We have previously reported that phytoceramide and phytosphingosine (PHS) stimulated the transcriptional activity of peroxisome proliferator-activated receptor γ (PPARγ) in cells. PPARγ is a therapeutic target for type 2 diabetes. We found in this study that an oral administration of PHS improved diet-induced glucose intolerance in mice. Since PHS is highly expressed in yeast, PHS in fermented foods may improve diabetes.

Key words: phytoceramide; phytosphingosine; sphingolipid β(4)-desaturase/C4-hydroxylase isoform 2 (DES2); sphingolipid; PPAR

Sphingolipids are ubiquitous components of the plasma membrane in all animals, plants and fungi, and consist of a sphingoid base [sphingosine, sphinganine, phytosphingosine (PHS), or other species]. Sphingosine is the most common sphingoid base in mammalian cells. While PHS is a minor sphingoid base in mammalian cells, it is the major sphingoid base in yeast and fungi. Phytoceramide (phCer) is a fatty acid derivative of PHS, and is a common backbone of complex phyto-type sphingolipids. The daily intake of sphingolipids from foods has been estimated to be about 300–400 mg for humans in the United States of America. Although many subclasses of sphingolipid are present in food, a large proportion of the daily intake of phCer or PHS is derived from foods containing plants, yeasts, and fungi, particularly fermented foods. We have previously reported that phCer and PHS stimulated the transcriptional activities of peroxisome proliferator-activated receptor γ (PPARγ), using a dual-luciferase reporter system. PPARγ is a ligand-activated transcriptional factor belonging to the nuclear receptor superfamily that has important roles in glucose and lipid homeostasis. Such synthetic ligands of PPARγ, as rosiglitazone and pioglitazone are widely used to treat type 2 diabetes. We therefore investigated in this study the in vivo effects of phCer and PHS on diet-induced glucose intolerance, a major characteristic of type 2 diabetes. Several studies have shown that dietary sphingolipids were hydrolyzed to their components, namely sphingoid bases, fatty acids, and the polar head group, by intestinal enzymes and were then taken up by mucosal cells. The absorbed sphingoid base could then be re-converted into complex sphingolipids. Orally administered phCer can be hydrolyzed to PHS and fatty acids, so we tested whether the administration of PHS would increase the efficiency of absorption. Little is so far known about the absorption and disposition of PHS in vivo. Sphingolipid β(4)-desaturase/C4-hydroxylase isoform 2 (DES2) is an enzyme responsible for the synthesis of phyto-type sphingolipids, including phCer and PHS. Since DES2 knockout (KO) mice lack both phCer and PHS, we thought that these mice would provide a good animal model to examine the uptake and metabolism of dietary PHS. The DES2 KO mice were fed for 2 weeks on a normal diet (ND, AIN76A) supplemented or not with 0.2% PHS (Tokyo Chemical Industry, Tokyo, Japan). Lipids were extracted from the liver and small intestine of the mice, and the levels of free PHS and PHS-containing sphingolipids (phSPG) were measured as previously described. All animal procedures conformed to the Guidelines of Hokkaido University for the Care and Use of Laboratory Animals published by Animal Research Committee of Hokkaido University. As expected, the liver and small intestine of mice fed on the PHS-free diet contained no PHS or phSPG (data not shown). However, the respective concentrations of PHS and phSPG in the small intestine of mice fed on the 0.2% PHS diet were 154.9 pmol/mg of protein and 20,773 pmol/mg of protein (Table 1). These results indicate that PHS was taken up and converted to phCer, similar to other sphingoid bases. Surprisingly, PHS or reconstituted phSPG also reached the liver. We first confirmed the uptake and re-conversion of PHS into phCer by using DES2 KO mice. We next examined the in vivo effects of orally administered PHS, which was expected to be converted into phCer, on obesity and type 2 diabetes in diet-induced obese (DIO) mice. The DES2 KO mice were fed on the ND or high-fat diet (HFD) supplemented or not with 0.2% PHS (HFD + PHS) from 4 to 28 weeks of age. Figure 1A shows that the mean food intake was not significantly

Note

Improved High-Fat Diet-Induced Glucose Intolerance by an Oral Administration of Phytosphingosine

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different among the diets. The body weight of the mice was markedly higher in the HFD and PHS + HFD groups than in the ND group, although there were no significant differences between the HFD and PHS + HFD groups (Fig. 1B). We also measured the plasma triacylglycerol and total cholesterol levels in each group after a 16-h fast. As expected, HFD markedly increased the plasma total cholesterol level, consistent with the development of obesity (Fig. 1D). The presence of PHS hardly affected the triacylglycerol and total cholesterol levels (Fig. 1C and D). DIO is well known to cause hepatic dysfunction and HFD used here increased the levels of the markers of hepatic damage, including glutamate oxaloacetate dehydrogenase (GOT) and glutamic pyruvic transaminase (GPT) (Fig. 1E and F).

Although the GOT levels were significantly higher in the HFD group than in the ND group, there were no significant differences between the ND and PHS + HFD groups (Fig. 1E). We have already described synthetic ligands of PPARγ being used to treat type 2 diabetes and we have previously reported that phCer and PHS activated PPARγ.4) We therefore next examined the effect of PHS on glucose intolerance, a major characteristic of type 2 diabetes. The intraperitoneal glucose tolerance test (GTT) was performed as

Table 1. Uptake and Metabolism of Dietary PHS by the Small Intestine and Liver

<table>
<thead>
<tr>
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<th>Small intestine</th>
<th>Liver</th>
</tr>
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<tbody>
<tr>
<td>ph-SPG</td>
<td>20773.2 ± 3692.0</td>
<td>183.7 ± 145.6</td>
</tr>
<tr>
<td>PHS</td>
<td>154.9 ± 104.2</td>
<td>19.2 ± 6.6</td>
</tr>
</tbody>
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Lipids were extracted from the liver and the small intestine of mice. Free PHS and PHS-containing sphingolipids (phSPG) were measured as previously described.11)

Fig. 1. Effects of PHS Supplementation on the Body Weight, Plasma Neutral Lipids, and Hepatic Damage.

DES2 knockout (KO) mice were fed with a high-fat diet (HFD; D123492, Research Diet, New Brunswick, NJ, USA) supplemented or not with 0.2% PHS (Tokyo Chemical Industry, Tokyo, Japan) or a normal diet (ND; AIN76A, Nihon Nohsan Kogyo Co., Yokohama, Japan) from 4 to 22 weeks of age. A, Food intake was measured at 22 weeks of age as previously described.12) B, Body weight was measured weekly. C–F, The plasma triacylglycerol (C), total cholesterol (D), glutamate oxaloacetate dehydrogenase (GOT; F), and glutamic pyruvic transaminase (GPT; F) levels were measured by using a Triglyceride E-test Wako kit (Wako, Japan), Cholesterol E-test Wako kit (Wako, Japan), and Transaminase CII-test Wako kit (Wako, Japan) after a 16-h fast, as previously described.12) Values are presented as the mean ± SD of 6–7 mice/group. *p < 0.05 and **p < 0.01 vs. the ND group. The p value was calculated by one-way ANOVA with the Tukey-Kramer post-test for multiple comparisons.
previously described. As expected, the blood glucose levels associated with chronic and low-grade inflammation. Adipokines, such as MCP-1 and TNF, while decreasing the expression of adiponectin. The early stages of obesity and diabetes are characterized by an inflammatory event in adipose tissue, preventing the development of glucose intolerance and obesity.

The administration of PHS has previously been reported to decrease the plasma cholesterol and triacylglycerol concentrations in APOE-/-Leiden mice, this being mediated by inhibiting the intestinal absorption of cholesterol and triacylglycerol. In contrast, the administration of PHS has reportedly improved the fasting plasma glucose level without affecting the plasma triacylglycerol level. These reports suggest that administration of PHS did not only inhibit the intestinal absorption of neutral lipids, but also had an other function to improve glucose intolerance. Such a synthetic ligand of PPARγ, as troglitazone acts as an insulin sensitizer and is used to treat type 2 diabetes. However, troglitazone itself has not improved adiposity or weight gain. We found in this present study that PHS improved the glucose intolerance associated with DIO, but could not reduce the weight gain induced by HFD, similar to the effects of troglitazone.

We have reported in our previous study that PHS derived from brewer’s yeast activated PPARγ. PHS and phCer are the major sphingolipids in yeast and fungi, and are thus present in the fermented food consumed almost exclusively by the Japanese. Our results therefore indicate that PHS and phCer in traditional Japanese fermented food might help to prevent the development of type 2 diabetes. We have recently reported that 4,8-sphingadienine enhanced ceramide production in the skin by activating PPARs. Since many other subclasses of sphingolipid are present in food, we should determine in a future study which type of sphingolipid is important in daily food.

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**References**


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**Fig. 2.** Attenuation of HFD-Induced Glucose Intolerance and Changes in the Expression Levels of Genes Associated with Type 2 Diabetes by PHS Supplementation. A, Mice were fed with ND or HFD supplemented or not with PHS, and glucose tolerance tests were performed as previously described. After a 16-h fast, the mice were intraperitoneally injected with 2 g/kg body weight of glucose. The glucose levels were measured in blood collected from the tail vein at the indicated times. B, RNA was isolated from epididymal adipose tissue of mice fed with HFD supplemented or not with PHS. The experimental conditions for real-time PCR and the primers used have been described elsewhere. Values are presented as the mean ± SD of 6–7 mice/group. The p value was calculated by one-way ANOVA with the Tukey-Kramer post-test for multiple comparisons (*p* < 0.05 and **p** < 0.01).
Adsorption and Metabolism of Dietary Phytosphingosine


