Effect of Dietary Phosphatidylinositol on Cholesterol Metabolism in Zucker (fa/fa) Rats

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Abstract: Recent studies have shown that dietary phospholipids, especially phosphatidylcholine and phosphatidylserine, have various beneficial biological effects. However, there are not enough data concerning the physiological function of dietary phosphatidylinositol (PI). The metabolic syndrome, a cluster of metabolic abnormalities such as dyslipidemia, diabetes mellitus, and hypertension, is widespread and increasingly prevalent diseases in industrialized countries. In the present study, we evaluated that the effect of dietary PI on cholesterol metabolism in metabolic syndrome model Zucker (fa/fa) rats. For 4 weeks, rats were fed semisynthetic diets containing either 7% soybean oil or 5% soybean oil plus 2% PI. Dietary PI prevented the mild hypercholesterolemia and hepatic cholesterol accumulation in Zucker (fa/fa) rats. These effects were attributable to an increased fecal bile acid excretion and to the tendencies of decreased ACAT1 mRNA level and increased CYP7A1 mRNA level in the liver. Additionally, dietary PI markedly increased microsomal PI content in the liver of Zucker (fa/fa) rats. Our study suggests that dietary PI normalizes cholesterol metabolism through the enhancement of fecal bile acid excretion in the metabolic syndrome model rats.

Key words: phosphatidylinositol, cholesterol metabolism, bile acids, Zucker (fa/fa) rats

1 INTRODUCTION

Lifestyle-related diseases, such as obesity, hyperlipidemia, atherosclerosis, type 2 diabetes, and hypertension, are widespread and increasingly prevalent in industrialized countries. Accompanied by the rapid increase in the number of elderly people, this becomes a medical and a socioeconomic issue. A clustering of metabolic disorders in an individual, defined as metabolic syndrome, is known to increase cardiovascular morbidity and mortality¹. Although the pathogenesis of metabolic syndrome is complicated and precise details of the underlying mechanisms are not known, it has been suggested that the quality of dietary fats may be an important modulator in terms of the risks associated with this syndrome².

Although triglycerides (TGs) make up the majority of dietary fat, phospholipids (PLs) compose about 3-8% of the daily intake of total dietary fats³⁴. Growing evidence indicates that dietary PLs, especially phosphatidylcholine (PC), phosphatidylethanolamine (PE) and phosphatidylserine (PS), have beneficial effects compared with dietary TGs. For example, dietary PC, PE and PS have been reported to have lipid-lowering effects and improve brain function, respectively⁵⁻¹⁰. Despite the fact that the use of PC and PS for nutritional and therapeutical purposes has increased in recent years, reports examining the physiological functions of dietary Phosphatidylinositol (PI) are scarce. PI is a minor component of dietary PLs and is found in legumes and seeds such as soybeans, peanuts, rapeseeds, and sunflower seeds¹¹. It has been known that PI in biological membranes plays key roles in mediating cellular responses to external stimuli¹²⁻¹³. Recently, we also reported that dietary PI prevents the development of nonalcoholic fatty liver disease (NAFLD) through the enhancement of serum adiponectin level in a rat model of the metabolic syn-
drome\(^{4}\).

In the present study, we evaluated the effect of dietary PI on cholesterol metabolism in Zucker (fa/fa) rats. Zucker (fa/fa) rats develop a syndrome with multiple metabolic and hormonal disorders that share many features with human obesity\(^{15-17}\). Zucker (fa/fa) rats have hyperphagia, because they have a missense mutation on the leptin receptor gene, and become obese, developing diabetes, NAFLD, dyslipidemia.

2 MATERIALS AND METHOD

2.1 Animals and diets

All aspects of the experiment were conducted according to the guidelines provided by the ethical committee of experimental animal care at Saga University. Male Zucker (fa/fa) rats aged 5 weeks were purchased from Japan SLC, Shizuoka, Japan. The rats were housed individually in metal cages in a temperature-controlled room (24°C) under a 12-h light/dark cycle. After a 1-week adaptation period, the rats were assigned to two groups (six rats each) that were fed one of two diets: a semisynthetic diet supplemented with 7% soybean oil (TG group) or a semisynthetic diet supplemented with 5% soybean oil plus 2% soybean PI (PI group). The basal semisynthetic diets were prepared according to recommendations of the AIN-76\(^{18}\) and contained the following (in weight %): casein, 20; cornstarch, 15; cellulose, 5; mineral mixture (AIN-76), 3.5; vitamin mixture (AIN-76), 1; DL-methionine, 0.3; choline bitartrate, 0.2; fat, 7; and sucrose, 48. Soybean PI (contained 81.3% PI, 14.2% PC, and 4.5% PE) was prepared from soybean PLs by enzymatic methods utilizing the phospholipase B having a poor hydrolytic activity on PI\(^{19}\). The rats consumed 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.

2.2 Measurement of hepatic and serum cholesterol levels

Serum cholesterol level was measured using commercial enzyme assay kits (Wako Pure Chemicals, Tokyo, Japan). Liver lipids were extracted and purified according to the method of Folch et al.\(^{20}\), and hepatic cholesterol level was measured by the method of Sperry and Webb\(^{21}\).

2.3 Analysis of mRNA expression

Total RNA was extracted from 50 mg of liver, using a RNeasy Lipid Tissue Mini Kit (Qiagen, Tokyo, Japan). A TaqMan Universal PCR Master Mix (Applied Biosystems, Tokyo, Japan); Assay-on-Demand, Gene Expression Products (Rn00579605_m1 for acyl-CoA: cholesterol acyltransferase1 (ACAT1), Rn00564065_m1 for cholesterol 7α-hydroxylase (CYP7A1), Rn00598438_m1 for low-density-lipoprotein (LDL)-receptor, Rn01759149_m1 for liver receptor homologue-1 (LRH-1), Hs99999901_s1 for 185 RNA, Applied Biosystems), and TaqMan MGB Gene Expression Kits for 3-hydroxy-3-methyl-glutaryl-CoA (HMG-CoA) reductase and sterol regulatory element binding protein-2 (SREBP-2) were used for the quantitative real-time RT-PCR analysis of ACAT1, CYP7A1, LDL-receptor, LRH-1, 185 RNA, HMG-CoA reductase, and SREBP-2 expression in the liver. The details of the TaqMan MGB Gene Expression Kits were as follows: HMG-CoA reductase (forward primer, 5’-AGTGATTGTGCTAGATTTATTGTTGGAAG-3’; reverse primer, 5’-GCTGTGCTGAAGATGTGAAAGC-3’; and TaqMan MGB probe, 5’-FAM-TTGCTGTTGTATGTTAAAGT-MGB-3’); SREBP-2 (forward primer, 5’-CCACGACCTTGTATACAGACA-3’; reverse primer, 5’-GCATTGCCATGGCGTCT-3’; and TaqMan MGB probe, 5’-FAM-TCAAATGGCAAACCTT-3’-MGB). The amplification was performed with a real-time PCR system (ABI Prism 7000 Sequence Detection Systems; Applied Biosystems). Results were quantified with a comparative method and were expressed as a relative value after normalization to the 185 RNA expression.

2.4 Measurement of fecal bile acids level

Feces were collected for 2 days at the end of feeding period and lyophilized for 48 h. Lyophilized feces were powdered using a food processor (TML-15, Tescom Denki, Co., Ltd., Tokyo, Japan). Fecal steroids were extracted three times with ethanol (10 mL × 3) for 1 h at 75°C from 100 mg of powdered lyophilized-feces, and total bile acid levels were determined using commercial enzyme assay kit (Wako Pure Chemicals, Tokyo, Japan).

2.5 Analysis of hepatic microsomal PI content by HPLC

The hepatic microsomal PI was determined by an HPLC method (Toyo Kensa Center Co., Ltd, Shizuoka, Japan)\(^{22}\). The preparation of hepatic subcellular fractions was conducted as described previously\(^{23}\). The total lipids in the microsome were extracted by the method of Bligh and Dyer\(^{23}\) and dissolved in hexane:isopropyl alcohol (0.2 M acetate buffer (pH 4.2) (8:8:1, v/v)). PL classes were separated by an HPLC (Shimadzu Co., LC-9A, Kyoto, Japan) equipped with a 4.6 × 250 mm silica column (YMC Co. Ltd., SIL-06, Kyoto, Japan). The mobile phase consisted of hexane:isopropyl alcohol (0.2 M acetate buffer (pH 4.2) (8:8:1, v/v)) and the flow rate was 2 mL/min. Detection was monitored at 206 nm (Shimadzu Co., SPD-6A, Kyoto, Japan). The content of hepatic microsomal PI was calculated using the following equation; [The peak area of PI/All peak areas] × 100 (%).

2.6 Statistical analysis

All values are expressed as means ± SEM. The signifi-
cance of differences between means for two groups was determined by Student’s $t$ test. Differences were considered to be significant at $P<0.05$.

3 RESULTS

3.1 Effect of dietary PI on growth parameters

Although there were no significant differences in initial body weight, final body weight, food intake, and WAT weight between the groups, the relative liver weight was significantly decreased in PI-fed rats. The data from the same rats were indicated in detail in our earlier report\textsuperscript{14}.

3.2 Effect of dietary PI on hepatic and serum lipid levels

As reported previously, the PI diet significantly decreased hepatic cholesterol levels (TG group, 2.84 ± 0.07; PI group, 2.19 ± 0.04 mg/g liver, $P<0.05$) and serum cholesterol levels (TG group, 191 ± 7; PI group, 155 ± 6 mg/dL, $P<0.05$) by 23% and 19%, respectively\textsuperscript{14}.

3.3 Effect of dietary PI on mRNA levels of cholesterol metabolism related enzymes in the liver

The two groups of rats did not differ in the mRNA levels of HMG-CoA reductase, LDL-receptor, SREBP-2, and LRH-1 in the liver of rats (data not shown). However, the mRNA level of ACAT1 tended to decrease (63%, $P = 0.234$) in the liver of PI-fed rats compared with TG-fed rats. In contrast, the mRNA level of CYP7A1 tended to increase (2.3-fold, $P = 0.101$) in the liver of PI-fed rats compared with TG-fed rats (Fig. 1).

3.4 Effect of dietary PI on fecal bile acids level

Although there was no significant difference in weights of feces among the groups (data not shown), the excretion of bile acids into feces markedly increased (2.3-fold) by the PI diet compared with the TG diet (Fig. 2).

3.5 Effect of dietary PI on the content of hepatic microsomal phosphatidylinositol

The content of hepatic microsomal phosphatidylinositol significantly increased (1.2-fold) by the PI diet compared with the TG diet (Fig. 3).

4 DISCUSSION

Our previous report showed that dietary PI prevents the development of NAFLD through the enhancement of serum adiponectin level in a rat model of the metabolic syndrome\textsuperscript{14}. Now we report the hypocholesterolemic effect of dietary PI in Zucker ($fa/fa$) rats. After 4 week of feeding, Zucker ($fa/fa$) rats fed the TG diet had severe hepatic steatosis and mild hypercholesterolemia\textsuperscript{14}. The relative

![Hepatic mRNA levels](image)

**Fig. 1** Effect of Dietary PI on mRNA Levels of Cholesterol Metabolism-related Enzymes in the Liver of Zucker ($fa/fa$) Rats. Rats were fed TG diet or PI diet for 4 weeks. Values are expressed as means ± standard error of six rats. Asterisk shows significant difference at $P<0.05$.

![Bile acids](image)

**Fig. 2** Effect of Dietary PI on Fecal Bile Acid Levels in Zucker ($fa/fa$) Rats. Rats were fed TG diet or PI diet for 4 weeks. Values are expressed as means ± standard error of six rats. Asterisk shows significant difference at $P<0.05$.

![Microsomal phosphatidylinositol](image)

**Fig. 3** Effect of Dietary PI on Hepatic Microsomal Contents of Phosphatidylinositol in Zucker ($fa/fa$) Rats. Rats were fed TG diet or PI diet for 4 weeks. Values are expressed as means ± standard error of six rats. Asterisk shows significant difference at $P<0.05$.  

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liver weight was 21% less in PI-fed rats, and this was partly associated with a reduction in the cholesterol accumulation in the liver. As a consequence, serum cholesterol level was also decreased in PI-fed rats compared with TG-fed rats. ACAT1, a rate-limiting enzyme of cholesterol esterification, CYP7A1, a rate-limiting enzyme of bile acid synthesis, HMG-CoA reductase, a rate-limiting enzyme of cholesterol synthesis, and LDL-receptor relate cholesterol homeostasis. The gene expression of cholesterol biosynthetic enzymes and receptor, such as HMG-CoA reductase and LDL-receptor, are regulated by SREBP-2, a transcriptional factor. Given the results that the PI diet did not affect the mRNA levels of HMG-CoA reductase, LDL-receptor, and SREBP-2 compared with the TG diet, the cholesterol-lowering effect of dietary PI was not attributable to the reduction of cholesterol biosynthesis. 

On the other hand, ACAT1 mRNA level tended to decrease by the PI diet, whereas CYP7A1 mRNA level tended to increase by the PI diet (Fig. 1). Additionally, the PI diet increased levels of fecal bile acids compared with the TG diet (Fig. 2). Previous studies showed that dietary PC and PE revealed hypcholesterolemic effects through the increased fecal excretion of neutral steroid, not bile acids, compared with the TG diet. Thus, we suppose that the hypocholesterolemic effect of PI diet was attributable to suppressed lipoprotein synthesis and enhanced bile acid synthesis in the liver, and was a unique mechanism differs from that revealed by other PLs.

LRH-1 is an orphan nuclear receptor expressed in the liver, pancreas, intestine and ovary. In these tissues, LRH-1 plays a predominant role in development, reverse cholesterol transport and bile acids homeostasis. LRH-1 binds to a binding element of LRH-1 (LRH-RE) and is necessary for transactivation of CYP7A1 gene. In the present study, LRH-1 mRNA levels did not differ among the groups. A recent study, however, suggests that phosphatidylinositol phosphates such as PI(3,5)P2 and PI(3,4,5)P3 act as ligands for LRH-1. In addition, Iwaki et al. found LRH-RE in adiponectin promoter, and our previous report showed that dietary PI increased serum adiponectin levels in Zucker (fa/fa) rats. Therefore, we suppose that dietary PI might be associated with the normalization of cholesterol metabolism and the increase of serum adiponectin by inducing LRH-1 transactivation through the increase of microsomal PI content in Zucker (fa/fa) rats.

In conclusion, our study shows that dietary PI decreases hepatic and serum cholesterol levels through the enhancement of fecal bile acids excretion in the metabolic syndrome model rats. Although composition of dietary fatty acids can affect CYP7A1 gene expression and bile acid production in the liver, there was no significant difference of fatty acid composition between TG diet and PI diet in this study. Therefore, we suppose that the beneficial effects demonstrated in this study were attributable to the physiological functions of PI itself or its constituent base inositol. Given that dietary PC and PE have lipid-lowering effects, comparison concerning physiological effects on the development and prevention of metabolic syndrome among PLs, such as PC, PE, and PI, would be of great interest for future study.

References


Effect of Dietary PI on Lipid Metabolism in Obese Rats

(1986).


