Physiological Effects of Dietary PIPS Soybean-Derived Phospholipid in Obese Zucker (fa/fa) Rats

Bungo Shirouchi,1 Koji Nagao,1 Kenta Furuya,1 Masatoshi Shiojiri,2 Xiaoli Liu,2 and Teruyoshi Yanagita1,3

1Department of Applied Biochemistry and Food Science, Saga University, 1 Honjo, Saga 840-8502, Japan
2Bio/Fine Chemicals Development, Nagase ChemteX Corporation, 2-2-3 Marotani, Nishi-ku, Kobe 651-2241, Japan

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The effects of soybean-derived phospholipid, PIPS NAGASE™ (PIPS), on obesity-induced diseases were studied in obese rats. Dietary PIPS alleviated hepatic steatosis and fatty liver in the rats. These effects were attributable to reduced lipogenesis and enhanced lipolysis in the liver. The results suggest that PIPS can be useful as a dietary component that would reduce the risk of lifestyle-related diseases.

Key words: phosphatidylinositol; phosphatidylserine; fatty liver; Zucker (fa/fa) rat

Since diet, especially dietary fat, has been recognized as contributing to the development and progression of obesity, the influence of the quantity and quality of dietary fat on the pathogenesis of obesity-induced diseases has been studied.6) Although triglycerides make up the majority of dietary fat, phospholipids comprise about 3–8% of the daily intake of total dietary fat.7,8) Growing evidence indicates that dietary phospholipids, especially phosphatidylcholine (PC) and phosphatidylethanolamine (PE), have beneficial effects compared with dietary triglycerides.9–12) We have recently reported that dietary phosphatidylinositol (PI), a minor component of dietary phospholipids, alleviated obesity-induced diseases in obese rats through an increase in the serum levels of adiponectin and the enhancement of hepatic fatty acid beta-oxidation and fecal bile acid excretion.13,14) It is also known that dietary phosphatidylserine (PS), a minor component of dietary phospholipids, improved the brain function.15) The effects of dietary PS on the pathogenesis of obesity-induced diseases, however, have not been fully evaluated.

We evaluated in the present study the effects of the soybean-derived phospholipid, PIPS NAGASE™ (PIPS; Nagase ChemteX Corp., Kobe, Japan), on lipid metabolism in obese Zucker (fa/fa) rats. PIPS was prepared from soybean lecithin and t-serine by an enzymatic transphosphatidylation method utilizing phospholipase D and was composed mainly of PI and PS. Zucker (fa/fa) rats develop a syndrome with multiple metabolic and hormonal disorders that shares many features with human obesity.16–18) Zucker (fa/fa) rats have hyperphagia because they have a missense mutation on the leptin receptor gene, and become obese, developing hyperlipidemia, type-2 diabetes, and fatty liver.

Five-week-old male Zucker (fa/fa) rats were purchased from Japan SLC (Shizuoka, Japan). The rats were individually housed in metal cages in a temperature-controlled room (24°C) under a 12-h light/dark cycle. After a 1-week adaptation period on a powdered diet (CE-2; Clea Japan, Tokyo), they were assigned to two groups of six rats each, each group being fed one of two diets: (i) a semi-synthetic diet containing (in weight %) casein, 20; soybean oil, 7; cornstarch, 15; vitamin mixture (AIN-76™), 1; mineral mixture (AIN-76™), 3.5; dl-methionine, 0.3; choline bitartrate, 0.2; cellulose, 5; and sucrose, 48 (the control group); and (ii) a semi-synthetic diet supplemented with 2% PIPS at the expense of soybean oil (the PIPS group). PIPS contained (in weight %) phospholipids, 82.1 (PS, 21.1; PI, 20.4; phosphatidic acid (PA), 15.3; PE, 13.4; PC, 7.6; and others, 4.3); triglyceride, 0.3; moisture, 0.8; and unknown soybean-derived acetone-insoluble components, 16.8. The animals received the diets for 4 weeks. All the rats were then killed by aortic exsanguination under diethyl ether anesthesia. The perirenal, epididymal, omental, and waist subcutaneous white adipose tissues (WATs) and the liver were excised for analysis. All aspects of the experiment were conducted according to the guidelines established by the ethical committee on experimental animal care of Saga University. After the 4-week feeding period, the amount of food intake, final body weight and total WAT weights had not
been significantly altered by dietary PIPS in the Zucker \((fa/fa)\) rats (data not shown). In contrast, the liver weight was significantly decreased in the PIPS-fed rats (control, 4.14 ± 0.15; PIPS, 3.40 ± 0.07 g/100 g of body weight; \(p < 0.05\)).

To further examine the effects of dietary PIPS on the liver, we analyzed the concentration of hepatic triglyceride and the activity of the hepatic injury marker in the serum. Liver lipids were extracted and purified by the method of Folch et al.,\(^\text{19}\) and the concentration of triglyceride was measured as previously described.\(^\text{20}\) As shown in Fig. 1, the hepatic triglyceride accumulation was markedly alleviated by dietary PIPS in the Zucker \((fa/fa)\) rats. Consistent with the alleviation of hepatomegaly and fatty liver by dietary PIPS, the activity of aspartate aminotransferase (AST), a marker of hepatic injury, was markedly lower in the serum of the PIPS-fed rats (Fig. 1). These results suggest that dietary PIPS could prevent the development of obesity-induced hepatic injury in obese Zucker \((fa/fa)\) rats.

To gain insight into the effects of dietary PIPS on the regulation of hepatic lipid metabolism, we analyzed the activities of enzymes related to fatty acid synthesis and fatty acid beta-oxidation. The activities of fatty acid synthase (FAS), glucose 6-phosphate dehydrogenase (G6PDH), peroxisomal beta-oxidation, and carnitine palmitoyltransferase (CPT) were measured as previously described.\(^\text{21–24}\) As is shown in Fig. 2, the activity of FAS, a key enzyme of fatty acid synthesis, was significantly reduced by dietary PIPS in the Zucker \((fa/fa)\) rats. Additionally, the activity of G6PDH, which provides NADPH required for fatty acid synthesis, was significantly decreased by dietary PIPS. Although the activity of peroxisomal beta-oxidation was not different between the groups (data not shown), dietary PIPS significantly enhanced the activity of CPT, a key enzyme in mitochondrial fatty acid beta-oxidation, as compared with the control diet, in the Zucker \((fa/fa)\) rats (Fig. 2). These results suggest that the alleviation of hepatomegaly and fatty liver by dietary PIPS was attributable to the reduction of FAS and G6PDH activities and enhancement of the CPT activity in the liver. We have previously reported that dietary PI enhanced fatty acid beta-oxidation, but did not suppress fatty acid synthesis in the liver.\(^\text{3}\) PIPS, however, contains some acetone-insoluble components other than PI and PS. We have previously reported that dietary PC, which PIPS contained in a small amount, alleviated fatty liver by enhancing fatty acid beta-oxidation and suppressing fatty acid synthesis in the liver of rats.\(^\text{4}\) On the other hand, a previous study has shown that dietary PE did not affect the hepatic triglyceride levels when compared with dietary soybean oil.\(^\text{5}\) In addition, none of the previous studies made have evaluated the effect of PS, PA, and other soybean-derived acetone-insoluble components on the hepatic triglyceride levels in rats. Further studies are necessary to clarify the effect of more purified phospholipids containing only PI and PS on lipogenesis and lipolysis in the liver. It has recently been reported that adiponectin had a protective effect against fatty liver.\(^\text{6}\) The serum adiponectin level in this study was significantly higher in the PIPS-fed rats (Fig. 3). We
therefore consider that the increase in serum adiponectin level caused by dietary PIPS also contributed to alleviating the development of obesity-induced hepatic injury in obese Zucker (fa/fa) rats.

In conclusion, our results indicate that dietary PIPS alleviated the development of such obesity-induced diseases as hepatomegaly and fatty liver in obese Zucker (fa/fa) rats and suggest that PIPS can be useful as a dietary component that will reduce the risk of lifestyle-related diseases. Given that dietary PC, PE, and PI have beneficial effects, a comparison of the physiological effects on the development and prevention of lifestyle-related diseases among such phospholipids as PC, PE, PI, and PS would be of great interest for future study.

References