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Dietary Phospholipids Ameliorate Fructose-Induced Hepatic Lipid and Metabolic Abnormalities in Rats[1,2]

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Abstract

Overconsumption of fructose results in hepatic dyslipidemia, which has a documented correlation with metabolic syndrome. We examined whether the ingestion of phospholipids (PL) from soybeans prevents fructose-induced metabolic abnormalities. Rats were fed either a fructose-free diet (C), a 60% fructose diet (F), or a 60% fructose plus 3% PL diet (F-PL) for 10 wk. At wk 8, plasma glucose concentrations after glucose loading were significantly higher in rats fed the F diet than in rats fed the C and F-PL diets, which did not differ from one another. The concentrations of hepatic TG, diglycerides, ceramides, and oleates in rats fed the F diet for 10 wk was significantly higher than those in rats fed the C diet. The increases were prevented by concurrent PL ingestion; concentrations did not differ between the F-PL and C groups. Dietary fructose increased the mRNA expression of *SREBP1, ChREBP*, and genes related to lipogenesis. PL completely inhibited these increases. Furthermore, reflecting the difference at the mRNA level, lipogenic enzyme activities were greater in rats fed the F diet than in rats fed the C diet, and PL ingestion suppressed the increased activities by fructose feeding. Treatment of cultured Hep-G2 cells with fructose for 24 h increased the levels of SREBP1 and ChREBP nuclear proteins, which were suppressed by culture with purified PL components, especially phosphatidylethanolamine and phosphatidylinositol. These findings indicate that PL prevents fructose-induced metabolic abnormalities in association with alterations of the hepatic lipid profile by inhibiting de novo lipogenesis. J. Nutr. 141: 2003–2009, 2011.

Introduction

The prevalence of metabolic syndrome and the risk of developing diabetes mellitus are increasing worldwide (1). Insulin resistance is a central pathophysiological feature of diabetes mellitus. Various physiological conditions, such as the excessive accumulation of lipids in adipose or nonadipose tissues, are related to the development of insulin resistance (2,3). Hepatic steatosis is strongly associated with insulin resistance (4–6). Short-term high-fat feeding results in increased hepatic lipids without lipid accumulation in other tissues and insulin resistance (7,8). A variety of lipids, such as DG and ceramides, and the fatty acid composition in the liver are also related to insulin resistance (3,9,10).

Environmental factors such as diet cause metabolic abnormalities, including insulin resistance and hepatic steatosis. In recent decades, an increase in the total energy intake and a shift in the types of nutrients ingested have been observed (11). The consumption of fructose have increased, mainly due to the increased consumption of soft drinks (11,12). High fructose intake induces metabolic abnormalities such as insulin resistance, hypertriglyceridemia, and hepatic steatosis in humans and rodents (13–15). Fructose ingestion increases mRNA expression of the genes coding for lipogenic enzymes via the activation of sterol regulatory element-binding protein 1 (SREBP1)[3] and carbohydrate response element binding protein (ChREBP) in the liver (16,17). These metabolic derangements in the liver may play a central role as causes of metabolic abnormalities induced by fructose feeding, and the results of recent studies with gene knockout animals suggest that lipogenic enzymes are a therapeutic target for metabolic abnormalities (18,19).

PL are components of the lipid bilayers of the cell membrane. PL from soybeans have hypolipidemic effects in humans and rodents (20,21). PL ingestion also suppresses mild hepatic TG accumulation through the inhibition of lipogenesis in the fasted-refed model (22,23). These findings suggest that the ingestion of PL is effective against fructose-induced hepatic steatosis and

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[2] Supplemental Figure 1 and Table 1 are available from the "Online Supporting Material" link in the online posting of the article and from the same link in the online table of contents at jn.nutrition.org.

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[3] Abbreviations used: ACC, acetyl-CoA carboxylase; C-4 to C-12 straight chain; C, fructose-free diet; *ChREBP*, carbohydrate response element binding protein; DG, diglyceride; *ELOVL*, the elongation of long-chain fatty acids; FAS and F, 60% fructose diet; F-PL, 60% fructose plus 3% PL diet; OGTT, oral glucose tolerance test; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PL, phospholipid; *SREBP*, sterol regulatory element-binding protein; TC, total cholesterol; WAT, white adipose tissue.

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subsequent insulin resistance, but the effects of PL are not well understood.

In this report, we examined whether dietary PL prevents fructose-induced metabolic abnormalities, such as hypertriglycemia, hepatic steatosis, and insulin resistance, and investigated the underlying mechanisms.

Materials and Methods

Materials. Fructose was obtained from Wako Pure Chemicals Industries. Corn oil and soybean PL (SLP-white) were purchased from Oriental Yeast and Tsuji Oil Mill, respectively. The composition of PL was 34.4% PC, 26.6% PE, 18.7% PI, 4.4% phosphatidic acid, and 15.9% others. Purified PL components (PC, PE, and PI) from soybean were purchased from Sigma-Aldrich.

Animals and diet. Male Wistar rats were obtained from Clea Japan at 6 wk of age and maintained at 23 ± 2°C under a 12-h-light/-dark cycle (lights on from 0700 to 1900 h). The rats were fed a laboratory diet (CE-2; Clea Japan) for 1 wk to stabilize their metabolic conditions. All rats were randomly divided into 3 groups (6 rats/group) and consumed ad libitum water and one of the following diets: *1*) fructose-free diet (C); *2*) 60% fructose diet (F); or *3*) 60% fructose plus 3% PL diet (F-PL) (**Table 1**). The rats were maintained at 3 rats/cage on the experimental diets for 10 wk. Body weight and food intake were measured every 2 or 3 d. Food intake [g/(rat·d)] was determined by subtracting the remaining food weight from the initial food weight per cage and dividing by the number of rats housed in the cage. The energy intake [kJ/(rat·d)] was calculated from the food intake and the macronutrient composition of each diet. This study was approved by the Animal Care Committee of the Kao Tochigi Institute.

Blood analysis. At wk 5 of the experiment, blood samples were drawn from the tail vein from 4-h feed-deprived rats. Plasma TG and glucose were determined using enzyme assay kits L-type Wako TG-H and L-type Wako Glu2 (Wako). Plasma insulin was measured using the insulin EIA kit (Morinaga).

At wk 8 of the experiment, an OGTT was performed. The rats were feed-deprived for 12 h prior to the oral glucose load (2 g/kg body weight). Blood samples were drawn from the tail vein at 0, 15, 30, 60, and 120 min after glucose loading. Plasma glucose and insulin levels were measured as described above.

On the final day of the experiment, rats were anesthetized by diethyl ether, and blood was collected into tubes containing heparin from the rats under ad libitum feeding conditions via the abdominal aorta. Plasma TC, nonesterified fatty acid, and PL were determined using enzyme assay kits L-type Wako CHO-H, L-type Wako NEFA-C, and L-type Wako PL-H (Wako). Plasma leptin was measured using a leptin EIA kit (Morinaga).

TABLE 1 Composition of the experimental diets[1]

Ingredient	C	F	F-PL
		g/kg	
Corn oil	50	50	50
Phospholipids	—	—	30
Fructose	—	600	600
Sucrose	50	—	—
Casein	200	200	200
Cellulose powder	75	75	75
Mineral mixture[2]	35	35	35
Vitamin mixture[2]	10	10	10
Potato starch	580	30	—
Energy, *kJ/100g*	1610	1610	1560

[1] C, fructose-free diet; F, 60% fructose diet; F-PL, 60% fructose plus 3% PL diet.
[2] AIN-76 (42).

Liver lipid and glycogen analysis. After blood collection, rats were bled from the inferior vena cava under diethyl ether anesthesia between 0900 and 1200 h and a portion of the liver was frozen in liquid nitrogen and maintained at –80°C until assayed. Total lipid extraction and determination of hepatic TG, TC, and PL were performed as previously described (24). DG and ceramides were measured according to the method of Turinsky et al. (25). [^{32}P]Phosphatidic acid (corresponding to DG content) and ceramide-1-phosphate (corresponding to ceramide content) were identified by autoradiography then analyzed with a BAS2500 Bioimage Analyzer (Fuji Photo Film). The fatty acid composition was measured by GC (26). The methylated fatty acids were extracted and analyzed using a GC-18A gas chromatograph (Shimadzu) and HR-SS-10 capillary column (Shinwa Chemical Industries). The liver glycogen content was measured using an enzymatic technique (27).

Quantitative real-time PCR analysis. Isolation of total RNA, production of cDNA, and real-time PCR were performed as described previously (24). The primers listed in **Supplemental Table 1** were used for quantitative real-time PCR analysis. For quantitative precision, the same amount of total RNA was consistently used for each expression analysis and the expression amount of each gene was normalized by the expression of the housekeeping gene, ribosomal protein, large, P0 (RPLP0/36B4).

Measurement of enzyme activity. The liver was homogenized with 0.1 mol/L potassium phosphate buffer (pH 7.4). Subcellular fractionation was performed according to the methods described by Shimomura et al. (28). The cytosolic fraction was used for the measurement of fatty acid synthase (FAS) and acetyl-CoA carboxylase (ACC) activity. The microsomal pellet was used for the measurement of stearoyl-CoA desaturase (SCD) and the elongation activity of long-chain fatty acids (ELOVL). Protein concentrations were determined using a Micro BCA protein assay kit (Pierce).

FAS and ACC activities were measured spectrophotometrically with a UV-1650PC (Shimadzu) by observing the consumption of NADPH at 340 nm (29,30), with minor modification. In the measurement of ACC activity, the cytosolic fraction passed through a Sephadex G-50 column (Bio-Rad Laboratories).

SCD activity was assayed by measuring the oleates produced from [1-^{14}C]stearate according to the method described by Shimomura et al. (28) and Miyazaki et al. (31). ELOVL activity was assayed by measuring [2-^{14}C]malonyl-CoA incorporation into exogenous acyl-CoA according to the method described by Matsuzaka et al. (32).

Cell culture and treatment. Hep-G2 human hepatocarcinoma cells (American Type Culture Collection) were maintained in culture with low glucose (5.5 mmol/L) DMEM (Sigma-Aldrich) containing 10% (v:v) FBS (Gibco) and 1% (v:v) antibiotics (Gibco) on collagen-coated plate at 37°C in 5% CO_2. Cells were seeded in 6-well plates at a density of 1.0×10^6 for 24 h. The cells were then rinsed with PBS and cultured in serum-free DMEM containing 25 mmol/L fructose, 250 μmol/L BSA, and various purified PL components (PC, PE, PI, 10 mmol/L final concentration). After 24 h of culture, the cells were collected and subjected to quantitative real-time PCR or immunoblotting.

Immunoblotting. Nuclear extracts were prepared from Hep-G2 cells using NE-PER Nuclear and Cytoplasmic Extraction Reagents (Pierce) following the manufacturer's instructions. The nuclear extracts (40 μg) were subjected to 7.5% SDS-PAGE. Proteins were then transferred to a nitrocellulose membrane (Hybond-ECL; GE Healthcare UK). Western-blot analysis performed using antibodies against SREBP1 or ChREBP (Santa Cruz Biotechnology). Detection was achieved using the enhanced chemiluminescence kit (GE Healthcare).

Statistical analysis. All values are presented as means ± SD. All data were first tested for homogeneity of variance with Bartlett's test. When the variance was equal, the data were analyzed using a 1-factor ANOVA and then differences between individual group means were analyzed using the Tukey-Kramer test. If the variance was unequal, the Steel-Dwass test was used. The Tukey-Kramer and Steel-Dwass tests were

performed using R version 2.5.1 (33). OGTT data were tested for time and diet effects by repeated-measures ANOVA with the Tukey-Kramer post hoc test. $P < 0.05$ was considered significant.

Results

Physiological variables. Feed-deprived rats that consumed the F diet for 5 wk had significantly higher plasma TG (+141%) compared with rats fed the C diet (**Table 2**). The plasma TG concentration of rats fed the F-PL diet was 57% greater than that of rats fed the C diet ($P = 0.09$) and was significantly lower than that of rats fed the F diet. Plasma glucose and insulin concentrations did not differ among groups, but plasma glucose tended to be higher in rats fed the F diet compared with those fed the C diet ($P = 0.06$).

All groups had similar body weights over the experimental period (**Supplemental Fig. 1**). Consistent with body weight, the epididymal, perirenal, and retroperitoneal WAT weights did not differ among the groups. Rats fed the F diet had significantly greater liver weights than rats fed either the C diet or F-PL diet.

Rats fed the F diet for 10 wk had significantly higher plasma TG (+188%), glucose (+23%), and PL (+51%) concentrations compared with rats fed the C diet (Table 2). The plasma TG and glucose concentrations of rats fed the F-PL diet tended to be higher compared with those of rats fed the C diet ($P = 0.09$ and 0.06, respectively). Plasma insulin concentrations tended to be higher in rats fed the F diet compared with rats fed the C diet ($P = 0.10$), but this increase was absent in rats fed the F-PL diet. Plasma leptin concentrations did not differ significantly among the groups.

TABLE 2 Body weight and plasma components of rats fed a C, F, or F-PL diet for 5 and 10 wk[1]

	C	F	F-PL
Wk 5[2]			
Body weight, *g*	372 ± 16	377 ± 17	372 ± 21
Plasma components			
TG, *mmol/L*	2.60 ± 0.41[a]	6.27 ± 1.14[b]	4.08 ± 0.89[a]
Glucose, *mmol/L*	6.34 ± 0.67	8.11 ± 1.45	6.98 ± 0.44
Insulin, *nmol/L*	0.29 ± 0.04	0.28 ± 0.07	0.30 ± 0.05
Wk 10[3]			
Body weight, *g*	410 ± 17	420 ± 19	417 ± 24
Body weight gain, *g/10 wk*	151 ± 18	160 ± 17	157 ± 27
Energy intake,[4] *kJ/(rat · d)*	304 ± 30	313 ± 43	302 ± 23
Epididymal WAT, *g*	6.99 ± 0.85	6.94 ± 1.12	6.57 ± 1.38
Perirenal WAT, *g*	3.21 ± 0.16	3.00 ± 0.55	2.89 ± 0.44
Retroperitoneal WAT, *g*	10.3 ± 0.7	9.8 ± 1.8	8.9 ± 1.8
Liver, *g*	14.5 ± 0.9[a]	20.1 ± 2.0[b]	18.3 ± 1.5[b]
Plasma components			
TG, *mmol/L*	1.78 ± 0.25[a]	5.13 ± 1.26[b]	4.24 ± 1.80[ab]
TC, *mmol/L*	2.47 ± 0.50	3.18 ± 0.56	2.38 ± 0.91
Glucose, *mmol/L*	7.88 ± 0.72[a]	9.66 ± 0.61[b]	9.10 ± 1.16[ab]
NEFA, *mmol/L*	0.46 ± 0.13	0.71 ± 0.17	0.56 ± 0.24
Phospholipids, *mmol/L*	11.7 ± 3.4[a]	17.6 ± 4.5[b]	11.7 ± 2.6[a]
Insulin, *nmol/L*	0.51 ± 0.08	0.70 ± 0.16	0.51 ± 0.17
Leptin, *mg/L*	2.41 ± 0.66	2.44 ± 0.83	2.03 ± 0.80

[1] Values are means ± SD, $n = 6$. Means in a row with superscripts without a common letter differ, $P < 0.05$. C, fructose-free diet; F, 60% fructose diet; F-PL, 60% fructose plus 3% PL diet; NEFA, nonesterified fatty acid.
[2] Data were obtained from rats that had been feed-deprived for 4 h.
[3] Data were obtained from fed rats.
[4] Energy intake data are the means calculated from 30 measurements that were performed per cage every 2 or 3 d during the 10 wk of experiment.

OGTT. After oral glucose loading, the plasma glucose and insulin concentrations of rats fed the F diet were significantly higher than those of rats fed the C diet (**Fig. 1***A*,*C*). Also, the AUC of glucose and insulin were 34 and 142% greater in rats fed the F diet than in rats fed the C diet ($P < 0.05$) (Fig. 1*B*,*D*). Glucose concentrations in rats fed the F-PL diet were significantly lower than those in rats fed the F diet between 15 and 120 min and were comparable to those in rats fed the C diet. At 60 min, insulin concentrations were significantly lower in rats fed the F-PL diet than in rats fed the F diet. $AUC_{glucose}$ and AUC

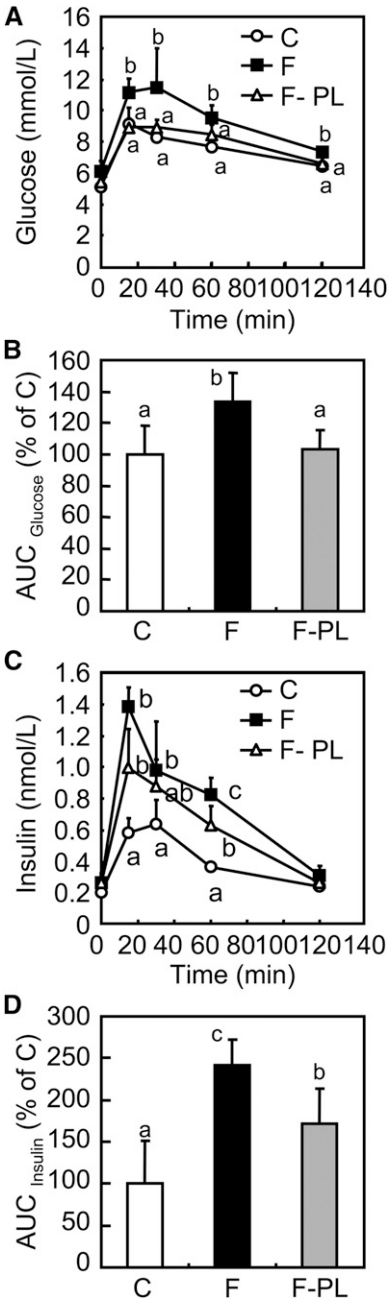

FIGURE 1 Plasma glucose (*A*) and insulin (*C*) concentrations and relative AUC of glucose (*B*) and insulin (*D*) during an OGTT of rats fed the C, F, or F-PL diets for 8 wk. Values are means ± SD, $n = 6$. Means (*B*,*D*) or means at a time (*A*,*C*) without common letter differ, $P < 0.05$. C, fructose-free diet; F, 60% fructose diet; F-PL, 60% fructose plus 3% PL diet; OGTT, oral glucose tolerance test.

$_{insulin}$ in rats fed the F-PL diet were 23 and 29% lower than those in rats fed the F diet ($P < 0.05$).

Hepatic lipid accumulation. Hepatic TG, ceramide, and TC concentrations were significantly higher by 45, 36, and 36%, respectively, in rats fed the F diet compared with rats fed the C diet (**Table 3**). On the other hand, the concentrations were significantly lower by 44, 51, and 37%, respectively, in rats fed the F-PL diet than in rats fed the F diet. Hepatic DG concentration was also 73% higher ($P < 0.05$) in rats fed the F diet compared with rats fed the C diet and significantly lower in rats fed the F-PL diet compared with rats fed the F or C diet. The hepatic glycogen concentration was significantly higher in rats fed the F-PL diet compared to rats fed the F or C diet.

The hepatic oleate [18:1(n-9)] concentration was significantly greater in rats fed the F diet compared with rats fed the C diet, whereas the oleate concentration in rats fed the F-PL diet was significantly lower than in rats fed the F diet (**Table 4**). The ratios of palmitoleate [16:1(n-7):palmitate (16:0) and 18:1(n-9): stearate (18:0)], indicative of desaturation by SCD-1 activity, were significantly higher in the liver of rats fed the F diet compared with rats fed the C diet. In rats fed the F-PL diet, these ratios were significantly lower than those in rats fed the F diet, and [16:1(n-7)):(16:0)] was significantly lower than that in rats fed the C diet. The ratio of 18:0:16:0, indicative of ELOVL activity, was significantly higher in rats fed the F-PL diet than in rats fed the C or F diet.

Hepatic gene expression. The mRNA expression levels of the genes related to de novo lipogenesis (*FASN*, *ACACA SCD1*, and *ELOVL6*) in rats fed the F diet were significantly higher than those in rats fed the C diet (**Table 5**). The mRNA expression levels of these genes in rats fed the F-PL diet were significantly lower than those in rats fed the F diet. In addition, the mRNA expression levels of *SREBP1* and *ChREBP* targeting lipogenic genes were also significantly higher in rats fed the F diet than in rats fed the C diet and significantly lower in rats fed the F-PL diet than in rats fed the F diet. These expression levels of genes of fatty acid synthesis and transcriptional factors other than *ELOVL6* in rats fed the F-PL diet did not differ significantly from those in rats fed the C diet. The mRNA expression level of *ELOVL6* was significantly higher in rats fed the F-PL diet than in rats fed the C diet. Furthermore, the mRNA expression of glucose-6-phosphate dehydrogenase X-linked (*G6PDX*), the regulatory enzyme of the pentose phosphate pathway in which xylulose-5-phosphate is produced and ChREBP translocation to the nucleus is promoted, was significantly higher in rats fed the F diet than in rats fed the C diet. The expression level of this gene in rats fed the F-PL diet was significantly lower than that in rats fed the C or F diet. There were no differences in the mRNA expression of *PPARa* and PPARα-targeting genes such as acyl-CoA oxidase (*ACOX*) and acyl-CoA dehydrogenase, C-4 to C-12 straight chain (*ACADM*) related fatty acid catabolism.

TABLE 3 Hepatic lipid and glycogen concentrations of rats fed a C, F, or F-PL diet for 10 wk[1]

	C	F	F-PL
TG, *μmol/g*	16.4 ± 1.3[a]	23.9 ± 5.5[b]	16.6 ± 1.1[a]
DG, *μmol/g*	0.67 ± 0.08[b]	1.15 ± 0.31[c]	0.33 ± 0.06[a]
Ceramides, *μmol/g*	0.44 ± 0.05[a]	0.61 ± 0.05[b]	0.41 ± 0.04[a]
TC, *μmol/g*	10.1 ± 0.3[a]	13.8 ± 1.3[b]	10.1 ± 0.6[a]
PL, *μmol/g*	77.5 ± 19.8[b]	50.6 ± 9.0[a]	74.1 ± 10.4[b]
Glycogen, *mg/g*	26.3 ± 5.2[a]	23.8 ± 4.8[a]	41.3 ± 5.5[b]

[1] Values are means ± SD, $n = 6$. Means in a row with superscripts without a common letter differ, $P < 0.05$. C, fructose-free diet; DG, diglyceride; F, 60% fructose diet; F-PL, 60% fructose plus 3% PL diet; PL, phospholipid TC, total cholesterol.

TABLE 4 Fatty acid composition, desaturation, and elongation ratio of fatty acid in the liver of rats fed a C, F, or F-PL diet for 10 wk[1]

	C	F	F-PL
Fatty acid composition, *nmol/g liver*			
14:0	0.81 ± 0.15[a]	1.30 ± 0.46[b]	0.70 ± 0.17[a]
16:0	23.3 ± 3.0[b]	27.1 ± 6.4[b]	19.9 ± 2.1[a]
16:1(n-7)	3.70 ± 1.04[b]	5.70 ± 2.21[b]	1.68 ± 0.61[a]
18:0	12.8 ± 0.4	13.3 ± 0.9	14.1 ± 2.3
18:1(n-9)	12.1 ± 1.8[a]	22.5 ± 6.4[b]	9.6 ± 2.1[a]
18:2(n-6)	14.6 ± 2.2[a]	16.8 ± 1.5[a]	21.3 ± 3.5[b]
18:3(n-6)	0.28 ± 0.02[a]	0.35 ± 0.04[b]	0.28 ± 0.04[a]
18:3(n-3)	0.08 ± 0.07[a]	0.15 ± 0.02[a]	0.33 ± 0.08[b]
Ratio of desaturation			
16:1(n-7):16:0	0.16 ± 0.03[b]	0.21 ± 0.03[c]	0.08 ± 0.03[a]
18:1(n-9):18:0	0.95 ± 0.13[a]	1.69 ± 0.46[b]	0.70 ± 0.20[a]
Ratio of elongation			
18:0:16:0	0.55 ± 0.06[a]	0.51 ± 0.09[a]	0.72 ± 0.14[b]
18:1(n-9):16:1(n-7)	3.42 ± 0.69[a]	4.05 ± 0.34[ab]	6.39 ± 2.50[b]

[1] Values are means ± SD, $n = 6$. Means in a row with superscripts without a common letter differ, $P < 0.05$. C, fructose-free diet; F, 60% fructose diet; F-PL, 60% fructose plus 3% PL diet.

Enzyme activity-related de novo lipogenesis. The enzyme activities of FAS, ACC, SCD, and ELOVL in the liver were higher in rats fed the F diet than in rats fed the C diet (**Table 6**). These activities were significantly lower in rats fed the F-PL diet (43, 35, 53, and 55% decreases compared with rats fed the F diet, respectively) and did not differ from those of rats fed the C diet.

Active component of PL and effect of PL treatment on SREBP1 and ChREBP protein. To identify the active component of PL, we treated Hep-G2 cells with fructose with or without

TABLE 5 Expression of genes in the liver of rats fed a C, F, or F-PL diet for 10 wk[1]

Gene	C	F	F-PL
Fatty acid synthesis		*Fold of C*[2]	
FASN	1.00 ± 0.30[a]	3.85 ± 0.82[b]	1.96 ± 0.75[a]
ACACA	1.00 ± 0.20[a]	2.38 ± 0.28[b]	1.40 ± 0.39[a]
SCD1	1.00 ± 0.25[a]	1.58 ± 0.39[b]	0.98 ± 0.37[a]
ELOVL6	1.00 ± 0.43[a]	8.40 ± 1.88[c]	3.01 ± 1.07[b]
Transcription factors			
SREBP1	1.00 ± 0.27[a]	1.46 ± 0.33[b]	0.99 ± 0.23[a]
ChREBP	1.00 ± 0.15[a]	1.36 ± 0.04[b]	1.10 ± 0.12[a]
PPARa	1.00 ± 0.17	1.07 ± 0.07	0.99 ± 0.24
Pentose phosphate pathway			
G6PDX	1.00 ± 0.23[b]	2.18 ± 0.39[c]	0.63 ± 0.14[a]
Fatty acid catabolism			
ACOX	1.00 ± 0.08	1.19 ± 0.11	1.04 ± 0.09
ACADM	1.00 ± 0.05	0.85 ± 0.09	0.95 ± 0.13

[1] Values are means ± SD, $n = 6$. Means in a row with superscripts without a common letter differ, $P < 0.05$. C, fructose-free diet; F, 60% fructose diet; F-PL, 60% fructose plus 3% PL diet.
[2] The amount of mRNA was normalized to that of Rplp/36B4 mRNA and expressed relative to the C diet group.

TABLE 6 Enzyme activities associated with de novo lipogenesis in the liver of rats fed a C, F, or F-PL diet for 10 wk [1]

Enzyme	C	F	F-PL
	pmol (min · mg protein)		
FAS	17.6 ± 1.7[a]	25.2 ± 3.1[b]	14.3 ± 2.3[a]
ACC	63 ± 15[a]	112 ± 10[b]	72 ± 13[a]
SCD	1240 ± 190[b]	1670 ± 280[c]	690 ± 290[a]
ELOVL	12.3 ± 2.4[a]	20.4 ± 4.2[b]	9.7 ± 2.2[a]

[1] Values are means ± SD, $n = 6$. Means in a row with superscripts without a common letter differ, $P < 0.05$. C, fructose-free diet; F, 60% fructose diet; F-PL, 60% fructose plus 3% PL diet.

purified PL components. Treatment with fructose increased the mRNA expression levels of *FASN*, *ACACA*, and pyruvate kinase, liver and RBC (*PKLR*), a gene under direct control of ChREBP, in Hep-G2 cells (**Table 7**). In addition, fructose also significantly increased the mRNA expression of *SREBP1* and tended to increase the mRNA expression of *ChREBP* ($P = 0.09$). The addition of PE or PI, the main components of soybean PL, decreased the fructose-induced mRNA expression of *FASN* and *ACACA*. Adding PC, PE, or PI decreased the fructose-induced increase in *SREBP1* mRNA expression. On the other hand, only PE significantly suppressed the mRNA expression of *ChREBP* and *PKLR*. SREBP1 and ChREBP proteins in the nucleus were significantly increased by fructose treatment of Hep-G2 cells, consistent with the mRNA expression pattern (**Fig. 2**). The addition of PC significantly decreased the fructose-induced elevation of SREBP1 protein in the nucleus, although PC did not suppress the increases of the mRNA expression of *SREBP1* and genes targeting SREBP1 by fructose stimulation. The addition of PE and PI, however, significantly suppressed the fructose-induced elevation of SREBP1 and ChREBP protein in the nucleus.

Discussion

PL ingestion improved fructose-induced metabolic abnormalities such as hyperlipidemia, hepatic steatosis, and impaired glucose tolerance. Fructose induced markers of de novo lipogenesis and PL ingestion effectively inhibited this process at the level of mRNA expression and enzyme activities. As a consequence of these metabolic changes, PL decreased the accumulation of TG, DG, ceramides, and oleate, the end-products of fatty acid synthesis, in the liver. These findings suggest an association between the suppression of the accumulation of hepatic lipids through the inhibition of de novo lipogenesis and the improvement of fructose-induced metabolic abnormalities by PL. Although the doses of fructose used in this study are widely used in studies of fructose, we have to note that they are above the physiological range.

Consistent with previous reports (22,23), PL ingestion modulated the expression of lipogenic genes in the liver; gene expression and activities of the enzymes involved in fatty acid synthesis, FAS and ACC, were lower in rats fed the F-PL diet than in rats fed the F diet. PL ingestion also decreased SCD-1 and ELOVL6, which are involved in the modification of fatty acids. Recent studies in mouse models of *SCD-1* and *ELOVL6* deficiency demonstrated the involvement of endogenous fatty acid modification in energy metabolism (18,19). Global deletion of *SCD-1* prevents obesity, hepatic steatosis, and insulin resistance. *ELOVL6* knockout mice have phenotypes similar to *SCD-1* deletion mice except for the antiobesity effect. In addition, liver-specific *SCD-1* knockout mice are protected from high-carbohydrate diet-induced adiposity and hepatic steatosis. These mice have a reduced oleate concentration in the liver and the phenotypes of *SCD-1* deficiency disappeared by the addition of oleate to the diet. These data suggest that oleate is a key fatty acid for the development of fructose-induced metabolic abnormalities. In the present study, PL ingestion dramatically decreased the liver oleate concentration induced by fructose feeding. These results suggest that PL may ameliorate fructose-induced metabolic abnormalities, including hypertriglycemia and hepatic steatosis, through the reduction of de novo lipogenesis and consequently a reduced oleate concentration in the liver.

The accumulation of lipids in the liver is the most consistent predictor of hepatic insulin sensitivity. In particular, DG and ceramides activate protein kinase C isoenzymes that can inhibit insulin receptor tyrosine kinase activity by initiating serine/threonine phosphorylation of the insulin receptor or insulin receptor substrate (7,34). In the present study, we showed that PL ingestion reduced fructose-induced hepatic lipid accumulation, including DG and ceramides. Therefore, PL may prevent glucose intolerance through the reduction of a series of lipids in the liver. On the other hand, the increased hepatic TG, DG, ceramides, and oleates caused by high-fat feeding are insufficient to cause insulin resistance in mice overexpressing diacylglycerol acyltransferase in the liver (35). Although our findings indicate that the accumulation of a variety of lipids in the liver relates to the development of insulin resistance in the case of fructose overconsumption, another mechanism might account for the

TABLE 7 Expression of genes on the Hep-G2 cells incubated for 24 h with 25 mmol/L fructose in the presence or absence of 10 μmol/L PL components[1]

Gene	C	F	F-PC	F-PE	F-PI
Fatty acid synthesis			*Fold of C*[2]		
FASN	1.00 ± 0.07[a]	2.58 ± 0.57[b]	2.11 ± 0.30[b]	1.14 ± 0.04[a]	1.30 ± 0.12[a]
ACACA	1.00 ± 0.01[a]	2.01 ± 0.24[b]	1.75 ± 0.20[b]	0.88 ± 0.08[a]	1.11 ± 0.26[a]
Carbohydrate metabolism					
PKLR	1.00 ± 0.04[ab]	1.89 ± 0.30[c]	1.49 ± 0.23[bc]	0.82 ± 0.10[a]	1.36 ± 0.42[bc]
Transcription factors					
SREBP1	1.00 ± 0.03[b]	1.33 ± 0.16[c]	1.10 ± 0.03[b]	0.79 ± 0.02[a]	0.67 ± 0.04[a]
ChREBP	1.00 ± 0.03[ab]	1.32 ± 0.26[b]	0.97 ± 0.14[ab]	0.80 ± 0.05[a]	0.96 ± 0.11[ab]

[1] Values are means ± SD of 3 independent experiments. Means in a row with superscripts without a common letter differ, $P < 0.05$. C, cells incubated without fructose; F, cells incubated with 25 mmol/L fructose; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol.
[2] The amount of mRNA was normalized to that of Rplp/36B4 mRNA and expressed relative to the cells without fructose (C).

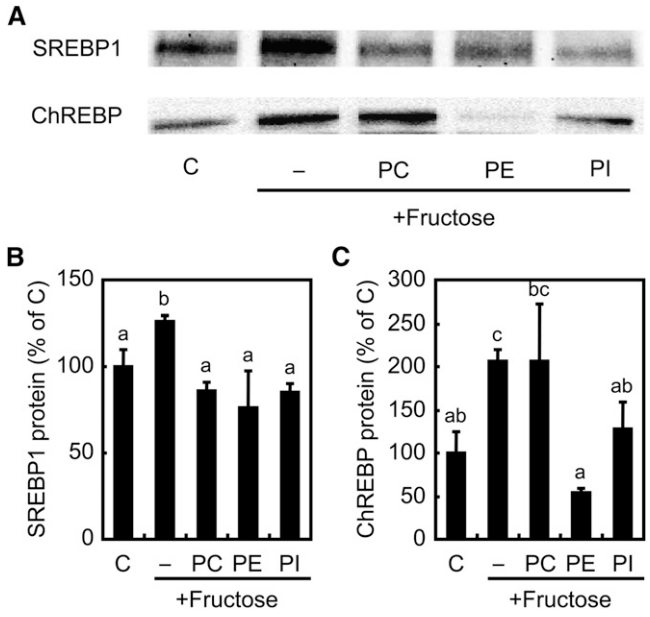

FIGURE 2 SREBP1 and ChREBP protein levels on Hep-G2 cells incubated for 24 h with 25 mmol/L fructose in the presence or absence of 10 nmol/L purified PL components. The images (*A*) shown are representative bands from 3 independent experiments. Values (*B,C*) are means ± SD of 3 independent experiments. Means without common letter differ, $P < 0.05$. C, fructose-free diet; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol.

relationship between hepatic lipids and the development of insulin resistance.

Dietary fructose regulates lipogenic gene expression via transcriptional and posttranscriptional effects on the transcriptional factors SREBP1 and ChREBP (16,17). The findings of the present study demonstrated that fructose feeding induced *SREBP1* and *ChREBP* expression in the liver, and PL ingestion decreased the increased mRNA expression of these genes. In Hep-G2 cells, fructose stimulated not only the mRNA levels but also the nuclear levels of SREBP1 and ChREBP protein and constituents of PL, especially PE and PI, reduced the mRNA and nuclear protein expression of these molecules. These findings indicate that the effect of PL on SREBP1 is independent of insulin, which is well documented to regulate SREBP1 expression (36,37). In addition, ChREBP nuclear translocation is regulated by the pentose phosphate pathway, particularly xylulose-5-phosphate. Xylulose-5-phosphate activates protein phosphatase 2A, which dephosphorylates ChREBP, thereby allowing its translocation into the nucleus (38,39). Ingestion of PL decreased the mRNA expression of *G6PDX*, which catalyzes a rate-limiting step of the pentose phosphate pathway, suggesting that the reduction of the nuclear expression of ChREBP by PE and PI is involved in the ChREBP translocation from the cytosol to the nucleus.

Some previous reports suggested that dietary PC and PI improve metabolic abnormalities, including hepatic steatosis and hyperlipidemia, by decreasing fatty acid synthesis and activating fatty acid β-oxidation, respectively (40,41). In the present study, PC suppressed the increased expression levels of nuclear SREBP1 protein induced by fructose stimulation in Hep-G2 cells. PE and PI decreased the expression levels of both nuclear SREBP1 and ChREBP protein. Our results suggest that the positive effects of purified constituents of PL on metabolic abnormalities are mediated, at least in part, through the suppression of SREBP1 and/or ChREBP. In addition, PE and PI suppressed the mRNA expression of target genes of SREBP1 and ChREBP such as *FASN* and *ACACA*, but PC did not affect the expression, suggesting an important role of ChREBP in the regulation of lipogenesis induced by fructose stimulation.

In summary, PL ingestion ameliorates hypertriglycemia, hepatic steatosis, and glucose intolerance in rats fed a fructose diet in association with the reduction of a variety of lipids, including TG, DG, ceramides, and oleates in the liver through the prevention of de novo lipogenesis. These findings might help to elucidate the impact of an altered hepatic lipid profile on metabolic abnormalities and suggest a possible therapeutic nutritional approach by PL in the prevention of fructose-induced metabolic abnormalities through the reduction of a variety of lipids in the liver.

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