JOURNAL OF

AGRICULTURAL AND FOOD CHEMISTRY

Effect of Dietary Omega 3 Phosphatidylcholine on Obesity-Related Disorders in Obese Otsuka Long-Evans Tokushima Fatty Rats

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Recent reports have shown that dietary phosphatidylcholine (PC) has various beneficial biological effects. Omega 3 polyunsaturated fatty acids (ω3 PUFAs) have also been reported to have lipid-lowering effects in animal models and human studies. In the present study, we investigated the effect of ω3 PUFAs containing PC (ω3-PC) on obesity-related disorders in Otsuka Long-Evans Tokushima fatty (OLETF) rats. Rats were fed semisynthetic diets that contained either 5% corn oil and 2% egg-PC or 5% corn oil and 2% ω3-PC for 4 weeks. During this 4 week feeding of the ω3-PC, the OLEFT rats showed a decrease of omental white adipose tissue weight. In addition, the ω3-PC diet significantly decreased liver weight and hepatic lipid levels in OLETF rats. These changes were attributable to the significant suppression of fatty acid synthase activity and significant enhancement in the activities of carnitine palmitoyltransferase and peroxisomal β-oxidation. Moreover, the ω3-PC diet reduced serum glucose levels concomitant with the increase of serum adiponectin levels. These results show that compared with egg-PC, ω3-PC can prevent or alleviate obesity-related disorders through the suppression of fatty acid synthesis, enhancement of fatty acid β-oxidation, and increase of the serum adiponectin level in OLETF rats.

KEYWORDS: ω3 polyunsaturated fatty acids; docosahexaenoic acid; eicosapentaenoic acid; phosphatidylcholine; obesity-related disorders; adiponectin; Otsuka Long−Evans Tokushima fatty rats

INTRODUCTION

Lifestyle-related diseases, such as hyperlipidemia, arteriosclerosis, diabetes mellitus, and hypertension, are widespread and increasingly prevalent diseases in industrialized countries and contribute to the increase in cardiovascular morbidity and mortality (*1*, *2*). Accompanied by the rapid increase in the number of elderly people, this becomes important not only medically but also socioeconomically. Although the pathogenesis of lifestyle-related diseases is complicated and the precise mechanisms have not been elucidated, obesity has emerged as one of the major cardiovascular risk factors according to epidemiologic studies (*3*−*5*).

Because diet, especially dietary fat, has been recognized as contributing to the development and prevention of obesity, the influence of quantities and qualities of dietary fats on the pathogenesis of obesity-related disorders has been studied (*6*−*8*). Although it is obvious that the majority of dietary fat is triglyceride, there is about a 3−8% daily intake of phospholipids in total dietary fats (*9*, *10*). Growing evidence indicates that dietary phospholipids have beneficial effects compared with

dietary triglyceride. For example, phosphatidylcholine (PC), which is a major component of dietary phospholipids, has been reported to improve brain function in animals (*11*, *12*) and alleviate orotic acid-induced fatty liver in rats (*13*). Furthermore, differential effects have arisen with respect to individual fatty acids. Omega 3 polyunsaturated fatty acids (ω3 PUFAs), such as eicosapentaenoic acid (EPA; 20:5) and docosahexaenoic acid (DHA; 22:6), are abundant in fish, shellfish, and sea mammals. Evidence from animal models and human studies have suggested that ω3 PUFAs have lipid-lowering effects (*14*, *15*).

In the present study, we investigated the effect of dietary ω3-PC, extracted from fish roe and containing ω3 PUFAs in its fatty acid composition, on obesity-related disorders in Otsuka Long-Evans Tokushima fatty (OLETF) rats. OLETF rats develop a syndrome with multiple metabolic and hormonal disorders that shares many features with human obesity (*16*−*19*). OLETF rats have hyperphagia, because they lack receptors for cholecystokinin and become obese, developing hyperlipidemia, fatty liver, and diabetes.

MATERIALS AND METHODS

Animals and Diets. Male OLETF rats aged 4 weeks were provided by Tokushima Research Institute (Otsuka Phamaceutical, Tokushima, Japan). The rats were housed individually in metal cages in an air-conditioned room (24 °C) under a 12 h light/dark cycle. After a 1 week

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Table 1. Fatty Acid Composition of Egg-PC and ω3-PC

	fatty acid composition, wt (%)	
	egg-PC[a]	ω3-PC[b]
14:0	1.4	1.4
16:0	29.9	15.0
16:1	1.4	1.2
18:0	17.5	13.9
18:1	27.9	10.5
18:2	15.4	0.7
20:5ω3	c	12.0
22:5ω3	c	5.2
22:6ω3	c	28.1
others	7.8	12.0
total	100	100

[a] Contained 80% PC, 17% PE, 3% others. [b] Contained 74% PC, 13% PE, 13% others. [c] Not detected.

adaptation period, the rats were assigned to two groups (six rats each). The basal diets were prepared according to recommendations of the AIN-76. Dietary fats were composed of a mixture of 5% corn oil and 2% egg-PC in the control diet and a mixture of 5% corn oil and 2% ω3-PC in the ω3-PC diet. Egg-PC and ω3-PC were extracted from chicken egg and salmon roe, respectively. The fatty acid compositions of egg-PC and ω3-PC are given in **Table 1**. Rats were fed the diets for 4 weeks. At the end of the feeding period, the rats were sacrificed by aortic exsanguination under diethyl ether anesthesia after a 9 h starvation period. White adipose tissue (WAT) and livers were excised immediately, and serum was separated from the blood. All aspects of the experiment were conducted according to the guidelines provided by the Ethical Committee of Experimental Animal Care at Saga University.

Analysis of Hepatic Lipids and Serum Parameters. Liver lipids were extracted according to the method of Folch et al. (*20*), and the concentrations of triglyceride and cholesterol were measured by the methods of Fletcher (*21*) and Sperry and Webb (*22*), respectively. Triglyceride and cholesterol in serum were measured using enzyme assay kits from Wako Pure Chemicals (Tokyo, Japan). Adiponectin, plasminogen activator inhibitor type 1 (PAI-1), and leptin levels in serum were measured using commercial rat enzyme-linked immunosorbent assay kits (Otsuka Pharmaceutical Co. Ltd., Tokyo, Japan; American Diagnostica Inc., Stamford, CT; Yanaihara Institute Inc., Fujinomiya, Japan, respectively).

Preparation of Hepatic Subcellular Fractions. A piece of liver was homogenized in six volumes of a 0.25 M sucrose solution that contained 1 mM EDTA in a 10 mM Tris-HCl buffer (pH 7.4). After the nuclei fraction was precipitated, the supernatant was centrifuged at 10000*g* for 10 min at 4 °C to obtain the mitochondria fraction. The resulting supernatant was recentrifuged at 125000*g* for 60 min to precipitate microsomes, and the remaining supernatant was used as the cytosol fraction. The protein concentration was determined according to the method of Lowry et al. (*23*), with bovine serum albumin used as the standard.

Assays of Hepatic Enzyme Activity. The activity of fatty acid synthase (FAS) in the hepatic cytosomal fraction was measured according to the method of Kelley et al. (*24*). The reaction mixture consisted of 0.5 M potassium phosphate buffer (pH 7.0) that contained 200 μM reduced nicotinamide adenine dinucleotide phosphate (NADPH), 66 μM acetyl-coenzyme A (CoA), and 200 μM malonyl-CoA. This mixture was placed in a cuvette and mixed with the enzyme. The reaction was observed spectrophotometrically at 340 nm for 5 min. A control reaction without substrate was also done. The plots of absorbance versus time were converted to nanomoles of NADPH reduction per minute, using a millimolar extinction coefficient of 6.22.

The activity of carnitine palmitoyltransferase (CPT) in the hepatic mitochondrial fraction was measured according to the method of Markwell et al. (*25*). The reaction mixture contained 58 mM Tris-HCl buffer (pH 8.0), 1.25 mM EDTA, 0.1% Triton X-100, 0.25 mM 5,5′-dithiobis-(2-nitrobenzoic) acid, and 37.5 μM palmitoyl-CoA. This mixture was placed in a cuvette and mixed with the enzyme. The

reaction was started by the addition of 1.25 mM L-carnitine and was observed spectrophotometrically at 412 nm for 5 min. A control reaction without substrate was also done. The plots of absorbance versus time were converted to nanomoles of CoA-SH release per minute, using a millimolar extinction coefficient of 13600.

The activity of peroxisomal β-oxidation was measured according to the method of Lazarow (*26*). The reaction mixture contained 940 μL of 50 mM Tris-HCl buffer (pH 8.0), 10 μL of 20 mM nicotinamide adenine dinucleotide (NAD), 3 μL of 0.33 M dithiothreitol, 5 μL of 1.5% BSA, 5 μL of 2% Triton X-100, 10 μL of 10 mM CoA, 10 μL of 1 mM flavin adenine dinucleotide and 10 μL of 100 mM KCN. This mixture was placed in a cuvette and mixed with 20 μL of mitochondrial fraction. The reaction was started by the addition of 10 μL of 1 mM palmitoyl-CoA and was observed spectrophotometrically at 340 nm for 5 min. A control reaction without substrate was also done. The plots of absorbance versus time were converted to nanomoles of NAD reduced per minute, using a millimolar extinction coefficient of 6.22.

Analysis of Fatty Acid Composition. Total lipids of serum and omental WAT were extracted according to the methods of Bligh and Dyer (*27*) and Folch et al. (*20*), respectively. Extracted lipids were methylated according to the method of Park et al. (*28*). Methyl esters of fatty acids were analyzed by gas−liquid chromatography.

Analysis of mRNA Expression. Total RNA was extracted from 100 mg of liver, using a TRI ZOL Reagent (Invitrogen). A TaqMan Universal PCR Master Mix (Applied Biosystems, Tokyo, Japan); Assay-on-Demand, Gene Expression Products (Rn00573474_m1 for acetyl-CoA carboxylase (ACC), Rn00595644_m1 for acetyl-CoA oxidase 2 (ACO2), Rn00580702_m1 for CPT1a, Rn00563995_m1 for CPT2, Rn00569117_m1 for FAS, Rn00566193_m1 for peroxisome proliferators activated receptor-alpha (PPAR-α), Rn00565707_m1 for PPAR-δ, Rn00440945_m1 for PPAR-γ, Hs99999901_s1 for 18S RNA, Applied Biosystems, Tokyo, Japan), and TaqMan MGB Gene Expression Kits for stearoyl-CoA desaturase 1 (SCD1) and sterol regulatory element binding protein-1c (SREBP-1c) were used for the quantitative real-time RT-PCR analysis of ACC, ACO2, CPT1a, CPT2, FAS, PPAR-α, PPAR-δ, PPAR-γ, 18S RNA, SCD1, and SREBP-1c expression in the liver. The details of the TaqMan MGB Gene Expression Kits were as follows: SCD1 (forward primer, 5′-AGCCTGTTCGTCAGCAC-CTT-3′; reverse primer, 5′-CACCCAGGGAAACCAGGAT-3′; and TaqMan MGB probe, 5′-FAM-CACTCTGGTGCTCAAC-MGB-3′); SREBP-1c (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 were expressed as a relative value after normalization to the 18S RNA expression.

Statistical Analysis. All values are expressed as means ± standard error. The significance of differences between means for two groups was determined by Student's *t* test. Differences were considered to be significant at $p < 0.05$.

RESULTS

Effect of Dietary ω3-PC on Growth Parameters. Table 2 summarizes the growth parameters of OLETF rats after a 4 week feeding period. Although there was no significant difference in final body weight or food intake between the groups, the ω3-PC diet significantly decreased liver weight and omental WAT weight of rats.
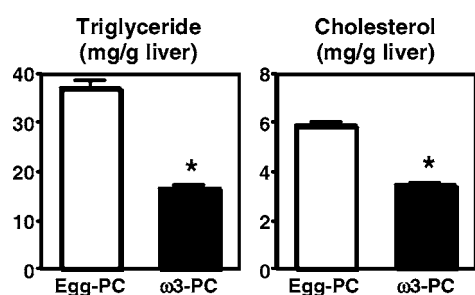
Effect of Dietary ω3-PC on Hepatic Lipid Levels. Figure 1 shows hepatic triglyceride and cholesterol levels of OLETF rats after 4 week feeding of the diets. Compared with the egg-PC diet, the ω3-PC diet markedly decreased hepatic triglyceride and cholesterol levels by 55% and 42%, respectively.

Effect of Dietary ω3-PC on Activities of Hepatic Enzymes Related to Lipid Metabolism. Figure 2 shows enzyme activities in the livers of OLETF rats after 4 weeks feeding of the diets. The activity of FAS, a key enzyme of fatty acid

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Table 2. Growth Parameters of OLETF Rats Fed the Egg-PC and ω3-PC Diets[a]

	egg-PC	ω3-PC
initial body weight (g)	112 ± 2	112 ± 3
final body weight (g)	329 ± 3	322 ± 5
body weight gain (g)	217 ± 3	209 ± 5
food intake (g)	611 ± 5	607 ± 4
food efficiency (g gain/g intake)	0.355 ± 0.006	0.345 ± 0.006
liver weight (g/100 g body weight)	4.20 ± 0.11	3.35 ± 0.06[b]
White Adipose Tissue Weight (g/100 g Body Weight)		
total	9.82 ± 0.27	9.25 ± 0.28
abdominal	6.17 ± 0.12	5.73 ± 0.24
epididymal	1.79 ± 0.06	1.62 ± 0.07
perirenal	2.82 ± 0.08	2.70 ± 0.13
omental	1.56 ± 0.03	1.40 ± 0.05[b]
subcutaneous	3.65 ± 0.16	3.52 ± 0.09

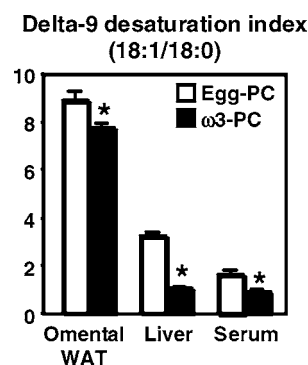[a] Values are expressed as mean ± standard error of six rats. [b] Significant difference at $p < 0.05$.

Figure 1. Effect of dietary ω3-PC on levels of hepatic lipids. Rats were fed semisynthetic diets that contained either 5% corn oil and 2% egg-PC or 5% corn oil and 2% ω3-PC for 4 weeks. Values are expressed as mean ± standard error of six rats. See Materials and Methods for composition of diets. Asterisk shows significant difference at $p < 0.05$.
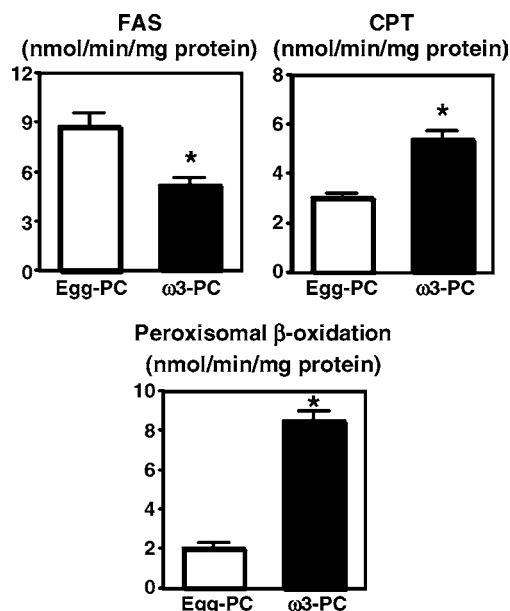
Figure 2. Effect of dietary ω3-PC on activities of hepatic enzymes related to triglyceride metabolism. Rats were fed semisynthetic diets that contained either 5% corn oil and 2% egg-PC or 5% corn oil and 2% ω3-PC for 4 weeks. Values are expressed as mean ± standard error of six rats. See Materials and Methods for composition of diets. Asterisk shows significant difference at $p < 0.05$.

synthesis, was markedly suppressed (41%) by the ω3-PC diet, as compared with the egg-PC diet. In contrast, mitochondrial β-oxidation, whose rate-limiting enzyme is CPT, and peroxi-

Table 3. Hepatic Fatty Acid Composition in OLETF Rats Fed the Egg-PC and ω3-PC Diets[a]

	fatty acid composition, wt %	
	egg-PC	ω3-PC
14:0	0.606 ± 0.048	0.395 ± 0.027[b]
16:0	21.6 ± 0.5	22.7 ± 0.5
16:1	5.04 ± 0.39	2.23 ± 0.12[b]
18:0	9.55 ± 0.34	17.0 ± 0.3[b]
18:1	30.6 ± 0.7	17.5 ± 0.4[b]
18:2ω6	15.4 ± 0.6	15.8 ± 0.3
18:3ω6	0.191 ± 0.014	0.116 ± 0.003
18:3ω3	0.0885 ± 0.0066	0.0712 ± 0.0050
20:3ω6	0.396 ± 0.024	0.996 ± 0.050[b]
20:4ω6	13.5 ± 0.7	13.1 ± 0.2
20:5ω3	0.0674 ± 0.0054	1.48 ± 0.06
22:4ω6	0.478 ± 0.025	c
22:5ω6	0.432 ± 0.035	c
22:5ω3	0.142 ± 0.014	1.04 ± 0.05
22:6ω3	1.94 ± 0.14	7.68 ± 0.31[b]

[a] Values are expressed as mean ± standard error of six rats. [b] Significant difference at $p < 0.05$. [c] Not detected.

Figure 3. Effect of dietary ω3-PC on delta-9 desaturation indices in omental WAT, liver, and serum. Rats were fed semisynthetic diets that contained either 5% corn oil and 2% egg-PC or 5% corn oil and 2% ω3-PC for 4 weeks. Values are expressed as mean ± standard error of six rats. See Materials and Methods for composition of diets. Asterisk shows significant difference at $p < 0.05$.

somal β-oxidation were significantly enhanced (1.8-fold and 4.2-fold, respectively) by the ω3-PC diet, as compared with the egg-PC diet.

Effect of Dietary ω3-PC on Fatty Acid Composition in the Liver. Table 3 summarizes fatty acid composition in the liver of OLETF rats after 4 weeks feeding of the diets. Compared with the egg-PC diet, the ω3-PC diet significantly increased the levels of stearic acid (18:0) and dihomo-γ-linolenic acid (20:3ω6) and decreased the levels of palmitoleic acid (16:1) and oleic acid (18:1). EPA and DHA levels were markedly increased (22-fold and 4-fold, respectively) by the ω3-PC diet, as compared with the egg-PC diet.
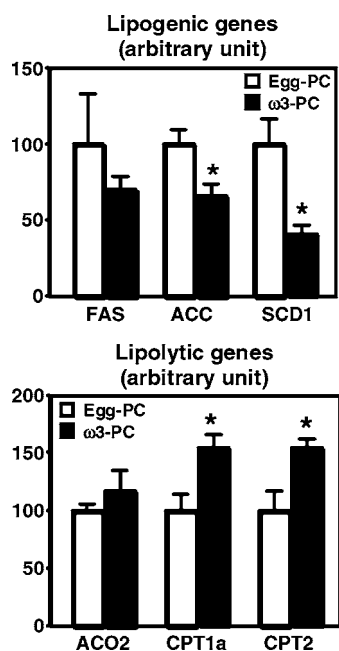
Effect of Dietary ω3-PC on Delta-9 Desaturation Indices in Omental WAT, Liver, and Serum. Figure 3 shows delta-9 desaturation indices in omental WAT, liver, and serum of OLETF rats after 4 weeks feeding of the diets. Delta-9 desaturation indices in omental WAT, liver, and serum were significantly decreased (13%, 68%, and 45%, respectively) by the ω3-PC diet, as compared with the egg-PC diet.

Effect of Dietary ω3-PC on Serum Parameters. Table 4 summarizes serum parameters of OLETF rats after 4 weeks feeding of the diets. Although the difference was not significant, the ω3-PC diet tended to decrease serum triglyceride level compared with the egg-PC diet. Serum cholesterol level was

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Table 4. Serum Parameters of OLETF Rats Fed the Egg-PC and ω3-PC Diets[a]

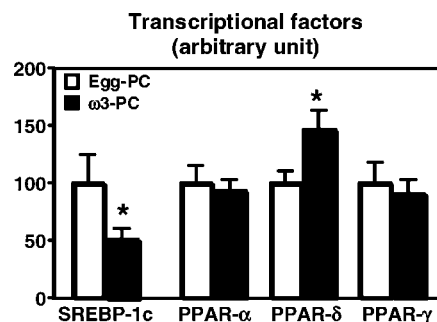|	egg-PC	ω3-PC
triglyceride (mg/dL)	135 ± 30	77.0 ± 16.5
cholesterol (mg/dL)	139 ± 4	106 ± 2[b]
glucose (mg/dL)	197 ± 6	166 ± 2[b]
adiponectin (μg/mL)	5.34 ± 0.23	6.54 ± 0.26[b]
leptin (ng/mL)	2.83 ± 0.55	2.85 ± 0.28
PAI-1 (ng/mL)	6.61 ± 3.19	4.01 ± 0.36

[a] Values are expressed as mean ± standard error of six rats. [b] Significant difference at $p < 0.05$.

Figure 5. Effect of dietary ω3-PC on mRNA levels of transcriptional factors in liver. Rats were fed semisynthetic diets that contained either 5% corn oil and 2% egg-PC or 5% corn oil and 2% ω3-PC for 4 weeks. Values are expressed as mean ± standard error of six rats. See Materials and Methods for composition of diets. Asterisk shows significant difference at $p < 0.05$.

and PPAR-γ, have been reported to relate to lipid and glucose metabolism. In the present study, the PPAR-δ mRNA level was markedly increased by the ω3-PC diet. On the other hand, mRNA levels of PPAR-α and PPAR-γ were not affected by the ω3-PC diet.

DISCUSSION

Recent reports have shown that dietary PC has various physiological effects (*11−13*). In the present study, we prepared PC from fish roe because of simultaneous supplementation of ω3 PUFAs, whose lipid lowering effects have been reported (*14, 15*), and investigated the effect of its feeding on obesity-related disorders in OLETF rats. After 4 weeks of feeding, the ω3-PC diet significantly decreased liver weight and hepatic triglyceride levels of OLETF rats. As a consequence, obesity-induced fatty liver was alleviated by the ω3-PC diet, as compared with the egg-PC diet. To further investigate the regulation of hepatic lipid metabolism, we analyzed the effect of dietary ω3-PC on the activities of enzymes related to fatty acid synthesis and fatty acid β-oxidation. As shown in **Figure 2**, the activity of FAS was markedly suppressed by the ω3-PC diet, whereas the activities of CPT and peroxisomal β-oxidation were markedly enhanced by the ω3-PC diet. Therefore, we suggested that the reduction of hepatic triglyceride levels by the ω3-PC diet was attributable to the suppression of fatty acid synthesis and the enhancement of fatty acid β-oxidation. Moreover, relevant data on expression of genes related to lipid metabolism in the liver are presented in **Figure 4**. The ω3-PC diet tended to decrease the mRNA level of FAS and markedly decreased the mRNA level of ACC. In contrast, CPT1a and CPT2 mRNA levels were markedly increased, and the ACO2 mRNA level tended to increase with the ω3-PC diet. These results indicated that the alteration of enzyme activities by ω3-PC feeding was regulated at the transcriptional level. It is known that gene expression of FAS is regulated by SREBP-1, a lipogenic transcriptional factor. The mature nuclear form of SREBP is produced from the membrane-bound precursor by proteolytic cleavage (*29*). Thus, the nuclear content of mature SREBP is under the regulation of gene expression and pro-teolytic cleavage of the precursor. In the present study, SREBP-1c mRNA level was markedly decreased by the ω3-PC diet, as compared with the egg-PC diet. Previous studies have shown that ω3 PUFAs have suppressive effects on SREBP-1 mRNA expression (*30*), which suggests that these fatty acids can decrease fatty acid synthesis through the transcriptional suppression of SREBP-1 signaling. Additionally, we previously

Figure 4. Effect of dietary ω3-PC on mRNA expression of genes related to lipid metabolism in liver. Rats were fed semisynthetic diets that contained either 5% corn oil and 2% egg-PC or 5% corn oil and 2% ω3-PC for 4 weeks. Values are expressed as mean ± standard error of six rats. See Materials and Methods for composition of diets. Asterisk shows significant difference at $p < 0.05$.

significantly decreased (24%) by the ω3-PC diet, as compared with the egg-PC diet. Compared with the egg-PC diet, the ω3-PC diet tended to decrease the serum PAI-1 level, whereas the serum adipocentin level was significantly increased by the ω3-PC diet. On the other hand, the serum leptin level was not affected by the ω3-PC diet.

Effect of Dietary ω3-PC on mRNA Expressions of Genes Related to Lipid Metabolism in the Liver. Figure 4 shows hepatic mRNA expression of genes related to lipid metabolism in OLETF rats after 4 weeks feeding of the diets. The mRNA expression of FAS tended to decrease in OLETF rats fed the ω3-PC diet. Expression of lipogenic genes, such as ACC and SCD1 was markedly decreased by the ω3-PC diet. In contrast, the mRNA expression of ACO2, the enzyme involved in peroxisomal β-oxidation, tended to increase in OLETF rats fed the ω3-PC diet. Expression of lipolytic genes, such as CPT1a and CPT2 was markedly increased by the ω3-PC diet.

Effect of Dietary ω3-PC on mRNA Expressions of Transcriptional Factors in the Liver. Figure 5 shows hepatic mRNA expression of transcriptional factors in OLETF rats after 4 weeks feeding of the diets. The mRNA expression of SREBP-1c, a lipogenic transcriptional factor, was markedly decreased by the ω3-PC diet. Transcriptional factors, PPAR-α, PPAR-δ,

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reported that dietary PC suppresses fatty acid synthesis through the decreased expression of FAS mRNA (*13*). Therefore, we speculate that ω3 PUFAs enhanced the suppressive effect of PC on fatty acid synthesis in the liver. On the other hand, the gene expression of the lipolytic enzymes are regulated by ligand-inducing transcription factors called PPAR-α (*31*). ω3 PUFAs have been reported to act as ligands for PPAR and to enhance the gene expression of the lipolytic enzymes (*32*). In addition, we previously reported that dietary PC enhances fatty acid β-oxidation (*13*). Therefore, we suggest that ω3 PUFAs and PC synergistically enhanced fatty acid β-oxidation. In the present study, there was no significant difference in mRNA level of PPAR-α among groups. Thus, we suggest that ω3-PC might act on the activity of PPAR-α as ligands. Recently, it has been demonstrated that PPAR-δ involves glucose and lipid metabolism (*33*). The ω3-PC diet markedly increased the mRNA level of PPAR-δ in this study. Therefore, we suggest that the increased PPAR-δ mRNA level by the ω3-PC diet might be associated with the normalization of glucose and lipid metabolism in OLETF rats.

The present study showed that the ω3-PC diet also decreased hepatic cholesterol levels. Previous studies have shown that fish oil suppresses the activity of 3-hydroxy-3-methyl-glutaryl-CoA reductase, a rate-limiting enzyme of cholesterol synthesis (*34*). Additionally, it has been reported that dietary PC decreased hepatic cholesterol levels through the enhancement of the secretion of bile cholesterol in hypercholesteolemic rabbits (*35*). Further studies are necessary to clarify the effect of dietary ω3-PC on the synthesis and excretion of cholesterol in the liver of OLETF rats.

Consistent with previous studies showing that ω3 PUFAs decrease blood lipid levels via the reduction of the synthesis and secretion of lipoprotein in the liver (*36*), the ω3-PC diet lowered serum lipid levels in these obese rats. Since blood lipids provide energy into the adipose tissue, we consider that the reduction of serum lipid levels contributes to a decreased omental WAT weight in ω3-PC fed OLETF rats.

To gain insight into the effect of dietary ω3-PC on lipid metabolism, we analyzed the fatty acid composition in the tissue of OLETF rats. Accompanied by the feeding of ω3-PC, fatty acid composition in the tissue was changed, and ω3 PUFAs were incorporated into tissues. Consistent with previous reports showing that ω3 PUFAs inhibit the delta-5 desaturation *in vitro* and *in vivo* (*37*−*39*), dihomo-γ-linolenic acid (20:3ω6) level was significantly increased in the liver of rats fed the ω3-PC diet. In addition, the ω3-PC diet markedly lowered monounsaturated fatty acid (MUFA) levels in tissues. Changes of fatty acid composition by the ω3-PC diet were shown not only in liver but also in omental WAT and serum. It has been recognized that MUFAs are the major fatty acid form in fat depots (*40*). The increased MUFA levels *in vivo* have been implicated in various disease states including obesity, diabetes, and cardiovascular disease (*41*−*43*). A key enzyme involved in the cellular synthesis of MUFA from saturated fatty acid is the membrane-bound SCD, which inserts a cis-double bond in the delta-9 position of fatty acid substrates. Previous studies of SCD1 knockout mice indicated that SCD1 deficiency suppresses the development of obesity and diabetes through the enhanced fatty acid oxidation in liver and the increased glucose uptake in muscle (*44*, *45*). SCD1 mRNA level was markedly decreased by the ω3-PC diet. As a consequence, the ω3-PC diet significantly decreased delta-9 desaturation indices, the ratio of oleic acid (18:1) versus stearic acid (18:0), in omental WAT, liver, and serum compared with the egg-PC diet. We suggest

that the reduction of omental WAT weight and the alleviation of fatty liver by the ω3-PC diet were, at least in part, attributable to the suppression of delta-9 desaturation.

Recently, it has been recognized that adipose tissue not only functions as a storage depot for triglycerides but also plays important roles in regulating glucose and lipid homeostasis by secreting a variety of bioactive molecules into the circulatory system (*46*, *47*). Those molecules are collectively called adipocytokines. Among those, adiponectin is the most abundant adipose-specific protein. The expression of adiponectin is reduced in obesity, and blood levels are negatively correlated with visceral fat accumulation (*47*, *48*). Several reports indicate that adiponectin enhances glucose uptake, glucose utilization, and fatty acid oxidation and suppresses gluconeogenesis and fatty acid synthesis by activating AMP-activated protein kinase and PPAR-α in liver and muscle (*49*−*52*). In the present study, the ω3-PC diet significantly decreased omental WAT weight, whereas it increased the serum adiponectin level in OLETF rats. Therefore, we suggest that the alleviation of glucose and lipid metabolism by the ω3-PC diet was attributable to the increased serum adiponectin levels. None of the studies have shown that dietary PC increases blood adiponectin levels. On the other hand, it has been reported that fish oil containing ω3 PUFAs increases blood adiponectin levels (*53*). According to the analysis of fatty acid composition in tissues, DHA, but not EPA, was detected in omental WAT in OLETF rats (data not shown). Therefore, it is strongly suggested that DHA independently contributes to the increased secretion of adiponectin from adipose tissue. As for other adipocytokines, it has been known that leptin regulates energy balance by suppressing appetite (*54*, *55*), and PAI-1 increases the risk of vascular diseases by inducing inflammation (*56*). The ω3-PC diet did not change serum leptin levels but tended to decrease serum PAI-1 levels. Therefore, it is suggested that the ω3-PC diet may alleviate obesity-induced inflammation.

In conclusion, our results show that, compared with egg-PC, ω3-PC can prevent or alleviate obesity-related disorders through the suppression of fatty acid synthesis, enhancement of fatty acid β-oxidation, and increase of the serum adiponectin level in OLETF rats. Possible findings on the effect of the form, such as PC vs triglyceride, used for ω3 PUFAs administration would be of great interest for future study.

ABBREVIATIONS USED

ACC, acetyl-CoA carboxylase; ACO, acetyl-CoA oxidase; CoA, coenzyme A; CPT, carnitine palmitoyltransferase; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; FAS, fatty acid synthase; MUFA, monounsaturated fatty acid; NAD, nicotinamide adenine dinucleotide; NADPH, nicotinamide adenine dinucleotide phosphate; ω3, omega 3; OLETF, Otsuka Long-Evans Tokushima fatty; PAI-1, plasminogen activator inhibitor type 1; PC, phosphatidylcholine, PPAR, peroxisome proliferator activated receptor; PUFA, polyunsaturated fatty acid; SCD, stearoyl-CoA desaturase; SREBP, sterol regulatory element binding protein; WAT, white adipose tissue.

LITERATURE CITED

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