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Dietary Sphingolipids Ameliorate Disorders of Lipid Metabolism in Zucker Fatty Rats

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Dietary sphingolipids (SL) inhibit colon carcinogenesis, reduce serum cholesterol, and improve skin barrier function and are considered to be "functional lipids". For comparative determination of the effects of SL with different chemical compositions on lipid metabolism and its related hepatic gene expression, Zucker fatty rats were fed pure sphingomyelin (SM) of animal origin and glucosylceramide (GC) of plant origin. After 45 days, the SM and GC diets led to significant reductions in hepatic lipid and plasma non-HDL cholesterol. Both SM and GC diets decreased plasma insulin levels, whereas only the GC diet increased the plasma adiponectin level. Hepatic gene expression analysis revealed increased expression of adiponectin receptor 2 (Adipor2), peroxisome proliferator-activated receptor alpha (PPARα), and pyruvate dehydrogenase kinase 4 (Pdk4). However, expression of stearoyl CoA desaturase (Scd1) was significantly decreased. These results suggest that dietary SL, even of different origins and chemical compositions, may prevent fatty liver and hypercholesterolemia through improvement of adiponectin signaling and consequent increases in insulin sensitivity.

KEYWORDS: Sphingolipids; sphingomyelin; glucosylceramide; Zucker rat; metabolic disorder; gene expression

INTRODUCTION

Food consumption induces leptin secretion from adipose tissue to suppress the intake of excess energy. However, chronic consumption of high-energy diets leads to the accumulation of lipids in nonadipose tissues and increased leptin secretion. The establishment of obesity results in hyperleptinemia, which induces leptin resistance (*1−3*). Hypertrophic adipose cells increase oxidative stress (*4*) and induce excess fatty acid secretion (*5*). Moreover, insulin and adiponectin resistance caused by obesity also induce various metabolic disorders, such as fatty liver and hyperlipemia, due to stimulation of lipogenesis in the liver (*6*, *7*). Although metabolic syndrome can be treated by lifestyle improvements, such as daily meals and exercise, people often seek solutions in routine drug and supplement use.

Sphingolipids (SL), a ubiquitous component of eukaryote cell membranes, consist of ceramide as a basic frame. Animal and plant cell membranes generally possess sphingomyelin (SM, ceramide phosphorylcholine) and glucosylceramide (GC), respectively, as major neutral SL. Following oral administration, these SL are hydrolyzed by intestinal enzymes into sphingoid base and fatty acid via ceramide (*8−10*). It was reported that absorption of 8-unsaturated sphingoid bases derived from plant GC is inferior to that of 4-*trans*-sphingenine (8-saturated type) derived from animal sources (*11*, *12*). Although many questions remain regarding nutritional requirements for SL, studies in experimental animals have shown that consumption of SL inhibits colon carcinogenesis (*13−16*), reduces serum cholesterol (*9*, *17*, *18*), and increases high-density lipoproteins (*9*). It has also been reported that dietary plant GC can improve skin barrier function (*19*, *20*). These suggest that SL are "functional lipids". Therefore, we evaluated whether dietary pure SM and GC, with completely different chemical compositions, show positive effects on lipid metabolism in Zucker fatty rat with leptin functional disorder.

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MATERIALS AND METHODS

Preparation and Detection of Highly Purified Sphingolipids. Animal SM was prepared from the skin of old laying hens according to a modification of the method described previously (*21*). We used chicken skin as a source of SM, as it has a high content of SM (*21*) and is available in large quantities from old laying hens (culled chicken). Chicken skin was collected from laying hens (Enu-Chicken Agricultural Union Corp., Chiran-cho, Japan) and minced. The minced skin was defatted with

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special superheated steam (hi-LOHS-R; Taiyo Seisakusyo, Hakodate, Japan) and lyophilized. After extraction with ethanol, pure SM was prepared according to the method described previously (*21*). GC material prepared from corn was a kind gift from Nippon Flour Mills Co. Ltd. (Atsugi, Japan) and further refined in our laboratory. Briefly, acetone and hexane/acetone precipitations (*22*) were carried out to remove glycerolipids to obtain a mixture fraction of sterylglucoside (SG) and GC. Then, ethanol precipitation was performed to separate the two glycolipid classes. This treatment effectively divided GC (ethanol-soluble) and SG (ethanol-insoluble). SM and GC were confirmed by comparison with HPLC retention time of standard SM (from bovine brain) and GC (lower spot from bovine brain), purchased from Sigma (St. Loius, MO), and analyzing the chemical composition by GC-MS, as described below. The purity was determined by calibration curve of standard compound. Normal-phase HPLC analysis was performed according to the method described by Mawatari et al. (*23*). TLC conditions were chloroform/methanol/water (65:25:4, v/v) for SM detection and chloroform/methanol (95:12, v/v) for GC detection. Detection was conducted by spraying 50% sulfuric acid followed by heating. SL composition was analyzed as reported previously (*24*). Composition (mol %) of fatty acid and sphingoid base was calculated as peak area/molecular weight.

Animals and Diets. Male Zucker rats, 5 weeks old, were purchased from Japan SLC. Eighteen rats were fed AIN-76 (Japan Clair, Tokyo, Japan) for 1 week (*25*) and divided into three groups of six rats. The animal room was kept at a constant temperature of 23 ± 1 °C, and the relative humidity was kept at 65 ± 5%. Room lighting consisted of 12 h periods of light and dark.

A normal diet based on AIN-76 (control group) and diets plus 0.5 wt % SM (SM group) and 0.5 wt % GC (GC group) were used as experimental diets. The other dietary ingredients were all diluted by the addition of SL. The rats were given the diets for 45 days. The final mean intakes of SL, calculated from the daily intake of diets and the body weight, were 350 mg/kg/day in the SM and GC groups.

Experimental Design. At the end of feeding trials, all rats were fasted for 24 h before being anesthetized with diethyl ether. After the abdominal cavity had been opened, blood was collected from the heart with a heparinized syringe and put into ice-cold tubes. Then, the livers were removed and weighed. A part of the livers was incubated in an RNA later (Takara Bio, Ohtshu, Japan) for the night at 4 °C before being stored at −80 °C. The residual livers were frozen with liquid nitrogen and stored at −80 °C. The plasma was separated by centrifugation at 1000*g* for 20 min at 4 °C and stored at −80 °C until required for analysis.

All aspects of the experiment were conducted according to the Guidelines for Experimental Animals of the Obihiro University of Agriculture and Veterinary Medicine.

Hepatic Lipid Analyses. Total lipids (TL) were extracted from the liver according to the method of Folch (*26*). Cholesterol content of TL was measured using a Cholesterol-E-test (Wako Pure Chemical Industries, Osaka, Japan). The inorganic phosphorus content of TL was determined according to the method of Bartlett (*27*). Fatty acid methyl esters were prepared from TL and analyzed with GC as described previously (*21*).

Determination of Plasma Components. Plasma triglyceride, cholesterol, HDL-cholesterol, phospholipid, and glucose were determined using a biochemical autoanalyzer (AU640 Olympus; Sapporo Clinical Laboratory, Sapporo, Japan). Free fatty acid (FFA) was determined using the NEFA-C-test (Wako Pure Chemical Industries, Osaka, Japan). Plasma insulin was determined by ELISA (Morinaga, Yokohama, Japan). Plasma adiponectin was determined by ELISA (AdipoGen, Incheon, South Korea).

Gene Microarray Analysis. Total RNA from the liver was prepared using an RNeasy Mini Kit in accordance with the manufacturer's instructions (Qiagen, Tokyo, Japan). For oligo DNA microarray analysis, RNA samples from groups given SL were used as controls. 3D-Gene Rat Oligo chip 12k (Toray Industries Inc., Tokyo, Japan) was used (12034 distinct genes). For efficient hybridization, this microarray has three dimensions; that is, it is constructed with a well as the space between the probes and cylinder stems with 70-mer oligonucleotide probes on the top. Total RNA was labeled with Cy3 or Cy5 using an Amino Allyl MessageAMP II aRNA Amplification Kit (Applied Biosystems, Foster City, CA). The Cy3- or Cy5-labeled aRNA was pooled in hybridization buffer and hybridized for 16 h according to the supplier's protocols (http://www.3d-gene.com). Hybridization signals were scanned using a ScanArray Express Scanner (PerkinElmer, Waltham, MA) and processed using GenePixPro version 5.0 (Molecular Devices, Sunnyvale, CA). Detected signals for each gene were normalized by a global normalization method (Cy3/Cy5 ratio median = 1).

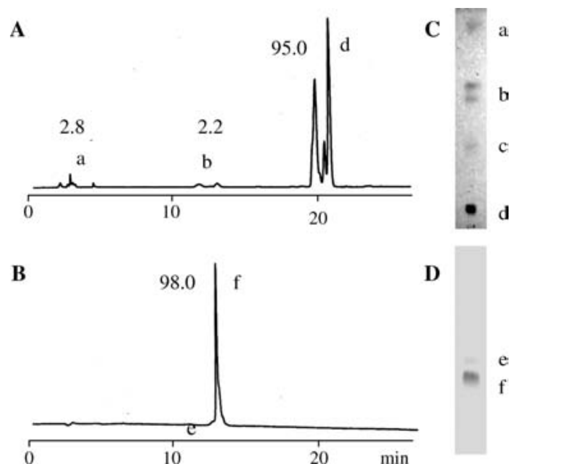

Figure 1. Confirmation of the purity of SM (**A**) and GC (**B**) by HPLC analysis and detection of SM (**C**) and GC (**D**) by TLC analysis. The values shown indicate percent of each peak area. Lower case letters indicate ceramide (a), glycosylceramide (b), sulfatide (c), sphingomyelin (d), sterylglucoside (e), and glucosylceramide (f).

Statistical Analysis. Values are means ± SD. The significance of differences between the control group and groups fed SL were determined by ANOVA using Excel-Toukei 2008 (SSRI, Tokyo, Japan) as previously described (*28*), and $p < 0.05$ was taken to indicate significance. In microarray analysis, gene expression significance was assessed by ANOVA as previously described (*28*).

RESULTS

Chemical Compositions of Sphingolipids. SM and GC were analyzed by HPLC and TLC (**Figure 1**). The purity of SM was 95%, and other minor components were GC, free ceramide, and sulfatide (cerebroside sulfate), which generally consisted of SL (**Figure 1A,C**). The purity of GC was 98%, which showed one spot on TLC (**Figure 1B,D**).

The chemical compositions of SM and GC are shown in **Table 1**. Chicken skin SM consisted of 16 fatty acids from C14:0 to C25:1, with C16:0 being the predominant type followed by C18:0 and C24:1. The acyl composition with shorter chain length, compared to those reported previously, will depend on the individual differences among chickens (*21*). The major sphingoid base was 4-*trans*-sphingenine (d18:1^{4t}), which is generally observed in mammals.

Corn GC consisted of 10 α-hydroxy fatty acids, with C20:0 as the predominant type followed by C24:0 and C18:0. The sphingoid base was rich in 8-*cis*-sphingadienine (d18:$2^{4t,8c}$), 8-*trans*-sphingadienine (d18:$2^{4t,8t}$), and 4-hydroxy-8-*cis*-sphingenine, similar to the data reported previously (*11*).

Body and Organ Weights and Hepatic Lipid Profiles. Significant differences were not observed among test groups in initial (control group, 119 ± 9 g; SM group, 119 ± 6 g; GC group, 122 ± 8 g) or final body weights (control group, 407 ± 3 g; SM group, 411 ± 4 g; GC group, 407 ± 16 g).

Lipid profiles of the liver are shown in **Table 2**. Total lipid content was 26.3 g/100 g for the control group, but decreased significantly by 24.3 and 17.5% for SM and GC groups, respectively. Phosphorus content derived from phospholipids was increased in a manner related to the decrease in total lipid content. There were no significant differences in cholesterol contents of the

Table 1. Fatty Chain Composition (Mole Percent) of the Experimental Sphingolipids[a]

fatty chain	SM	GC
acyl group	nonhydroxy	α-hydroxy
C16:0	44.1	3.0
C18:0	20.1	12.9
C18:1	3.8	
C20:0	4.1	50.8
C22:0	6.8	10.8
C22:1	0.2	
C23:0	1.8	0.6
C24:0	6.0	18.0
C24:1	8.3	
C24:2	1.8	
C26:0		2.5
others[b]	3.0	1.4
sphingoid base[c]		
d18:0	1.7	0.1
d18:1^{4t}	98.3	0.2
d18:1^{8c}		0.3
d18:1^{8t}		0.3
d18:2^{4t,8c}		72.0
d18:2^{4t,8t}		13.4
t18:0		0.6
t18:1^{8c}		12.6
t18:1^{8t}		0.5

[a] Values are means of three independent experiments. [b] Others consist of 14:0, 17:0, 19:0, 21:0, 25:0, and 25:1 for SM and 19:0, 21:0, and 25:0 for GC. [c] d, dihydroxy base; t, trihydroxy base.

Table 2. Hepatic Lipid Content and Fatty Acid Composition (Mole Percent) in Zucker Rats Fed SM and GC[a]

variable	control	SM	GC
TL (g/100 g of liver)	26.3 ± 1.2	19.9 ± 1.4**	21.7 ± 1.6**
P (mg/g of TL)	3.4 ± 0.3	4.8 ± 0.2***	4.2 ± 0.4*
14:0	1.6 ± 0.2	1.7 ± 0.1	1.5 ± 0.1
16:0	42.9 ± 0.4	41.4 ± 1.0*	40.0 ± 0.7***
16:1(n-7)	9.8 ± 0.3	9.9 ± 0.2	10.1 ± 0.7
18:0	4.5 ± 0.3	5.2 ± 0.2*	5.1 ± 0.6
18:1(n-9)	31.2 ± 1.3	28.9 ± 1.2	30.4 ± 0.2
18:1(n-7)	2.5 ± 0.2	2.5 ± 0.0	2.5 ± 0.1
18:2(n-6)	4.3 ± 0.6	5.4 ± 0.4*	5.7 ± 0.4**
20:4(n-6)	2.1 ± 0.3	3.2 ± 0.3**	2.9 ± 0.2**
22:6(n-3)	0.4 ± 0.1	0.8 ± 0.1**	0.7 ± 0.1**
others[2]	0.8 ± 0.1	1.0 ± 0.1	0.9 ± 0.1
n-6	6.6 ± 0.8	9.0 ± 0.7*	9.0 ± 0.4**
n-3/n-6	0.07 ± 0.01	0.09 ± 0.01**	0.08 ± 0.01

[a] Values are means ± SD ($n = 6$). TL and P indicate total lipid and inorganic phosphorus contents, respectively. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$ (versus control group).

liver (data not shown). With regard to component fatty acids, both SL diets had significantly lower palmitic acid and increased levels of polyunsaturated fatty acids (PUFA), such as linoleic, arachidonic, and docosahexaenoic acids, compared to the control group. The ratio of n-3/n-6 fatty acids was significantly increased in the SM group.

Plasma Lipid, Insulin, and Adiponectin Levels. Analytical data of plasma lipids are shown in **Table 3**. SM and GC diets significantly lowered plasma total cholesterol levels by 23 and 27%, respectively, compared to the control group. The decrease in total cholesterol was caused by the decrease in the level of non-HDL cholesterol. Plasma phospholipids were also decreased in the SM and GC groups. There were no significant differences in plasma FFA and TG levels between the groups.

Table 3. Plasma Concentrations of Lipids, Glucose, Insulin, and Adiponectin in Zucker Rats Fed SM and GC[a]

variable	control	SM	GC
total cholesterol (mg/dL)	181.5 ± 11.2	139.5 ± 9.4**	133.3 ± 4.2***
non-HDL-cholesterol (mg/dL)	97.5 ± 12.4	66.0 ± 9.4**	54.0 ± 2.3***
TG (mg/dL)	171.4 ± 80.8	164.0 ± 38.9	191.5 ± 33.7
PL (mg/dL)	287.8 ± 24.7	243.8 ± 14.7*	253.0 ± 11.4*
FFA (mequiv/dL)	2.3 ± 1.5	1.6 ± 0.3	1.6 ± 0.3
glucose (mg/dL)	160.2 ± 14.3	169.8 ± 24.2	149.5 ± 38.1
insulin (ng/mL)	14.6 ± 1.9	11.5 ± 0.9*	7.5 ± 2.3**
adiponectin (μg/mL)	27.5 ± 1.6	27.2 ± 4.9	34.6 ± 0.9***

[a] Values are means ± SD ($n = 6$). *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$ (versus control group).

The plasma insulin level was high in the control group (14.5 ng/mL), whereas it was significantly decreased by 21% for the SM group and by 49% for the GC group (**Table 3**). The adiponectin level was increased only in the GC group. Fasting blood glucose levels were not significantly different between groups.

Hepatic mRNA Expression Analysis. DNA microarray analysis was carried out using the liver total RNA to analyze the changes in expression of lipid metabolism-related genes induced by dietary SL. With regard to cholesterol metabolism-related genes, the expression of Hmgcs1 was suppressed, but that of Hmgcr was not altered (**Table 4**). The expression of Pdk4, which inhibits the conversion of pyruvate to acetyl-CoA, was increased, and the effect was significant in the SM-fed groups. However, Cyp7a1 expression was suppressed in both SL-fed groups. With regard to cholesterol transport-related genes, Lrp4 expression was increased only in the SM groups, but was not altered in the GC groups. The expression of Apob, a core component of VLDL and LDL, was significantly suppressed, whereas Apoa5, a component protein of HDL, was increased. The plasma adiponectin level was increased (**Table 3**), and the level of expression of its receptor, Adipor2, was also increased. Moreover, expressions of PPARα, which is involved in adiponectin signaling, and β oxidation-related genes, such as Cpt2 and Acox3, which are involved in metabolism downstream of PPARα signaling, were also increased. Mlycd expression was increased. Expression of Acacb, a fatty acid synthesis-related gene, was suppressed in both SL-fed groups. Scd1 expression was significantly suppressed. Gluconeogenesis-related genes were not generally changed, except for the slight suppression of Fbp2.

Expression of Hk3, which catalyzes the initial phosphorylation of glucose in the glycolytic pathway, was significantly increased, although expression of the liver-specific gene, Gck (*29*), was hardly changed. The results indicated that there were little changes in glucose metabolism-related genes overall.

DISCUSSION

We evaluated the effects of two types of dietary SL with different chemical compositions, which were prepared from animal and plant sources, on lipid metabolism in a rat model of leptin resistance. The results showed that dietary SL prevents fatty liver and lowers plasma cholesterol levels. In contrast to our expectations, a number of common features were observed in dietary animal and plant SL-fed rats.

As actual meals eaten by humans contain both SM and GC as cell membrane components (*9*, *30*), which form a food matrix together with many other food components, the biological use of the pure SL used in this study may be different from intact SL in meals (*31*). Following oral administration, SM from chicken skin is partly converted to sphingosine and long-chain fatty acids via ceramide (**Table 1**). Moreover, it is considered that GC from corn

Table 4. Expression Change of Hepatic Genes Relevant to Lipid Metabolism[a]

gene name	accession no.	description	SM	GC	
		Lipid Metabolism			
Hmgcr	NM_013134.2	3-hydroxy-3-methylglutaryl-CoA reductase	1.1	1.0	ns
Hmgcs1	NM_017268.1	hydroxymethylglutaryl-CoA synthase	0.5	0.4	**
Pdk4	NM_053551.1	pyruvate dehydrogenase kinase isozyme 4	4.3	1.7	**
Cyp7a1	NM_012942.1	cytochrome P450 7A1	0.3	0.2	**
Lrp4	NM_031322.1	LDL receptor-related protein 4	2.2	0.9	†
Abcg1	NM_053502.1	ATP-binding cassette subfamily G, member 1	1.5	0.8	†
Abcg5	NM_053754.2	ATP-binding cassette subfamily G, member 5	2.8	2.4	*
Abcg8	NP _569098.2	ATP-binding cassette subfamily G, member 8	1.2	1.4	**
Lip1	NM_012732.3	lysosomal acid lipase	1.9	1.3	**
Acacb	NM_053922.1	acetyl-coenzyme A carboxylase β	0.6	0.5	†
Scd1	NM_139192.1	stearoyl-CoA desaturase 1	0.1	0.2	**
LXRα	NM_031627.1	liver X receptor α	0.5	0.7	**
Apoa5	NM_080576.1	apolipoprotein A5	1.5	1.7	*
Apob	NM_019287.1	apolipoprotein B	0.4	0.4	**
Adipor1	NM_207587.1	adiponectin receptor 1	1.3	1.0	ns
Adipor2	NM_001037979.1	adiponectin receptor 2	1.8	2.1	**
PPARα	NM_013196.1	peroxisome proliferator-activated receptor α	2.1	3.9	*
Cpt1a	NM_031559.1	carnitine *O*-palmitoyltransferase 1	1.4	0.9	ns
Cpt2	NM_012930.1	carnitine *O*-palmitoyltransferase 2	2.1	1.9	**
Acox3	NM_053339.1	acyl-coenzyme A oxidase 3	1.7	1.6	*
Mlycd	NM_053477.1	malonyl-CoA decarboxylase	1.6	1.9	**
		Gluconeogensis/Glycolysis			
Fbp2	NM_053716.1	fructose-1,6-bisphosphatase isozyme 2	0.6	0.6	*
Pck1	NM_198780.3	phosphoenolpyruvate carboxykinase	1.1	1.1	ns
G6pc3	NM_176077.3	glucose-6-phosphatase, catalytic, 3	0.8	0.9	ns
Hk3	NM_022179.2	hexokinase-3	2.0	3.3	**
Gck	NM_012565.1	glucokinase	1.5	1.4	**

[a] Data denote ratio (Cy3/Cy5) of global normalized value, signifiantly different between SL diet groups and control diet group at *, $P < 0.05$, and **, $P < 0.01$; †, $P < 0.1$; ns, not significant.

is partly converted to 4,8-sphingadiene and α-hydroxy fatty acids via ceramide (*11*). The extent to which various sphingoid bases are absorbed in vivo and reused in the epithelial cells is not still clear. In addition, the bioavailability of α-hydroxy fatty acids, specific components of sphingolipids as well as sphingoid base, is also not clear. However, it seems that these metabolites exert some actions in the intestinal tract and/or after being absorbed. Especially, as only GC elevated plasma adiponectin level, the existences of 8-unsaturated sphingoid bases and/or α-hydroxy fatty acids (plus ceramide) derived from GC not SM may exert some influence on adipose cells and promote adiponectin secretion.

SL administration prevented fat accumulation in the liver and increased the proportion of PUFA. This may be responsible for the suppression of de novo fatty acid synthesis from acetyl-CoA to reduce hepatic TG and not the promotion of PUFA synthesis from essential fatty acids. In fact, hepatic gene expression analysis suggested that a series of fatty acid metabolism-related gene alterations, such as suppression of acetyl-CoA synthesis by the increase in Pdk4 expression, suppression of malonyl-CoA synthesis from acetyl-CoA (Acac), promotion of decarboxylation of malonyl-CoA by the increase in Mlycd expression, and suppression of unsaturation from stearic acid to oleic acid by suppression of Scd1 expression, could contribute to improvement of the liver lipid profile. The suppression of Scd1 gene expression may increase insulin sensitivity and be effective for the prevention of obesity and in the treatment of various metabolic disorders (*32*). The promotion of adiponectin secretion in the GC group and adiponectin signaling by the increase in Adipor2 expression in GC and SM groups, with consequent increases of PPARα expression, may be responsible for normalization of lipid metabolic disorders (*33*, *34*). Adipor2 is expressed specifically in the liver, whereas Adipor1 is expressed in skeletal muscle (*35*). Malonyl CoA, the expression of which was transcriptionally suppressed, is an inhibitor of carnitine palmitoyl transferase (Cpt) (*36*). Furthermore, as activated PPARα increases Cpt activity (*37*), promotion of lipolysis in the liver may also contribute to prevention of fatty liver. It was assumed that suppression of sterol regulatory element binding protein-1c (SREBP-1c) expression via inactivation of LXR regulated the transcription of lipogenic genes in the liver (*38*), because the plasma cholesterol level was decreased. However, SREBP-1c was not detected because of its low level of Cy3 expression in the samples.

Plasma cholesterol, particularly non-HDL cholesterol, was significantly decreased by SL diets, although expression of Hmgcr was not altered. The expression of Cyp7a1, one of the enzymes contributing to plasma cholesterol level, was suppressed, which was related to the decrease in LXRα expression (*39*). LXRα also regulates the expression of Abcg5/Abcg8, which is a cholesterol excretion-related gene and a heterodimer expressed in the liver as well as the small intestine (*40*). However, Abcg5 expression was significantly increased in both SL-fed groups. Duivenvoorden et al. (*18*) referred to the plasma lipid lowering effect of SM resulting from complex formation in the intestinal tract between the positively charged primary amine of sphingoid base and fatty acid and between SL and cholesterol. Recently, it was reported that SM with longer acyl chains from C22 to C24 could more easily be excreted into feces without being hydrolyzed than C16 and C18 types (*41*). As chicken skin SM consists of some very long chain acyls as well as unsaturated types, these strongly suggest that a part of dietary SM, which was not hydrolyzed in the intestinal tract, influenced the bile salts profile and cholesterol reabsorption in the small intestine (*18*, *42*, *43*).

With regard to other possible mechanisms, metabolic improvements starting from acetyl-CoA nodes, such as inhibition of acetyl-CoA synthesis (Pdk4) and HMG-CoA synthesis (Hmgcs1), as described above, may contribute to the decreases in plasma cholesterol levels. Pdk4 is a key enzyme in lipid and glucose metabolism and regulates metabolism by switching the energy source from glucose to lipid (*44*, *45*). Pdk4 plays a role in the inhibition of glycolysis and promotion of gluconeogenesis to supply glucose in the fasting state. Moreover, the Pdk4 gene is known to be transcriptionally activated by ligand binding to PPARα (*46*). Therefore, it was suggested that dietary SL or their metabolites may activate PPARα with subsequent induction of Pdk4 gene expression to make use of lipids as an energy source, which resulted in the prevention of fatty liver and lowering of cholesterol level. It has been reported that sphingoid bases and not ceramide bind to PPARα (*47*). However, studies with Pdk4 knockout mice and Zucker diabetic fatty rats suggested that down-regulation of Pdk4 is effective for the treatment of type 2 diabetes (*48*, *49*). Therefore, overexpression of Pdk4 could lead to glucose metabolic disorder. This study revealed that dietary SL could induce normalization of adiponectin signaling followed by promotion of insulin sensitivity, as plasma insulin levels were decreased. This may improve glucose metabolism in the skeletal muscle and prevent worsening of blood glucose level. Moreover, the insulin-lowering effect of SL may have contributed to the increase in Pdk4 expression, as excess insulin inhibits Pdk4 (*50*).

In conclusion, dietary pure SL, regardless of type (i.e., from animal and plant sources), may ameliorate fatty liver, hypercholesterolemia, and insulin resistance through the promotion of adiponectin signaling.

ABBREVIATIONS USED

Abcg1, ATP-binding cassette, subfamily G, member 1; Abcg5, ATP-binding cassette subfamily G, member 5; Abcg8, ATP-binding cassette, subfamily G, member 8; Acacb, acetyl-coenzyme A carboxylase β; Acox3, acyl-coenzyme A oxidase 3; Adipor1, adiponectin receptor 1; Adipor2, adiponectin receptor 2; Apoa5, apolipoprotein A5; Apob, apolipoprotein B; Cpt1a, carnitine *O*-palmitoyltransferase 1; Cpt2, carnitine *O*-palmitoyltransferase 2; Cyp7a1, cytochrome P450 7A1; Fbp2, fructose-1,6-bisphosphatase isozyme 2; FFA, free fatty acids; G6pc3, glucose-6-phosphatase catalytic 3; GC, glucosylceramide; Gck, glucokinase; Hk3, hexokinase 3; Hmgcr, 3-hydroxy-3-methylglutaryl-coenzyme A reductase; Hmgcs1, hydroxymethylglutaryl-coenzyme A synthase, cytoplasmic; Lip1, lysosomal acid lipase; Lrp4, low-density lipoprotein receptor-related protein 4 precursor; LXRα, liver X receptor α; Mlycd, malonyl-CoA decarboxylase; P, inorganic phosphorus; Pck1, phosphoenolpyruvate carboxykinase; Pdk4, pyruvate dehydrogenase kinase isozyme 4; PL, polar lipids; PPARα, peroxisome proliferator-activated receptor α; PUFA, polyunsaturated fatty acids; Scd1, stearoyl-CoA desaturase 1; SG, sterylglycoside; SL, sphingolipids; SM, sphingomyelin; SREBP-1c, sterol regulatory element binding protein-1c; TG, triacylglycerol; TL, total lipids.

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