable source of n-3 PUFA. Utilization of these bioresources would result in a worldwide reduction in waste. Fishery by-products, such as squid skin, muscle, and connective tissues and the testis and ovaries of fish and shellfish are rich in DHA-bound phospholipids (PL)\textsuperscript{5}. Recently, n-3 PUFA-bound PL have attracted the attention of researchers and consumers for their superior intestinal absorption\textsuperscript{6} and easier incorporation into cell membranes\textsuperscript{7} over n-3 PUFA-bound TAG. These benefits may be due to the amphiphilic properties of n-3 PUFA-bound PL. In addition, n-3 PUFA-bound PL are functionally superior, biologically and nutritionally, to n-3 PUFA-bound TAG in terms of: reducing serum lipid concentration\textsuperscript{8}, increasing the bioavailability of DHA and EPA in plasma\textsuperscript{9}, and reducing inflammation\textsuperscript{10}.

Most of the PL prepared from fishery by-products bind
DHA and EPA at the sn-2 position and saturated fatty acids (SFA) at the sn-1 position\textsuperscript{10}. Therefore, DHA and EPA concentrations can be improved by removing the fatty acids (FA) residue at the sn-1 position. We previously reported on the partial hydrolysis of PL prepared from squid meal using sn-1,3 positional-specific lipase (Lipase A-10D, Nagase ChemteX Co., Osaka, Japan). We found that phospholipase A1 activity against PL increased DHA and EPA and decreased SFA concentrations, compared with the crude squid PL before partial hydrolysis\textsuperscript{10}. The partial hydrolysis of PL, lysophospholipids (LPL), is a commonly used emulsifier in food, pharmaceutical, and cosmetic industries\textsuperscript{11}. In addition to high-performance emulsifying properties, LPL have health-benefits when bound to DHA\textsuperscript{13}. Our previous study reported that a diet containing DHA- and EPA-bound LPL had a higher hypolipidemic effect than a diet containing comparable levels of DHA and EPA-bound TAG\textsuperscript{12}. Dietary docosahexaenoic acid-bound lysophosphatidylcholine (DHA-LPC) showed effective DHA accretion in the brain, and improved brain function in adult mice\textsuperscript{14}. Additionally, it was demonstrated that the transport of DHA-LPC across the blood-brain barrier (BBB) occurs via major facilitator superfamily domain-containing protein 2a (Mfsd2a)\textsuperscript{15}. For this reason, DHA-LPC is expected to improve brain function as well as DHA accretion in the brain. However, the health benefits of DHA-LPC, other than DHA accretion in brain, have not been studied. In this study, we prepared an oil rich in DHA-LPC (DHA-LPC rich oil) from fishery by-products, including squid (Todarodes pacificus) meal, through lipase-mediated partial hydrolysis followed by absorbing agent purification. The purpose of this study was to evaluate the effects of dietary DHA-LPC rich oil on serum and liver lipid concentrations, and serum and brain FA composition in male Wistar rats.

2 Experimental

2.1 Materials

Squid (T. pacificus) meal, including skin, muscle, and connective tissues, was provided by Iwasaki Suisan Co. Ltd. (Hokkaido, Japan). The components of the experimental diet were purchased from Oriental Yeast Co. Ltd. (Tokyo, Japan) and FUJIFILM Wako Pure Chemical Co. (Osaka, Japan). All other chemicals were of reagent grade and were obtained from Sigma-Aldrich Japan Co. Ltd. and NACALAI TESQUE, INC. (Kyoto, Japan).

2.2 Preparation of DHA-LPC rich oil

In order to extract the crude PL from the squid meal, the squid meal was dried and treated with 95% (v/w)/2-propanol for 4 hours at 55°C, then the solvent was evaporated. The crude PL was partially hydrolyzed with lipase from Rhizopus oryzae (Lipase A-10D, Nagase ChemteX Co., Osaka, Japan). The methodology used to prepare 20-30% LPL purity (crude LPL) from squid meal was described in detail previously\textsuperscript{12}. The prepared crude LPL was dissolved in 3-5 times weight of ethanol at 45-50°C. Then, 5% by weight active carbon was added to the crude LPL, and stirred for 30 min under ambient conditions. Filtration using a diatomaceous earth precoated filter press was carried out to remove insoluble materials and color. The decolored crude LPL obtained was loaded on an ion exchange resin (PA306S, Mitsubishi Chemical Co., Tokyo, Japan) column with 97% ethanol solution, then eluted with 85% ethanol. Then, the DHA-LPC rich fraction obtained was placed on a silica gel (D-100-60A, Asahi Glass SI- Tech, Co. Ltd., Fukuoka, Japan) column, and eluted with 85% ethanol. The eluent ethanol was evaporated and dried under vacuum at 40–50°C. The resulting product was DHA-LPC rich oil, which has more than 60% LPC purity. The yield of the DHA-LPC rich oil was 0.21% per wet squid meal weight. The prepared DHA-LPC rich oil was stored with nitrogen gas headspace at −35°C until used.

2.3 Analysis of lipid composition in DHA-LPC rich oil and experimental diets

After methylation of FA with boron trifluoride methanol, the FA composition of the squid crude PL, DHA-LPC rich oil, and experimental diets were determined using a gas chromatography (GC) system (GC-2014, Shimadzu Co., Kyoto, Japan) with Omegawax® capillary GC column (Merck KGaA, Darmstadt, Germany) as described previously\textsuperscript{16}. The PL contents of the squid crude PL and DHA-LPC rich oil were determined using phosphorus analyses as described previously\textsuperscript{17}. The PL class compositions of the squid crude PL and DHA-LPC rich oil were analyzed by two-dimensional thin-layer chromatography (TLC) using two solvent systems: chloroform/methanol/ammonia (first solvent, 65:35:8, v/v/v) and chloroform/acetic acid/water (second solvent, 5:2:1:1:0.5, v/v/v/v/v). Spots were visualized with 8% phosphoric acid containing 3% cupric acetate and the PL class composition was determined using JustTLC software (version 4.0.3, Sweden, Lund, Sweden). Cholesterol content of squid crude PL and DHA-LPC rich oil were analyzed using GC system (GC-2014, Shimadzu Co.) with DB-5 column (Agilent Technologies, California, USA), and 5α-cholestanol as an internal standard, as described previously\textsuperscript{18}.

2.4 Experimental diets and animals care

The experimental protocol was reviewed and approved by the Animal Ethics Committee of Kansai University and followed the “Guide for the Care and Use of Experimental Animals” issued by the Prime Minister’s Office of Japan. Four-week-old male Wistar rats obtained from Japan SLC,
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Inc. (Shizuoka, Japan) were kept in an air-conditioned room (temperature: 21-23°C; humidity: 50-70%; illuminated, 08:00-20:00), with free access to tap water and feed. Fourteen rats were divided into two dietary groups of seven rats each: the soybean oil diet (SO group) and DHA-LPC rich oil diet (DHA-LPC group) groups. The experimental diets were prepared according to the AIN-93G formula\(^{10}\) and contained 7% soybean oil (SO diet) or 4.5% soybean oil with 2.5% DHA-LPC rich oil, 20% casein, 3.5% AIN-93G mineral mixture, 1% AIN-93 vitamin mixture, 0.25% choline bitartrate, 0.3% L-cystine, 5% cellulose, 10% sucrose, 0.014% tert-butylhydroquinone, and 13.2% dextrinized corn starch. Corn starch was added to equal 100%.

Food consumption and body weights were recorded daily. After 28 days, rats were weighed and euthanized under isoflurane (Intervet K.K.; Osaka, Japan) anesthesia between 09:00 and 11:00. Blood was collected from the inferior vena cava without the use of anti-coagulants, and serum was obtained by centrifugation at 2,000 \(\times g\) for 15 min. The liver, cerebral cortex, hippocampus, brainstem, and abdominal white adipose tissue (WAT) from the epididymis, mesentry, perinephria, and retroperitoneum were removed rapidly, weighed, rinsed with saline, and then frozen in liquid nitrogen, followed by storage at \(-80\)°C until later analysis.

2.5 Analysis of lipid parameters in serum, liver, and feces

The serum lipids, including, TAG, PL, total-cholesterol, high-density lipoprotein (HDL)-cholesterol, non-HDL-cholesterol, and non-esterified fatty acid (NEFA) were measured using an Olympus AU5431 automatic analyzer (Olympus Co., Tokyo, Japan) by a commercial service (Japan Medical Laboratory, Osaka, Japan). The liver, cerebral cortex, hippocampus, brainstem, and abdominal white adipose tissue (WAT) from the epididymis, mesentry, perinephria, and retroperitoneum were removed rapidly, weighed, rinsed with saline, and then frozen in liquid nitrogen, followed by storage at \(-80\)°C until later analysis.

2.6 Analysis of hepatic enzyme activities

The liver was homogenized with ten volumes of 3 mM Tris-HCl buffer (pH 7.4) containing 0.25 M sucrose and 1 mM EDTA-2Na. The post-nuclear supernatant, mitochondrial fraction, and soluble fraction of liver homogenate were separated using a centrifugal separator, as described previously\(^{22}\). The activities of fatty acid synthase (FAS)\(^{21}\), acetyl-CoA carboxylase (ACC)\(^{22}\), glucose-6-phosphate dehydrogenase (G6PDH)\(^{23}\), and malic enzyme (ME)\(^{24}\) in the soluble fraction; carnitine palmitoyltransferase 2 (CPT-2)\(^{25}\) in the mitochondrial fraction; and acyl-Coenzyme A oxidase (ACOX)\(^{26}\) in the post-nuclear supernatant were measured spectrophotometrically. Protein contents in the three liver homogenate fractions were measured using the Pierce\(^{TM}\) Modified Lowry Protein Assay Kit (Thermo Fisher Scientific K.K., Kanagawa, Japan) and the Pierce\(^{TM}\) Bovine Serum Albumin Standard (Thermo Fisher Scientific K.K.).

2.7 Statistical analysis

Data showed the means and standard errors of the mean (SEM). Significance was evaluated using the Student’s \(t\)-test. Significance was set at \(p<0.05\) or \(p<0.01\). Analyses were performed using GraphPad Prism7 software (GraphPad Software, California, USA).

3 Results and Discussion

3.1 Lipid indices of the squid crude PL and DHA-LPC rich oil

Table 1 represents the FA composition, PL content, PL class composition, and cholesterol content of squid crude PL and DHA-LPC rich oil. DHA was increased to 79.3 mol% in the DHA-LPC rich oil and the concentrations of palmitic, palmitoleic, and stearic acid were reduced. Cholesterol content was also reduced in the DHA-LPC rich oil compared with the squid crude PL. We previously reported that the partial hydrolysate of PL prepared \(\text{via}\) enzymatic modification of squid crude PL, which was a partial hydrolysate of PL, referred to as crude LPL in this paper; contained 62.6 mol% DHA\(^{12}\). Most of the DHA in marine sources is located in \(sn-2\) position\(^{12}\). In this study, partial hydrolysis using lipase from \(R.\ oryzae\) was performed to cleave off the FA moiety bound at the \(sn-1\) position of the

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In the context of the document, it appears there are various sections discussing the methods and results of experiments involving the effects of docosahexaenoic acid-bound lysophosphatidylcholine on metabolism. The text describes the preparation of experimental diets, the collection of blood and tissue samples, and the analysis of lipid and enzyme activities. The results are discussed in the context of metabolic changes, with a focus on the effects of the DHA-LPC on various metabolic parameters such as enzyme activities and lipid compositions.
In recent years, the sn-1 and sn-2 positional isomers of LPC and the molecular species of PL have been analyzed by using \(^{31}P\) NMR\(^{27}\). Further studies are necessary to confirm the ratio of sn-1 and sn-2 position isomers of LPC in the DHA-LPC rich oil. High DHA concentration in LPL was obtained through this process and was then purified through active carbon, ion exchange resin, and silica gel treatments. Compared with the squid crude PL and partial hydrolysate of PL (described in detail in our previous study\(^{12}\)), the DHA-LPC rich oil (prepared in this study) contained higher DHA concentration and lower SFA concentration. The squid crude PL mainly consisted of phosphatidylcholine (PC) and phosphoethanolamine (PE). In contrast, DHA-LPC rich oil had more than 60% purity for LPC, and did not contain PE and lysophosphatidylethanolamine (LPE). In our previous report, crude LPL was composed of 23.8% LPE\(^{22}\). The reason DHA-LPC rich oil did not contain PE and LPE is that silica gel purification removed the lower polarity PL. We prepared an oil containing DHA-LPC via partial hydrolytic reaction using \(R.\) oryzae lipase followed by purification using resin that contained 79.3 mol% DHA and 66.5 wt% LPC.

### Table 1
Lipid indices of the squid crude phospholipids (PL) and docosahexaenoic acid-bound lysophosphatidylcholine (DHA-LPC) rich oil.

| FA composition (mol%) | Squid crude PL | DHA-LPC rich oil |
|-----------------------|----------------|------------------|
| C16:0                 | 31.8           | 1.7              |
| C16:1 n-7             | 4.1            | 0.3              |
| C18:0                 | 3.0            | 0.7              |
| C18:1 n-9             | 1.5            | 0.3              |
| C20:4 n-6 (ARA)       | 1.6            | 1.8              |
| C20:5 n-3 (EPA)       | 12.3           | 13.5             |
| C22:6 n-3 (DHA)       | 38.3           | 79.3             |
| Others                | 7.4            | 2.4              |

| PL class composition (wt%) |
|-----------------------------|
| LPC                         | 66.5           |
| PC                          | 41.5           |
| PE                          | 32.3           |
| SM                          | 7.7            |
| Others                      | 18.5           |
| Cholesterol concentration (mg/g) | 16.24          |

ARA, arachidonic acid; DHA-LPC, docosahexaenoic acid-bound lysophosphatidylcholine; EPA, eicosapentaenoic acid; FA, fatty acids; LPC, lysophosphatidylcholine; PC, phosphatidylcholine; PE, phosphoethanolamine; PL, phospholipids; SM, sphingomyelin.

### Table 2
Fatty acid composition of the experimental diets.

| Experimental diets | SO | DHA-LPC |
|--------------------|----|---------|
| C16:0              | 11.5 | 7.0    |
| C16:1 n-7          | –   | 0.1    |
| C18:0              | 3.7  | 2.3    |
| C18:1 n-9          | 21.5 | 12.5   |
| C18:2 n-6          | 53.3 | 30.8   |
| C18:3 n-3          | 5.8  | 3.4    |
| C20:4 n-6 (ARA)    | –   | 0.4    |
| C20:5 n-3 (EPA)    | –   | 2.9    |
| C22:6 n-3 (DHA)    | –   | 17.0   |
| Others             | 4.3  | 2.9    |

ARA, arachidonic acid; DHA, docosahexaenoic acid; DHA-LPC, docosahexaenoic acid-bound lysophosphatidylcholine; EPA, eicosapentaenoic acid; LPC, lysophosphatidylcholine; SO, soybean oil.
Table 3  Growth parameters, organ weights, and lipid contents in serum, liver, and feces.

| Groups      | SO                  | DHA-LPC             |
|-------------|---------------------|---------------------|
| Growth parameters |                   |                     |
| Initial body weight (g)   | 122.9 ± 1.8         | 124.0 ± 1.6         |
| Final body weight (g)    | 326.3 ± 5.2         | 333.0 ± 5.7         |
| Body weight gain (g/day) | 7.27 ± 0.17         | 7.47 ± 0.20         |
| Food intake (g/day)      | 16.1 ± 0.5          | 16.7 ± 0.6          |
| Food efficiency (g/g)   | 0.451 ± 0.010       | 0.448 ± 0.012       |
| Organ weights (g/100 g BW) |                   |                     |
| Liver           | 4.07 ± 0.15         | 4.14 ± 0.08         |
| Epididymal WAT  | 1.26 ± 0.12         | 1.06 ± 0.12         |
| Mesentery WAT   | 1.38 ± 0.14         | 1.29 ± 0.12         |
| Perirenal and retroperitoneal WAT | 1.64 ± 0.06 | 1.56 ± 0.07         |
| Total WAT\(^1\)    | 4.28 ± 0.26         | 3.92 ± 0.28         |
| Serum lipid concentrations |                   |                     |
| TAG (mg/dL)      | 97.0 ± 21.1         | 59.7 ± 8.2          |
| PL (mg/dL)       | 143 ± 6             | 121 ± 5*            |
| Total-cholesterol (mg/dL) | 75.1 ± 4.0         | 56.8 ± 3.7**        |
| HDL-cholesterol (mg/dL) | 50.0 ± 3.0         | 47.9 ± 3.4          |
| Non-HDL-cholesterol (mg/dL) | 25.1 ± 1.7         | 11.5 ± 1.3**        |
| NEFA (mEq/L)     | 547 ± 26            | 314 ± 43**          |
| Liver lipid concentrations (mg/g Liver) |                   |                     |
| TAG              | 56.7 ± 2.4          | 35.6 ± 2.6**        |
| Cholesterol      | 5.00 ± 0.18         | 2.15 ± 0.19**       |
| PL               | 22.7 ± 0.8          | 24.7 ± 0.7          |
| Fecal lipid concentrations (µmol/day) |                   |                     |
| Cholesterol      | 12.5 ± 0.9          | 15.0 ± 1.8          |
| Total bile acids | 11.5 ± 1.2          | 12.1 ± 0.7          |

Data show mean ± SEM (\(n = 7\)). Values were significantly different from the control group at * \(p < 0.05\) and ** \(p < 0.01\) using the Student’s t-test.

\(^1\) Total WAT represents the sum of WAT weights from the epididymis, mesentery, perinephria, and retroperitoneum.

Table 3 shows growth parameters, organ weights, serum, liver, and fecal lipid concentrations. Previous studies reported that LPL had an emulsifying property and promoted fat digestion, thus promoting growth, performance, and nutrient utilization in chickens\(^28,29\). Our previous report indicated that the intake of the partial hydrolysate of PL did

3.2 Fatty acid composition of experimental diets

Table 2 represents the FA composition of the two experimental diets (SO and DHA-LPC diet). The DHA-LPC diet contained 4.5% (w/w) SO and 2.5% (w/w) DHA-LPC rich oil, the DHA concentration in the DHA-LPC diet was 17.0 mol%.

3.3 Growth parameters and lipid indices of serum and liver

Table 3 shows growth parameters, organ weights, serum, liver, and fecal lipid concentrations. Previous studies reported that LPL had an emulsifying property and promoted fat digestion, thus promoting growth, performance, and nutrient utilization in chickens\(^28,29\). Our previous report indicated that the intake of the partial hydrolysate of PL did
not change growth parameters, including weight gain, food intake, or food efficiency in rats. We believe weight gain and food efficiency remained unchanged because the rats had free access to food. There were no significant differences in liver, epididymal, mesenteric, perirenal, and retroperitoneal WAT weights between the SO and DHA-LPC diet groups.

Compared with rats fed the SO diet, rats fed the DHA-LPC diet showed a significant reduction in serum PL, total-cholesterol, non-HDL-cholesterol, and NEFA concentrations. The hypotriglyceridemic effect on serum concentrations of DHA and EPA has been recognized in humans as well as in rodents. In this study, the group consuming the DHA-LPC rich oil diet tended to have lower serum TAG concentrations than the group consuming the SO diet; however, the difference was not significant (p = 0.15). Moreover, rats consuming the DHA-LPC rich oil had significantly lower liver TAG and cholesterol concentrations than those in the SO group. Figure 1 illustrates the main FA contents of the liver. Since the DHA-LPC diet reduced liver TAG concentration, contents of main FA, including C16:0, C16:1n-7, C18:0, C18:1n-9, C18:1n-7, C18:2n-6, C18:3n-3, and C20:4n-6, in the DHA-LPC group were significantly lower than those in the SO group. On the contrary, the liver concentrations of EPA (C20:5n-3) and DHA (C22:6n-3) in the DHA-LPC rich oil group were significantly increased compared with those in the SO group. High contents of monounsaturated fatty acids (MUFA) of serum and organs are known to be associated with obesity, cardiovascular disease, and diabetes. Intake of DHA-LPC rich oil reduced the TAG and cholesterol concentrations in the serum and liver and MUFA concentration in the liver in normal rats.

3.4 Fecal steroid contents and hepatic enzyme activity related to FA metabolism

Changes in hepatic cholesterol homeostasis and interruptions to cholesterol uptake in intestinal cells are factors in reducing cholesterol concentration through increased n-3 PUFA and PL intake. Intake of PL is known to inhibit cholesterol absorption. However, the LPL formed by partial hydrolysis of PL does not inhibit cholesterol absorption in the intestine. In this study, the DHA-LPC rich oil group showed no change in fecal cholesterol or total bile acid excretions compared with the SO group (Table 3). These data are supported by our previous observations, in which the dietary partial hydrolysat of PL did not affect fecal cholesterol excretion. Although, hepatic mRNA expression levels of genes encoding proteins related to cholesterol homeostasis were not measured in the present experiment; our previous study reported that intake of PL containing n-3 PUFA and a partial hydrolysate of PL decreased cholesterol synthesis through suppression of hepatic 3-Hydroxy-3-Methylglutaryl-Coenzyme A Reductase (HMGCR). It has been shown that DHA-LPC rich oil intake may cause a reduction in serum and liver cholesterol concentrations, at least in part through the reduced expression of HMGCR. The TAG concentrations in the serum and liver are known to be partly affected by the balance of liver FA β-oxidation and biosynthesis. Figure 2 illustrates the activity of rate-limiting enzymes in hepatic FA metabolism. The DHA-LPC diet suppressed the activities of ACC (a key enzyme in de novo synthesis of FA) and G6PDH (a key enzymes in NADPH production) in the liver compared with the SO diet. Moreover, the activities of CPT-2 and ACOX, which are FA β-oxidation enzymes in mitochondria and peroxisomes, were enhanced in the DHA-LPC rich oil group compared with SO group. There were no significant differences in hepatic FAS and ME ac-

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**Fig. 1** Liver fatty acid content in Wistar rats fed experimental diets. Data show mean ± SEM (n = 7). Values were found to be significantly different from those fed the control diet at * p < 0.05 and ** p < 0.01 using the Student’s t-test. White box = SO group; Black box = DHA-LPC group. C16:0, palmitic acid; C16:1n-7, palmitoleic acid; C18:0, stearic acid; C18:1n-9, oleic acid; C18:1n-7, vaccenic acid; C18:2n-6, linoleic acid; C18:3n-3, alpha-linolenic acid; C20:4n-6, arachidonic acid; C20:5n-3, eicosapentaenoic acid; C22:6n-3, docosahexaenoic acid; FA, fatty acids; LPC, lysophosphatidylycholine; SO, soybean oil.

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tivity between those two groups. Therefore, dietary DHA-LPC rich oil reduced TAG concentrations in serum and in liver at least in part due to the suppression in FA synthesis and the enhancement in FA oxidation. Previous studies reported that DHA suppressed FA synthesis and enhanced FA oxidation through sterol regulatory element-binding protein-1c (SREBP-1c) and peroxisome-proliferator-activated receptor-α (PPAR-α) involvement, which are a transcriptional factor and a nuclear receptor36,37. In addition, dietary PC reduced the TAG concentrations in serum and liver mainly through the suppression of FA synthesis and enhancement of FA oxidation in orotic acid-induced fatty liver in rats38. Our previous study reported that the hypotriglyceridemic effect of PL containing DHA and EPA does not require esterification of n-3 PUFA to glycerophosphate structures of PL, but has additive effects of n-3 PUFA and glycerophosphate structures39. Therefore, the reduction of TAG in serum and liver by the intake of DHA-LPC rich oil is considered to have the additive effects of DHA and glycerophosphate structures.

3.5 Fatty acid composition of brain and serum

Previous studies reported that daily intake of DHA improved the reference memory related learning ability in young and adult rats, at least in part due to DHA accretion and the suppression of lipid peroxidation in the cerebral cortex and hippocampus39,40. Other studies reported that dietary DHA maintained normal levels of acetylcholine and choline and elevate DHA concentration in the hippocampus and cerebral cortex of stroke-prone spontaneously hypertensive rats41. Moreover, dietary DHA-LPC efficiently elevated DHA content in the brain, and improved brain function in adult mice42. Figure 3 shows the FA composition in the cerebral cortex, hippocampus, and brainstem. The intake of DHA-LPC rich oil increased the DHA composition in the hippocampus compared with the intake of SO. In the cerebral cortex and brainstem, the two diets (SO and DHA-LPC) did not affect the DHA composition. The brain relies on a constant supply of circulating DHA in serum to maintain homeostasis including membrane fluidity and lipid mediator production42. Previous studies reported that DHA-LPC was preferentially recovered in the brain of young rats41. Wong et al. identified the Mfsd2a transporter as the primary transporter for the uptake of DHA-LPC across the BBB into the brain45. DHA in the serum is found to be bound to PL, LPL, cholesteryl esters, and TAG within lipoproteins; or as an LPL, non-bound FA, or FA-albumin complexes46. To examine factors that increase DHA in the hippocampus, the FA compositions of total lipid, PC, and LPC in serum, shown in Fig. 4, were analyzed. The DHA compositions in these fractions (total lipid, PC, and LPC) were elevated in the DHA-LPC rich oil group compared with SO group. In addition, DHA-LPC diet reduced the arachidonic acid composition and elevated the EPA composi-

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![Figure 2](image_url)

Fig. 2  Liver enzyme activities of fatty acid metabolism-related proteins in Wistar rats fed experimental diets. Data show mean ± SEM (n = 7). Values were significantly different from the control diet at * p < 0.05 and ** p < 0.01 using the Student’s t-test. White box = SO group; Black box = DHA-LPC group. ACC, acetyl-CoA carboxylase; ACOX, acyl-CoA oxidase; CPT, carnitine palmitoyl transferase; FAS, fatty acid synthase; G6PDH, glucose-6-phosphate dehydrogenase; LPC, lysophosphatidylcholine; ME, malic enzyme; SO, soybean oil.

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The elevation of DHA composition in the hippocampus may be due to the increase in DHA composition in serum LPC. However, the serum PL content was decreased in the DHA-LPC group, and the amount of serum DHA-LPC in the DHA-LPC group may not have changed compared with that in the SO group. The amount of serum DHA-LPC should be measured to determine the cause of the increased hippocampal DHA composition.

However, the results of this study have the following limitations: DHA-LPC rich oil was not compared with common DHA containing oils, such as DHA-bound TAG or EE form of DHA, only with SO. Murota et al. reported that dietary DHA-bound PL significantly elevated DHA content in lymph nodes.
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J. Oleo Sci. 68, (8) 781-792 (2019)

phatic PL, however total DHA absorption was not significantly different when compared with dietary DHA-bound TAG and non-bound DHAα. Thus, the increase in DHA compositions in serum LPC and the hippocampus upon the intake of DHA-LPC rich oil cannot be stated as a causality. Future studies must compare DHA accretions in the hippocampus and serum upon intake of DHA-LPC rich oil with other chemical forms of DHA, including DHA-bound TAG, EE form of DHA, and PL. This study was conducted in normal rats rather than in rats with high-fat diet or genetic disease model. Therefore, it is required to demonstrate the physiological function of DHA-LPC rich oil against lifestyle-related diseases using obese animals.

Fig. 4 Fatty acid composition of total lipids, phosphatidylcholine, and lysophosphatidylcholine in serum. Data show mean ± SEM (n = 7). Values were significantly different from the control diet at * p<0.05 and ** p<0.01 using the Student’s t-test. White box = SO group; Black box = DHA-LPC group. C16:0, palmitic acid; C16:1n-7, palmitoleic acid; C18:0, stearic acid; C18:1n-9, oleic acid; C18:1n-7, vaccenic acid; C18:2n-6, linoleic acid; C18:3n-3, alpha-linolenic acid; C20:4n-6, arachidonic acid; C20:5n-3, eicosapentaenoic acid; C22:6n-3, docosahexaenoic acid; FA, fatty acids; LPC, lysophosphatidylcholine; PC, phosphatidylcholine; SO, soybean oil.
4 Conclusion

We prepared DHA-LPC rich oil via a partial hydrolytic reaction using R. oryzae lipase and increased its purity to 79.3 mol% DHA and 66.5% (w/w) LPC through active carbon, ion exchange resin, and silica gel purification. The intake of DHA-LPC rich oil reduced the TAG concentrations in the serum and liver at least partially due to the enhancement of CPT-2 and ACOX activities and suppression of ACC and G6PDH activities. Moreover, the increased hippocampal DHA composition in the DHA-LPC diet group may be due to the elevation of DHA composition in serum LPC. These results suggest that DHA-LPC rich oil has hypolipidemic effect and moderate increase in hippocampal DHA composition in normal rats.

Acknowledgments

This work was partially supported by “A Scheme to Revitalize Agriculture and Fisheries in Disaster Area through Deploying Highly Advanced Technology” from the Ministry of Agriculture, Forestry and Fisheries of Japan.

Conflict of interests

The authors declare that there are no conflicts of interest.

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