Oral Ingestion of *Aloe vera* Phytosterols Alters Hepatic Gene Expression Profiles and Ameliorates Obesity-Associated Metabolic Disorders in Zucker Diabetic Fatty Rats

Eriko Misawa,* Miyuki Tanaka, Kouji Nomaguchi, Kazumi Nabeshima, Muneo Yamada, Tomohiro Toida, and Keiji Iwatsuki

Functional Food Research Department, Food Science & Technology Institute, Morinaga Milk Industry Co. Ltd., Higashihara 5-1-83, Zama, Kanagawa 228-8583, Japan

ABSTRACT: We investigated the effects of the oral administration of lophenol (Lo) and cycloartanol (Cy), two kinds of antidiabetic phytosterol isolated from *Aloe vera*, on glucose and lipid metabolism in Zucker diabetic fatty (ZDF) rats. We demonstrated that the administrations of Lo and Cy suppressed random and fasting glucose levels and reduced visceral fat weights significantly. It was also observed that treatments with Lo and Cy decreased serum and hepatic lipid concentrations (triglyceride, nonesterified fatty acid, and total cholesterol). Additionally, Lo and Cy treatments resulted in a tendency for reduction in serum monocyte chemotactic protein-1 (MCP-1) level and an elevation in serum adiponectin level. Furthermore, the expression levels of hepatic genes encoding gluconeogenic enzymes (G6 Pase, PEPCK), lipogenic enzymes (ACC, FAS), and SREBP-1 were decreased significantly by the administrations of aloe sterols. In contrast, Lo and Cy administration increased mRNA levels of glycolysis enzyme (GK) in the liver. It was also observed that the hepatic β-oxidation enzymes (ACO, CPT1) and PPARα expressions tended to increase in the livers of the Lo- and Cy-treated rats compared with those in ZDF-control rats. We therefore conclude that orally ingested aloe sterols altered the expressions of genes related to glucose and lipid metabolism, and ameliorated obesity-associated metabolic disorders in ZDF rats. These findings suggest that aloe sterols could be beneficial in preventing and improving metabolic disorders with obesity and diabetes in rats.

KEYWORDS: *Aloe vera*, phytosterols, type 2 diabetes, obesity, gene expression, ZDF

■ INTRODUCTION

Obesity is a condition of an excess accumulation of body fat caused by an imbalance of energy intake and expenditure, in addition to genetic background. Obesity, especially visceral fat obesity, is strongly associated with the development of metabolic syndrome, which includes insulin resistance, type 2 diabetes mellitus, hypertension, dyslipidemia and cardiovascular disease.[1] The prevalence of obesity is one of the most serious health problems around the world.

Studies of obesity in the fields of food and nutritional sciences have focused on the search for extracts of herbal plants and/or functional food ingredients that can prevent or ameliorate excess body fat accumulation. The antiobesity effects of many kinds of herbal products and plant extracts have been studied. For example, tea catechins,[2] saponin of Korean red ginseng,[3] and curcumin[4] have been demonstrated to exhibit antiobesity effects in rodent models with high-fat-diet-induced obesity.

Aloe barbadensis Miller (*Aloe vera*), a member of the genus *Aloe*, is a kind of traditional medicinal plant belonging to the family Liliaceae.[5] Among 360 *Aloe* species, it is the most widely used in the manufacture of food and drink products, pharmaceuticals, and cosmetics. *Aloe* species have been used for their antitumor, anti-infection, anti-inflammatory, antioxidant, and laxative effects around the world.[6−8] Several clinical and experimental studies have demonstrated the hypoglycemic effects of *A. vera*.[9−11] Additionally, there are some reports that *A. vera*-derived extracts showed a preventive effect against insulin resistance[12] and a lipid-lowering effect.[13] From these observations,

it is expected that *A. vera* can be beneficial for the prevention or improvement of metabolic-syndrome-related disorders.

In our previous study, we confirmed the antihyperglycemic effect of *A. vera* gel and succeeded in identifying five minor phytosterols, namely, lophenol, 24-methyllophenol, 24-ethyllophenol, cycloartanol, and 24-methylenecycloartanol, as antidiabetic compounds.[14] The effects of compounds presenting chemical structures similar to that of aloe sterol have also been reported. For example, antidiabetic effects of 5-campestenone (24-methylcholest-5-en-3-on) and cholesterol-lowering activity of oryzanol have been shown.[15−17] We also investigated the effects of two kinds of aloe sterols [lophenol (Lo) and cycloartanol (Cy)] in Zucker diabetic fatty [ZDF; ZDF/Crl-Lepr^{fa} (fa/fa)] rats and found that the administrations of these aloe sterols improved hyperglycemia and glucose intolerance and reduced intra-abdominal fat accumulation.[18]

In the present study, we investigated the levels of serum adipocytokines and serum and hepatic lipid profiles and analyzed the expressions of hepatic-gene-related glucose and lipid metabolisms in ZDF rats, to address the mechanisms of antidiabetic and body-fat-reducing effects conveyed by aloe sterol ingestion.
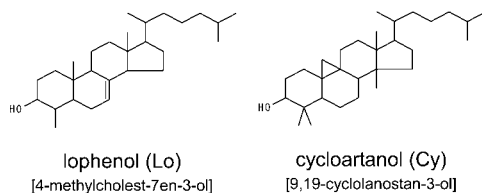
■ MATERIALS AND METHODS

Reagents. Lophenol (4-methylcholest-7-en-3-ol) and cycloartanol (9,19-cyclolanostan-3-ol) were isolated from *A. vera* (Figure 1).[14]

lophenol (Lo)
[4-methylcholest-7en-3-ol]

cycloartanol (Cy)
[9,19-cyclolanostan-3-ol]

Figure 1. Structures of lophenol (Lo, 4-methylcholest-7-en-3-ol) and cycloartanol (Cy, 9,19-cyclolanostan-3-ol).

These two compounds were dissolved in DMSO (Sigma-Aldrich, Tokyo, Japan) and the concentration was adjusted to 10 μg/mL with distilled water for the treatment sample. The final DMSO concentration was adjusted to 0.1%.

Experimental Animals. Six-week-old male Zucker diabetic fatty [ZDF/Crl-Lepr^{fa}(fa/fa)] rats and their lean littermates were purchased from Charles River Laboratories (Wilmington, MA). Rats were housed under a 12-h light/dark cycle with free access to food and water. They were given a high-fat diet containing 60 kcal % fat (D12492; Research Diets, NJ) throughout the study. The use of animals in this study was in accordance with a protocol that was approved by the Morinaga Milk Industry Co. Ltd. Animal Care Committee.

At 9 weeks of age, obese diabetic animals were divided into three groups [control, lophenol (Lo) and cycloartanol (Cy), n = 5 per group]. The ZDF rats were orally administered 1 mL of each solution of aloe sterols (25 μg/kg/day) once a day for 44 consecutive days. As a control, distilled water containing 0.1% DMSO was used. The rats had free access to food and water. The food intake of rats and their body weights were monitored during the treatment. The random and fasting blood glucose levels were measured at 0 (start), 3, and 5 weeks of treatments. The concentration of blood glucose was determined using a glucose meter (OneTouch Ultra; Johnson & Johnson, Tokyo, Japan). At the end of the experimental period (on the 45th day), the rats were anesthetized with diethyl ether after 18 h of fasting. Blood was obtained by cardiac puncture, and serum was prepared by centrifugation of blood at 1000g for 10 min at 4 °C and stored at −80 °C until analysis. Livers, intra-abdominal (epididymal, mesenteric and retroperitoneal) white adipose tissue (WAT), and gastrocnemius muscles were excised and weighed. Liver samples were frozen immediately with liquid nitrogen and stored at −80 °C until used for preparation of RNA and lipid.

Analytical Methods. Serum concentrations of triglyceride (TG), nonesterified fatty acid (NEFA), and total cholesterol (T-Cho) were determined using a kit obtained from Wako Pure Chemicals (Osaka, Japan). Serum insulin, leptin, and adiponectin levels were measured using ELISA kits (Revis insulin-rat kit, Revis leptin-rat kit and Revis high molecular adiponectin-mouse/rat kit; Shibayagi, Gunma, Japan). Serum monocyte chemotactic protein-1 (MCP-1) concentration was also determined using an ELISA kit (Biosource).

Liver lipids were extracted by homogenization in 1 mL of 2-propanol (Wako)/100 mg liver and then shaken for 45 min.[19] Samples were centrifuged at 3000g for 10 min, and the supernatant was assayed for TG, NEFA, and T-Cho contents using commercial kits (Wako).

Total RNA Preparation and Real-Time Quantitative RT-PCR. Frozen liver tissues were homogenized, and total RNA was isolated using Qiagen RNeasy mini kit (Qiagen Inc.). The quantity and quality of the RNA samples were assessed using Agilent Bioanalyzer (Agilent Technologies). The hepatic RNA sample was reverse-transcribed and quantitative PCR analysis was carried out with cDNA template using a 7500 Fast Real-time PCR system (Applied Biosystems).

The expressions of target mRNAs were measured using TaqMan methods (TaqMan Fast Universal PCR Master Mix, Applied Biosystems) or SYBR green dye (Fast SYBR Green Master Mix, Applied Biosystems). For TaqMan analysis, we used a set of predesigned primers and probes for each gene (TaqMan Gene Expression Assays, Applied Biosystems). The primer sets for target genes were glucose-6-phosphatase (G6 Pase) [G6pc; Rn00565347_m1], phosphopyruvate carboxykinase (PEPCK) [Pck1; Rn01529009_m1], acetyl-CoA carboxylase (ACC) [Acaca; Rn00573474_m1], fatty acid synthase (FAS) [Fasn; Rn00569117_m1], acyl-CoA oxidase (ACO) [Acox1; Rn00569216_m1], carnitine palmitoyl-transferase (CPT1) [Cpt1a; Rn00580702_m1], sterol regulatory element binding protein 1 (SREBP1) [Srepf1; Rn01495769_m1], SREBP2 [Srepf2; Rn01306296_m1], peroxisome proliferator-activated receptor alpha (PPARα) [Ppara; Rn00566193_m1], PPARγ; Pparg; Rn00440945_m1], peroxisome proliferator-activated receptor gamma coactivator 1 alpha (PGC-1α) [Ppargc1a; Rn00580241_m1], and β-actin [Actb; Rn00667869_m1]. For quantification of other genes using SYBR green, we purchased primer sets from Takara Bio Inc. (Shiga, Japan). The primer sets used were as follows: glucokinase (GK) [Gck; RA040941], hepatocyte nuclear factor-4 α (HNF4α) [Hnf4a; RA036661], pyruvate dehydrogenase kinase 4 (PDK4) [Pdk4; RA012286], and β-actin [Actb; RA006466].

The relative amounts of these mRNAs were normalized to the amount of β-actin mRNA.

Statistical Analysis. The results are presented as mean ± SEM. Two-tailed Student's t tests were used to compare between lean and obese ZDF-control groups. Among obese ZDF rats, the statistical differences were assessed by Dunnett's multiple comparison tests. Differences were considered to be significant when p values were less than 0.05.

■ RESULTS

Body Weight and Food Intake. Body weights of obese ZDF rats were significantly higher than those of lean littermates throughout the study (Table 1). After 6 weeks of treatment, the body weight gain in ZDF-control rats was 143.1 ± 7.8 g, versus 117.3 ± 3.4 g in the lean-control ($p < 0.05$). Among ZDF rats, body weights were not different between Lo and Cy administrations. Consistent with the results of body weight, lean rats showed lower food intake (Table 1). Additionally, there were no significant differences in the food intake of obese ZDF rats.

Hypoglycemic Effects of Lo and Cy. Figure 2 shows the changes in random (A) and fasting (B) blood glucose levels during the treatments in each group. In the lean animals, both random and fasting blood glucose concentrations remained within normal levels during the experiments. In contrast, untreated ZDF rats already showed mild hyperglycemia (random blood glucose levels of 200 mg/dL) at the beginning of the experiment, which slowly progressed. Compared with those in the ZDF-control group, both random and fasting blood glucose concentrations were suppressed markedly in the ZDF

Table 1. Body Weight and Food Intake of Male Zucker Diabetic Fatty (ZDF) Rats and Their Lean Littermates[a]

	lean	ZDF-control	ZDF + Lo	ZDF + Cy
initial body weight (g)	267.4 ± 4.0	371.9 ± 8.3**	372.6 ± 8.9	371.6 ± 6.6
final body weight (g)	386.8 ± 5.5	515.1 ± 14.2**	512.9 ± 12.2	524.3 ± 16.9
body weight gain (g/day)	2.79 ± 0.24	3.41 ± 0.19*	3.34 ± 0.11	3.64 ± 0.32
food intake (g/day)	15.9 ± 1.6	21.9 ± 1.5*	21.5 ± 1.6	22.5 ± 1.5

[a]Values are means ± SEM (n = 5). Significantly different from lean group at *$p < 0.05$ and **$p < 0.001$.

rats treated with Lo and Cy during the treatment period (Figure 2). After 5 weeks of treatment, random blood glucose
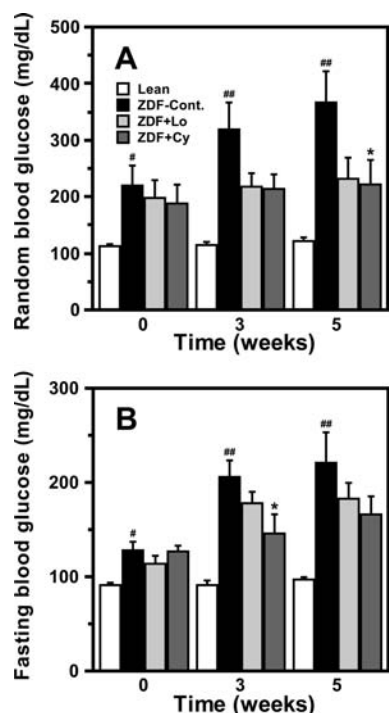
Figure 2. Effects of lophenol (Lo) and cycloartanol (Cy) treatments on random (A) and fasting (B) blood glucose levels in ZDF rats and lean rats. Blood glucose concentrations were monitored during the treatment. Values are means ± SEM ($n = 5$). Significantly different from lean group at $^{\#}p < 0.05$ and $^{\#\#}p < 0.01$. Significantly different from ZDF-control at $^{*}p < 0.05$.

levels in Lo- and Cy-treated rats were 39.6% and 37.2% lower than that in ZDF-controls, respectively (Figure 2A, $p < 0.05$ for Cy, Lo is not significant, but $p = 0.0537$). Consistent with blood glucose concentration, the HbA1c level of the ZDF-control group was significantly higher than that of lean rats, and it exhibited a lower value in both aloe sterol treated rats among the obese ZDF rats (lean, 2.6 ± 0.1; ZDF-control, 7.2 ± 0.7; Lo, 5.5 ± 0.5; Cy, 4.6 ± 0.4; $p < 0.05$ for Cy vs ZDF-control).[18]

Liver, Fat, and Muscle Weights. After 6 weeks of treatment, rats were sacrificed, and the absolute weights of liver, intra-abdominal (epididymal, mesenteric, and retroperitoneal) WAT, and gastrocnemius muscles were measured. Predictably, weights of livers showed a 1.99-fold increase and weights of all intra-abdominal adipose tissues a 2.85-fold increase in obese ZDF rats compared with those in the lean rats (Table 2). In contrast, the weights of gastrocnemius muscles in obese ZDF rats were reduced significantly compared with those in the lean littermates (by 26.7%, $p < 0.001$). Among ZDF rats, liver weights were slightly decreased in Lo- and Cy-treated rats. The total weights of intra-abdominal WAT were reduced by 27.7% and 26.3% in Lo- and Cy-treated groups, respectively, compared with those in the ZDF-control group. The weights of mesenteric and retroperitoneal WAT in the ZDF rats treated with Lo and Cy in particular were significantly lower than those of ZDF-control rats. Additionally, the weights of skeletal muscles were not affected by aloe sterol treatment.

Serum and Hepatic Lipids. As shown in Table 3, lipid (TG, NEFA, and T-Cho) concentrations in both serum and liver tissues of ZDF rats were significantly higher than those in the lean rats. Among ZDF rats, the serum TG levels in groups that underwent Lo and Cy treatments were 30.7% and 29.3% lower than those in the ZDF-control, respectively ($p < 0.05$ for both Lo and Cy). The concentrations of NEFA in serum were also reversed upon treatment with Lo and Cy (14.9 and 32.4%

Table 2. Tissue Weights of Male ZDF Rats and Their Lean Littermates[a]

tissue	tissue weight (g)			
	lean	ZDF-control	ZDF + Lo	ZDF + Cy
liver	10.14 ± 0.38	20.19 ± 1.13[##]	18.60 ± 1.30	19.28 ± 1.38
epididymal WAT	6.38 ± 0.46	12.80 ± 0.63[#]	11.77 ± 0.88	11.67 ± 0.39
mesenteric WAT	3.84 ± 0.33	6.82 ± 0.47[#]	4.48 ± 0.83*	3.70 ± 0.20**
retroperitoneal WAT	7.28 ± 0.48	30.33 ± 1.79[#]	20.55 ± 1.28**	20.76 ± 1.87**
total WAT	17.54 ± 1.24	49.95 ± 2.41[##]	36.79 ± 1.85**	36.13 ± 1.73**
gastrocnemius muscle	3.67 ± 0.22	2.69 ± 0.17[##]	2.83 ± 0.10	2.72 ± 0.09

[a]Values are means ± SEM ($n = 5$). Significantly different from lean group at $^{\#}p < 0.01$ and $^{\#\#}p < 0.001$. Significantly different from ZDF-control group at $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$.

Table 3. Serum and Hepatic Lipids of Male ZDF Rats and Their Lean Littermates

	lean	ZDF-control	ZDF + Lo	ZDF + Cy
		Serum		
TG (mg/dL)	138.5 ± 27.2	777.5 ± 97.6[#]	538.9 ± 81.7*	549.5 ± 63.2*
NEFA (mequiv/dL)	0.891 ± 0.063	3.421 ± 0.390[##]	2.910 ± 0.418	2.312 ± 0.288
T-Cho (mg/dL)	96.2 ± 7.0	245.6 ± 23.4[##]	229.7 ± 11.5	224.9 ± 20.5
		Liver		
TG (mg/g liver)	28.6 ± 4.3	105.6 ± 19.0[##]	56.2 ± 5.1*	58.2 ± 6.7*
NEFA (mequiv/g liver)	0.125 ± 0.006	0.535 ± 0.044[##]	0.344 ± 0.063	0.300 ± 0.026*
T-Cho (mg/g liver)	0.99 ± 0.47	5.48 ± 1.23[##]	3.32 ± 0.62	3.05 ± 0.26
[a]				

[a]Values are means ± SEM ($n = 5$). Significantly different from lean group at $^{\#}p < 0.05$ and $^{\#\#}p < 0.01$. Significantly different from ZDF-control group at $^{*}p < 0.05$.

decreases, respectively, $p < 0.05$ for Cy). However, serum T-Cho levels showed slight decreases upon Lo and Cy administrations. For the liver lipid concentrations, hepatic TG contents in Lo- and Cy-treated groups showed 46.5% and 44.9% reductions compared with those in the ZDF-control group ($p < 0.05$ for Lo and Cy). Similarly, hepatic NEFA levels were also decreased by Lo and Cy treatments (35.7% and 43.9% reductions, respectively, $p < 0.05$ for Cy). Moreover, reductions in T-Cho concentration were also observed in the liver of ZDF rats treated with Lo and Cy (39.4% and 44.3% reductions, respectively), whereas serum T-Cho levels did not show remarkable decreases by treatment with these aloe sterols.

Effects of Lo and Cy on Serum Parameters. Compared with lean rats, obese ZDF rats exhibited increases in serum insulin and leptin levels of 24.0- and 11.6-fold, respectively, whereas the concentrations of these serum parameters showed no marked differences among ZDF rats (Figure 3A,B). Similarly, the serum
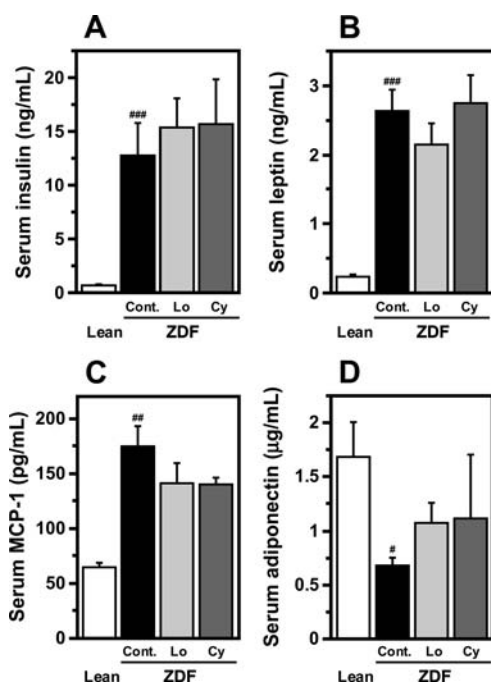
Figure 3. Effects of lophenol (Lo) and cycloartanol (Cy) on serum insulin (A), leptin (B), monocyte chemotactic protein-1 (MCP-1) (C), and adiponectin (D) in ZDF rats and lean rats. Values are means ± SEM ($n = 5$). Significantly different from lean group at [#]$p < 0.05$, [##]$p < 0.01$, and [###]$p < 0.001$.

MCP-1 levels in ZDF rats were 2.59-fold higher than those in the lean rats, but they tended to decrease in Lo- and Cy-treated ZDF rats (Lo, 0.85-fold; Cy, 0.83-fold; Figure 3C). In contrast, serum adiponectin levels were significantly higher in the lean rats compared with those in obese ZDF rats (lean, 2.51-fold; $p < 0.05$ vs ZDF-control; Figure 3D). Interestingly, treatments with Lo and Cy caused recovery of serum adiponectin concentrations, and 1.60- and 1.65-fold increases were observed, respectively, compared with those of ZDF-controls (Figure 3D).
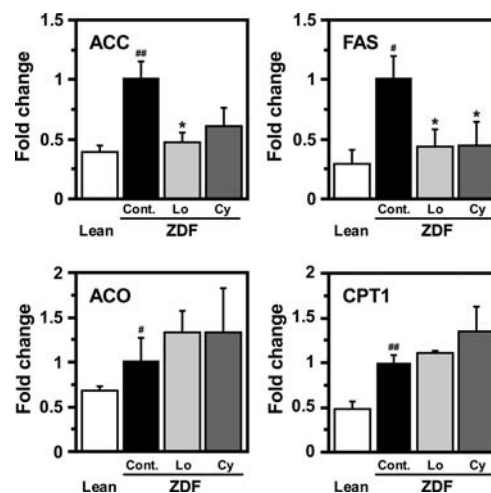
Effects of Lo and Cy on Hepatic Gene Expression. To investigate the effects of aloe sterols on glucose and lipid metabolism, changes in hepatic gene expressions in obese ZDF rats were assessed. The expressions of enzymes related to glucose metabolism (G6 Pase, PEPCK, GK, and PDK4) were investigated (Figure 4). In obese ZDF rats, the expression of the
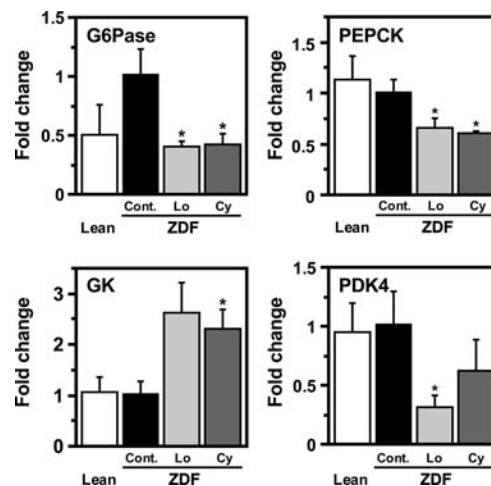
Figure 4. Effects of lophenol (Lo) and cycloartanol (Cy) treatments on expression of hepatic genes related to glucose metabolism. Values are means ± SEM ($n = 4$). Significantly different from ZDF-control at *$p < 0.05$.

G6 Pase gene showed a 2.04-fold increase, but the expressions of genes PEPCK, GK, and PDK4 were not changed compared with those of their lean littermates. Among ZDF rats, Lo and Cy administrations resulted in 2.59- and 2.27-fold increases in the expression of glycolysis (GK), respectively. Conversely, liver expressions of G6 Pase (Lo, 0.37-fold, $p < 0.05$; Cy, 0.43-fold, $p < 0.05$ vs ZDF-control) and PEPCK (Lo, 0.66-fold, $p < 0.05$; Cy, 0.69-fold, $p < 0.05$ vs ZDF-control) were decreased significantly by aloe sterol treatments (Figure 4). Additionally, PDK4 (Lo, 0.31-fold, $p < 0.05$; Cy, 0.31-fold) levels in the liver of Lo- and Cy-treated rats were also reduced compared with those of ZDF-control rats.

In Figure 5, we show the expressions of enzymes involved in lipid metabolism. ZDF rats had significantly higher levels of

Figure 5. Effects of lophenol (Lo) and cycloartanol (Cy) treatments on expression of hepatic genes related to lipid metabolism. Values are means ± SEM ($n = 4$). Significantly different from lean group at [#]$p < 0.05$ and [##]$p < 0.01$. * Significantly different from ZDF-control at $p < 0.05$.

mRNA for enzymes involved in fatty acid synthesis (ACC and FAS) and lipid catabolism (ACO and CPT1) than lean rats. Among ZDF rats, the expression levels of ACC (Lo, 0.47-fold,

$p < 0.05$; Cy, 0.61-fold vs ZDF-control) and FAS (Lo, 0.43-fold, $p < 0.05$; Cy, 0.44-fold, $p < 0.05$ vs ZDF-control) in Lo- and Cy-treated groups were lower than those in the ZDF-control rats. In contrast, the expressions of ACO (Lo, 1.33-fold; Cy, 1.33-fold) and CPT1 (Lo, 1.09-fold; Cy, 1.34-fold) demonstrated slight increases upon aloe sterol administration.

Furthermore, we addressed the expression of nuclear transcriptional factors (Figure 6). The expression of SREBP1 in
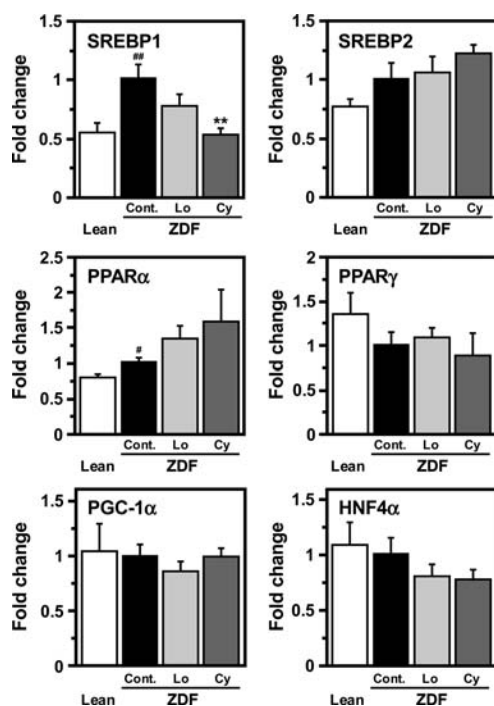

Figure 6. Effects of lophenol (Lo) and cycloartanol (Cy) treatments on expression of hepatic genes encoding transcriptional factors. Values are means ± SEM ($n = 4$). Significantly different from lean group at $^{\#}p < 0.05$ and $^{\#\#}p < 0.01$. Significantly different from ZDF-control at $^{**}p < 0.01$.

obese ZDF rats was increased significantly when compared with that in the lean rats. Among ZDF rats, liver SREBP1 (Lo, 0.77-fold; Cy, 0.52-fold, $p < 0.01$ vs ZDF-control) level was reversed by the treatment with aloe sterols among ZDF rats (Figure 6). The expression of PPARα in ZDF-controls was higher than that in their lean littermates. In contrast, both Lo and Cy administrations elevated the level of PPARα (Lo, 1.33-fold; Cy, 1.54-fold) gene expression compared with that in ZDF-controls (Figure 6). The SREBP2 level in ZDF rats was higher than that in lean rats, and it was slightly increased by Lo and Cy administrations (Figure 6). A modest decrease was observed in the expression of PPARγ in ZDF rats compared with that in lean rats, but there was no remarkable change among ZDF rats (Figure 6). The expression levels of PCG-1α and HNF4α did not exhibit obvious differences between lean and ZDF rats. Among ZDF rats, hepatic gene expressions for PCG-1α were slightly (Lo, 0.85-fold) and rarely (Cy, 0.98-fold) reduced, and those for HNF4α (Lo, 0.82-fold; Cy, 0.80-fold) showed lowering tendencies (Figure 6).

■ DISCUSSION

ZDF rats are animal models of type 2 diabetes mellitus, with a mutation in their leptin receptor. They spontaneously develop severe obesity, hyperglycemia, hyperlipidemia, and insulin resistance.[20] In this study, we examined the effects of Lo and Cy, antidiabetic phytosterols isolated from *A. vera*, on obesity-related metabolic disorders in ZDF rats.

We demonstrated improvements in hyperglycemia by Lo and Cy administrations without changes in body weights and food intakes in ZDF rats (Figure 2 and Table 1). Moreover, lower fat pad weights in mesenteric and retroperitoneal tissues were observed in aloe-sterol-treated ZDF rats (Table 2). There was no significant difference in skeletal muscle weight among the three groups of ZDF rats. It is suggested that aloe sterols suppress intra-abdominal fat accumulation without reducing skeletal muscle mass. In our previous study, we also performed oral glucose tolerance test (OGTT) and confirmed that the administration of aloe sterol could partially ameliorate glucose intolerance of ZDF rat.[18] Metabolic syndrome, an inducible state of cardiovascular diseases, is characterized by visceral fat obesity.[1,21] According to these observations, we considered that the ingestion of aloe sterol might be beneficial for the prevention of metabolic syndrome by suppressing visceral fat accumulation. In addition, we examined the lean littermates of ZDF rats fed with a 60% high-fat diet. They developed diet-induced obesity (DIO) (at 15 weeks of age, body weights of lean rats fed high-fat diet and normal diet were 386.8 ± 5.5 and 349.5 ± 6.5 g, respectively); however, the high-fat-diet-fed lean rats did not exhibit hyperglycemia and insulin resistance.[18]

Next, we measured the lipid (TG, NEFA, and T-Cho) concentrations of serum and liver of ZDF rats in a fasting condition. Since the levels of serum and hepatic lipids were decreased in aloe-sterol-treated groups, it is suggested that treatments with Lo and Cy could improve hyperlipidemia and hepatic steatosis (Table 3).

In recent reports, it has been demonstrated that chronic inflammation in WAT by infiltrating macrophages may contribute to the development of insulin resistance in obese diabetic animals.[22] Adipose tissue macrophages (ATMs) secrete proinflammatory cytokines, such as leptin, resistin, MCP-1, IL-6, and TNF-α, which lower insulin sensitivity. In contrast, adiponectin produced by adipocytes has anti-inflammatory and insulin-sensitizing effects.[23] Thus, we assessed the serum levels of adipocytokines. As shown in Figure 3, a reduction in MCP-1 and an increase in adiponectin were observed in serum of ZDF rats that underwent Lo and Cy treatments. As with serum MCP-1, serum insulin and leptin concentrations are significantly elevated in obese ZDF rats, compared with those in lean littermates. However, we observed that insulin and leptin levels were not changed by aloe sterol treatment among ZDF rats. Generally, young ZDF rats (5−7 weeks of age) exhibit normal fasting glucose with slightly elevated blood insulin levels and β-cells are increased 2-fold in ZDF rats compared with those of the age-matched lean controls. Then, they become progressively more hyperglycemic and insulin-resistant as circulating blood insulin levels are elevated and finally decreased by 20 weeks with pancreatic β-cell dysfunction.[20] Although we did not evaluate the islet morphology, it is considered that the β-cells of ZDF rats at 15 weeks of age would become hyperplasic in the middle phases of overt diabetes. We speculate that treatment of aloe sterol could improve hyperglycemia; however, hyperinsulinemia caused by time-dependent alteration of β-cells could not be reversed in ZDF rats.

Next, we performed gene expression analysis of liver tissue from ZDF rats in a fasting condition by quantitative real-time RT-PCR to evaluate the differences in lipid and carbohydrate metabolisms with aloe sterol ingestion.

First, we assessed the effect of aloe sterols on glucose metabolism. Our observations showed that the expression of GK, a member of the glycolytic pathway, was up-regulated in ZDF rats upon Lo and Cy administrations (Figure 4). In contrast, the expressions of gluconeogenic enzymes (G6 Pase and PEPCK) were reduced significantly by Lo and Cy treatments. From our results, it is considered that aloe sterols stimulated glucose catabolism and suppressed de novo glucose production. In addition, hepatic PDK4 expressions of aloe-sterol-treated rats were decreased compared with those of ZDF-control rats. PDK4 inhibits acetyl-CoA production from pyruvate catalyzed by pyruvate dehydrogenase complex (PDC), which is a process involving switching of the energy source from glucose to fatty acid. A previous report showed that PDK4 deficiency lowers blood glucose and improves tolerance and insulin sensitivity in DIO mice.[24] Therefore, we speculated that the reduction in PDK4 partially contributed to the hypoglycemic effect of aloe sterols in ZDF rats.

Second, we analyzed the expression of enzymes related to lipid metabolism. We found that expression levels of lipogenic enzymes (ACC and FAS) were significantly suppressed in ZDF rats upon Lo and Cy treatments (Figure 5). Additionally, we confirmed that the expressions of Elovl6 (long-chain fatty acid elongase family member 6) and SCD1 (sterol-CoA desaturase 1) showed similar patterns (data not shown). Previous studies on ACC, FAS, Elovl6, and SCD1 indicate that endogenous fatty acid synthesis is crucial for energy metabolism and insulin sensitivity in liver.[25−28] Our findings are consistent with these observations. Moreover, the mRNA expressions of ACO and CPT1 were elevated in ZDF rats upon Lo and Cy treatment; however, these changes were not significant (Figure 5). From our observations, it is suggested that Lo and Cy administrations induced down-regulation of fatty acid synthesis and a tendency for up-regulation of fatty acid oxidation in the liver. This phenomenon was considered as one of the reasons for the reduction in intra-abdominal fat and improvement of hyperlipidemia and liver steatosis in aloe-sterol-treated ZDF rats.

Third, we analyzed the expression of transcriptional genes. Wickert and Pfeiffer summarized the molecular network, such as transcriptional factors that link hepatic lipid and glucose metabolism.[29] In this review, they focused on some of the key molecules that may provide therapeutic targets for dietary or pharmacological interventions. Peroxisome proliferator-activated receptors (PPARs) are ligand-activated transcription factors that play an important role in lipid metabolism, and three subtypes (α, δ, γ) have been identified with different tissue-specific expression. PPARα, highly expressed in liver, stimulates β-oxidation by controlling their target genes (ACO and CPT1, etc.).[30] In contrast, SREBP1 is well-known as a regulator of the synthesis of fatty acids such as ACC and FAS.[31] Kamisuki et al. reported that fatostatin, an inhibitor of SREBP, blocked increases in body weight, blood glucose, and hepatic fat accumulation.[32] As we expected, expression of SERPB1 was reduced and PPARα level was elevated by Lo and Cy administrations in ZDF rats (Figure 6). A recent report showed that enhancement in the liver SREBP1/PPARα ratio, a condition that may favor lipogenesis over fatty acid oxidation, is associated with obesity, insulin resistance, and steatosis.[33] This observation is well-matched with our results that the SREBP1/PPARα ratio was decreased and metabolic-syndrome-related disorders were improved by the administration of aloe sterols to ZDF rats. Furthermore, we have preliminary data that the expression of LXRs (liver X receptors) was reduced in the livers of DIO mice treated with phytosterols. Since LXRs

modulate the expression of SREBP1, the reduction of LXR induced by phytosterol treatment might down-regulate SREBP1 genes.

In this study, we did not observe changes in PGC-1α and PPARγ expressions by aloe sterol treatment. We only assessed the gene expression in the liver, so the changes in PPARγ expression may not have been detectable. PGC-1α, a transcriptional coactivator, regulates cellular energy metabolic pathways in various tissues.[34] The expression of PGC-1α in liver is relatively low compared with that in other tissues under normal conditions, but starvation induces hepatic PGC-1α gene expression. In type 2 diabetes, PGC-1α activity is increased and glucose production is stimulated. There is a report that induction of gluconeogenic genes requires interaction of PGC-1α and HNF4α.[35] Additionally, hepatic gluconeogenic genes are also controlled by SREBP1 negatively via cross-talk with HNF4α interference with PGC-1α.[36] Our results showed that not only SREBP1 but also G6 Pase and PEPCK expressions were suppressed, whereas the expression of PGC-1α was not affected in ZDF rats upon aloe sterol administration. Because we found a reduction in HNF4α expression upon aloe sterol administration (Figure 6), the decrease in gluconeogenic enzymes may be partially caused by insufficient HNF4α.

In several studies, the changes in hepatic gene expressions by high-fat-diet feeding were reported.[37] For example, increased expression of genes, such as PPARα, SREBP1, ACO, CPT1, and ACC, and reduced expression of FAS, SCD, and others were confirmed in high-fat-diet-feeding animals compared with those in normal-diet-feeding animals. In the present study, all animals were fed with a high-fat diet and then analyzed in a fasting condition. Therefore, the changes in hepatic gene expressions that we observed should be considered the effects of high-fat-diet feeding and starvation.

Consistent with the modification of the transcriptional factor expression by aloe sterols, we have already found that Lo and Cy activated transcription of PPARs using a luciferase reporter assay.[38] Furthermore, in our preliminary experiment, we also confirmed that some orally administered Lo and Cy is absorbed by the small intestine and accumulated in the liver. Taking these observations together, we hypothesized that the absorbed phytosterols directly regulate the expression of transcriptional factors. Consequently, we supposed that the expression of transcription factors such as PPARα, SREBP1, and HNF4α might be regulated by aloe sterols initially, and subsequently, the expressions of lipolytic enzymes (ACO and CPT1), adipogenic enzymes (ACC, FAS, and SCD1), and gluconeogenic enzymes (G6 Pase and PEPCK) might be controlled secondarily.[29] In addition, Lo activated both PPARα and PPARγ, but Cy activated only PPARγ from our in vitro study.[38] However, we could not find prominent differences between Lo and Cy in our in vivo study. Further investigation will be needed to clarify the mechanisms of action of these compounds.

In conclusion, we demonstrated that oral ingestion of Lo and Cy prevented hyperglycemia, reduced hyperlipidemia, and improved liver steatosis and visceral fat accumulation in ZDF rats. Furthermore, we found that the expressions of gluconeogenic and lipogenic enzymes were suppressed, and the levels of enzymes related to glycolysis and lipolysis were increased in the livers of ZDF rats upon Lo and Cy treatments. The changes in these hepatic gene expressions were partially regulated by changes in transcriptional factors, such as SREBPs or PPARs. From our results, we predict that the administrations of Lo and Cy could prevent visceral fat obesity and improve

hyperglycemia, hyperlipidemia, and insulin resistance, among others. Furthermore, aloe sterols could be used as beneficial functional foods for the prevention of metabolic disorders accompanying diabetes and obesity.

■ AUTHOR INFORMATION

Corresponding Author

*Tel: 81-46-252-3070. Fax: 81-46-252-3049. E-mail: e_misawa@ morinagamilk.co.jp.

Notes

The authors declare no competing financial interest.

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