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ORIGINAL ARTICLE

Administration of phytosterols isolated from Aloe vera gel reduce visceral fat mass and improve hyperglycemia in Zucker diabetic fatty (ZDF) rats

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KEYWORDS
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Summary We examined the effects of lophenol (Lo) and cycloartanol (Cy), minor phytosterols of Aloe vera gel, in obese animal model of type II diabetes, Zucker diabetic fatty (ZDF) rats. Male ZDF rats were administered Lo and Cy at 25 μg/(kg day) daily for 44 days. Consecutive treatment of phytosterols suppressed the hyperglycemia, and random blood glucose levels after 35 days of treatment were 39.6 and 37.2% lower than the control, in Lo and Cy treatment groups, respectively. Consistent with the random blood glucose level, hemoglobin A1c (HbA1c) values of phytosterols treated rats were also lower than the control (Lo: 5.5 ± 0.8, Cy: 4.6 ± 0.7 vs. control: 7.2 ± 1.5). In the oral glucose tolerance test (OGTT) after 28 days of administration, the glucose intolerance was improved in phytosterols treatment groups. Additionally, the continuous administration of Lo and Cy also reduced the serum free fatty acid (FFA) and triglyceride (TG) levels except total cholesterol (T-Cho). Furthermore, the weights of total abdominal fat tissues were significantly lower than the control in ZDF rats with Lo (27.7%) and Cy (26.3%) treatment. These observations suggest that Aloe vera-derived phytosterols could reduce visceral fat accumulation, and would be useful for the improvement of hyperlipidemia and hyperglycemia.

Introduction

Aloe is a kind of traditional medical plant belonging to the family Liliaceae. Among 360 aloe species, *Aloe barbadensis Miller* (Aloe vera) is

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the most widely used in manufacture of food and drink products, pharmaceuticals, and cosmetics [1]. Aloe species have been used for their anti-tumor, anti-inflammatory, antioxidant, and laxative effects [2—4]. The anti-diabetic effects of Aloe have been also studied. There are some reports showed hypoglycemic effects of Aloe vera gel on streptozotocin-induced diabetic animals [5,6]. In the clinical trial, a research group of Mahidol University demonstrated the anti-diabetic activity of Aloe vera juice [7,8].

In our previous study, we demonstrated the anti-hyperglycemic effect of Aloe vera gel and identified five phytosterols, i.e., lophenol, 24-methyl-lophenol, 24-ethyl-lophenol, cycloartanol and 24-methylene-cycloartanol, as anti-diabetic compounds [9]. However, the effects of these compounds on the lipid metabolism have not been clear. Zucker diabetic fatty (ZDF; ZDF/Crl-Lepr^{fa}(fa/fa)) rats are used as type II diabetes model, and have a mutation in their leptin receptor and spontaneously develop obese, hyperglycemia, hyperinsulinemia and hyperlipidemia [10]. In this study, we administered two kinds of phytosterols (lophenol and cycloartanol) derived from Aloe vera gel to ZDF rats, in order to assess improving effects on hyperglycemia and hyperlipidemia.

Materials and methods

Reagents

Lophenol (4-methylcholest-7-en-3-ol) and cycloartanol (9,19-cyclolanostan-3-ol) were isolated from Aloe vera gel (Fig. 1) [9]. These two compounds are 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%.

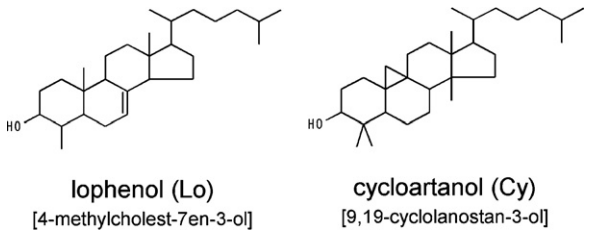

Figure 1 Structures of lophenol (Lo) and cycloartanol (Cy). Lo and Cy are phytosterols derived from Aloe vera gel.

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, USA). The obese animals were fed a high-fat diet containing 60 kcal% fat (Research Diets, New Brunswick, NJ, USA) throughout the study.

At 9 week of age, diabetic animals were divided into three groups (Control, Lophenol (Lo) and Cycloartanol (Cy), $n = 5$ per group). The ZDF rats were orally administered with 1 ml of each solution of anti-diabetic phytosterols (25 μg/(kg day)) once a day everyday with a sonde for 44 consecutive days. As control, distilled water containing 0.1% DMSO was used. During the treatment, body weight gain, food intake and random blood glucose levels were monitored. The concentration of blood glucose was determined using OneTouch Ultra (Johnson & Johnson company, Tokyo, Japan). On the 36th day from the start of the administration, hemoglobin A1c (HbA1c) levels were measured using DCA2000 system (Bayer Medical, Tokyo, Japan). On the 45th day, fasting rats were sacrificed and blood was collected by cardiac puncture. The intraperitoneal adipose tissues (epididymal fat, mesenteric fat and retroperitoneal fat) were excised as visceral fat and their weights were measured.

Glucose tolerance test

The oral glucose tolerance tests (OGTT) were performed in the fourth weeks after treatment begin (on the 29th day). Glucose at 1 g/kg of body weight was orally administered to ZDF rats fasted for 18 h overnight. Blood samples were obtained from the tail vein at times 0 (fasting levels), 15, 60, and 150 min after glucose administration, and the blood glucose concentration was immediately measured as above.

Serum measurements

Serum insulin concentration was measured by ELISA (Revis Rat Insulin kit, Shibayagi, Japan). Serum concentrations of free fatty acid (FFA), triglyceride (TG) and total cholesterol (T-Cho) were measured using a kit obtained from Wako Pure Chemicals (Osaka, Japan).

Statistical analysis

The results are presented as mean ± S.D. All statistical analyses were performed by Tukey—Kramer's

test. Differences were considered to be significant when p-values were less than 0.05.

Results

Hypoglycemic effects of Lo and Cy

Fig. 2 shows the changes in random blood glucose (A) during the treatments and the HbA1c (B) levels on the 36th day in each group. In the lean animals, the blood glucose concentrations were kept in normal levels during the experiments. In contrast, untreated ZDF rats already showed mild hyperglycemia (levels of 200 mg/dl) at the beginning of the experiment and slowly progressed. Compared to the ZDF-control group, the random blood glucose concentrations were suppressed in the rats treated with Lo and Cy during the experiments (Fig. 2A). On the 36th day from the start of the administration, random blood glucose levels were 39.6 and 37.2% lower than the ZDF-control rats, in groups of Lo and Cy treatments, respectively. The HbA1c level of ZDF-control group was significantly higher than that of lean rats. Among the ZDF rats, the HbA1c levels demonstrated lower value in both phytosterols treatment rats (Fig. 2B) (7.2 ± 1.5, 5.5 ± 0.8 and 4.6 ± 0.7, in control, Lo and Cy groups, respectively, $p < 0.05$ at Cy). These HbA1c data were consistent with the results of random blood glucose levels.

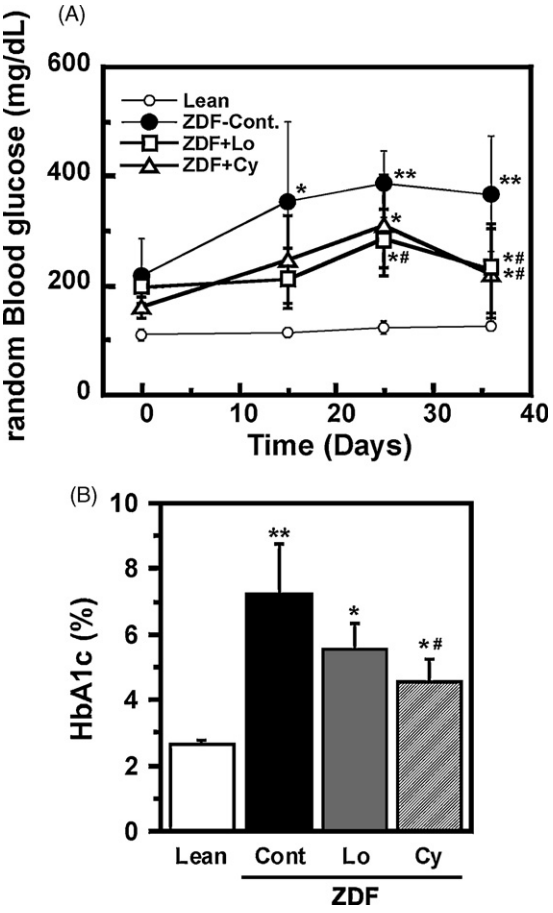

Figure 2 Effects of lophenol (Lo) and cycloartanol (Cy) on random blood glucose level (A) and HbA1c level (B) in Zucker diabetic fatty (ZDF) rats and lean rats. Random blood glucose levels were monitored during the treatment, and HbA1c levels were measured on the 36th day from the start of the administration. Values are means $\pm$ S.D. ($n = 5$). Significantly different from lean group at $^{*}p < 0.05$ and $^{**}p < 0.01$, respectively. $^{\#}$Significantly different from ZDF-control group at $p < 0.05$.

Glucose tolerance test

Following 28 days of phytosterols treatments, OGTT was performed. When compared to lean animals, the blood glucose levels were significant higher at all point during OGTT (Fig. 3A), and the blood glucose area under the curve (AUC) was also increased significantly in ZDF groups (Fig. 3C). Additionally, ZDF rats exhibited marked hyperinsulinemia (Fig. 3B). Among ZDF rats, blood glucose levels during OGTT were reduced in Lo- and Cy-treated rats at each time point (Fig. 3A). Particularly, at 150 min after glucose administration, the blood glucose levels in groups of Lo and Cy treatments indicated 71.8 and 72.5% of ZDF-control, respectively ($p < 0.05$ at Lo and Cy). As shown in Fig. 3B, initial blood insulin levels were not different among three groups of ZDF rats. However, higher insulin concentrations were observed in Lo- and Cy-treated groups after glucose loading (20.5 ± 6.8, 25.0 ± 1.8 and 25.4 ± 3.6 ng/ml in control, Lo and Cy groups, respectively at 15 min after glucose administration). In addition, the AUC of blood glucose was smaller in treated diabetic animals compared with ZDF-control rats (Fig. 3C) (46.9 ± 9.9, 36.3 ± 3.3, $34.1 \pm 5.1 \times 10^3$ mg/dl $\times$ min in control, Lo and Cy groups, respectively, $p < 0.05$ at Cy).

Effects of Lo and Cy on FFA and TG in serum

To clarify the effects of phytosterols on lipid metabolism, we measured the fasting serum FFA, TG and T-Cho levels in the ZDF rats after the consecutive treatments . It is well known that ZDF rats exhibit severe hyperlipidemia, and data of Fig. 4 indicates that all lipid parameters of ZDF rats were markedly higher than the lean rats. As shown in Fig. 4A, the serum FFA levels were 27.7 and 32.4% lower than ZDF-control rats in groups of Lo and Cy treatments, respectively ($p < 0.05$ at Cy). ZDF rats treated

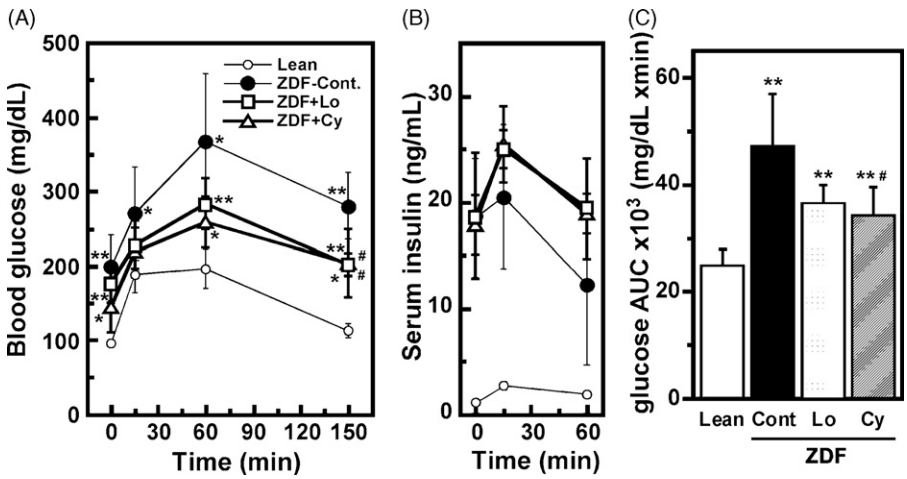

Figure 3 Effects of lophenol (Lo) and cycloartanol (Cy) treatment on blood glucose level (A) and serum insulin concentration (B) in Zucker diabetic fatty (ZDF) rats and lean littermates during oral glucose tolerance test (OGTT). Male ZDF rats were treated with Lo and Cy in ZDF rats at 4 weeks after beginning of administration. After oral administration of glucose (1 g/kg), the change of blood glucose level (A) and serum insulin concentration (B) was monitored, and the area under the curve of blood glucose (C) was calculated. Values are means ± S.D. ($n = 5$). **Significantly different from lean group at $p < 0.01$. #Significantly different from ZDF-control group at $p < 0.05$.

with Lo and Cy also exhibited 41.6 and 40.4% reduction in serum concentration of TG, compared with control rats ($p < 0.05$ at Lo and Cy, Fig. 4B). However, serum T-Cho levels were not affected by the phytosterol treatments (Fig. 4C).

Reduction of visceral fat mass by treatment of Lo and Cy

Next, we measured the absolute weights of epididymal fat, mesenteric fat and retroperitoneal fat as intraperitoneal visceral fat in ZDF rats with consecutive treatment of phytosterols. Predictably, all intraperitoneal adipose tissue was significantly increased in obese ZDF rats compared with the lean rats (Fig. 5). The weights of mesenteric fat and retroperitoneal fat in the ZDF rats treated with Lo and Cy were significantly lower than those of ZDF-control rats, but not the weights of epididymal fat. Compared with ZDF-control group, the total visceral fat masses were 27.7 and 26.3% lower in Lo- and Cy-treated groups, respectively. However, administration of Lo or Cy did not influence body weight gain in obese ZDF rats during the treatment (Table 1). Furthermore, whereas food intake of obese ZDF rats was significantly elevated compared with their lean littermates consistent with their greater body weights, there were no obvious differences in the average of food intake among the three groups of ZDF rats (Table 1). In addition, there were no adverse effects following the administration of Lo and Cy.

Discussion

In our previous report, we identified five minor phytosterols isolated from Aloe vera gel as the anti-diabetic compounds [9]. However, it was not clear the effects of these phytosterols on lipid metabolism. In the present study, to investigate the effects of these Aloe vera-derived phytosterols on both anti-diabetes and anti-hyperlipidemia, we administered Lo and Cy to male ZDF rats consecutively.

We observed that the random blood glucose levels and the HbA1c levels were significantly suppressed in phytosterol-administered rats compared with those in ZDF-control (Fig. 2A and B). As same with C57BL/KS-Lep$^{db/db}$ (db/db) mice [9], we demonstrated that the phytosterols obtained from Aloe vera gel have an ability to improve the hyperglycemia and control the blood glucose level consecutively in ZDF rats, too.

The result of OGTT showed that the glucose tolerances were apparently improved in ZDF rats with continuous treatment of Lo and Cy (Fig. 3A and C). Additionally, the serum insulin elevation was observed on OGTT in the Lo and Cy treatment groups, but this effect was not significant (Fig. 3B).

As shown in Fig. 4, administrations of Lo and Cy also reduced the serum FFA and TG levels, but not T-Cho. These results indicated that Lo and Cy might have improving effects of lipid metabolism. Moreover, it is considered that lowering effects

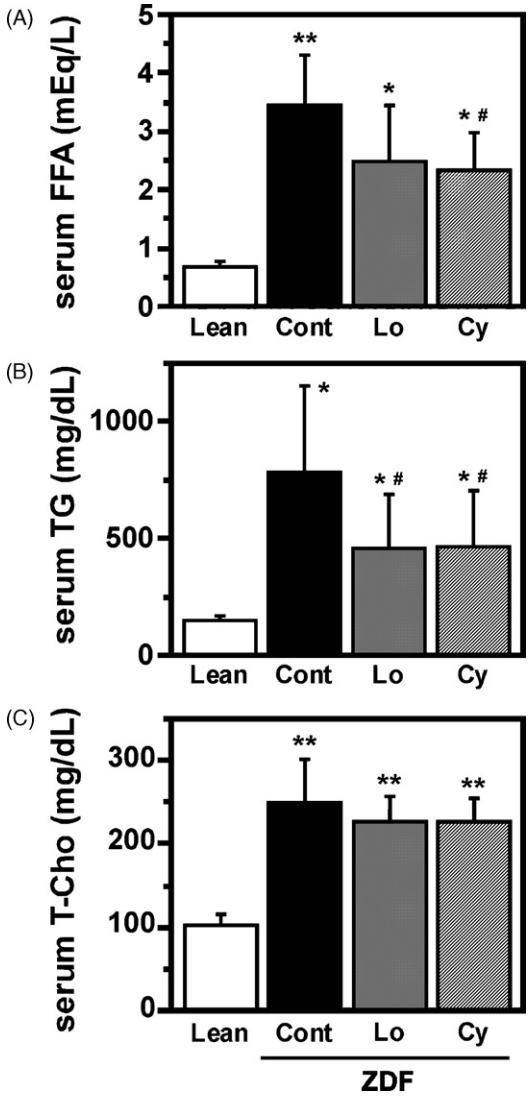

Figure 4 Effects of lophenol (Lo) and cycloartanol (Cy) treatment on serum free fatty acid (FFA) (A), triglyceride (TG) (B), and total cholesterol (T-Cho) (C) concentrations in Zucker diabetic fatty (ZDF) rats and lean rats. ZDF rats were administered cycloartanol and lophenol (25 μg/(kg day)) orally once a day everyday for 44 days. On the 45th day from the start of the administration, rats were sacrificed after overnight fasting and serum was collected. The concentrations of FFA, TG, and T-Cho in the serum were determined. Values are means ± S.D. ($n = 5$). **Significantly different from lean group at $p < 0.01$. #Significantly different from ZDF-control group at $p < 0.05$.

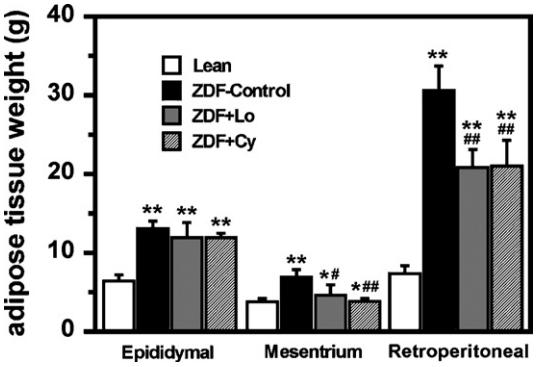

Figure 5 Absolute weights of abdominal adipose tissues of Zucker diabetic fatty (ZDF) rats and lean rats on the 45th day from the start of the administration. Values are means ± S.D. ($n = 5$). **Significantly different from lean group at $p < 0.01$. Significantly different from ZDF-control group at $^{\#}p < 0.05$ and $^{\#\#}p < 0.01$, respectively.

of serum FFA is beneficial for insulin sensitivity and insulin secretion, because FFA contributes the development of insulin resistance and promotion of pancreatic β-cell death [11—13].

Interestingly, the administrations of Lo and Cy significantly reduced abdominal fat such as mesenteric fat and retroperitoneal fat (Fig. 5) without a marked reduction in body weight gain (Table 1). Incidentally, reductions in visceral fat mass comprised only 2.65 and 3.55% of the body weight treatment with Lo and Cy, respectively. It was considered that the relative decrease in visceral fat mass was slight. Therefore, in ZDF rats, energy expenditure was probably stimulated by treatment with phytosterols [14], but food intake might have been sufficient excessive to prevent body weight reduction. Additionally, we have preliminary data that the phytosterols isolated from Aloe vera gel could reduce not only body fat (both abdominal fat and subcutaneous fat), but also body weight in diet-induced obesity (DIO) C57BL/6J mice (data not shown). However, ZDF rats are severe models of obesity and diabetes compared to DIO animals. So, we speculate that the dosage of Lo and Cy administered in this experiment might be insufficient to observe the changes of food intake and body weight. And we considered that higher dosage of phytosterols would be needed to detect the weight loss in the obese ZDF rat.

Recently, lifestyle-related diseases, such as diabetes mellitus, hyperlipidemia, hypertension, obese, etc. have been recognized as ''metabolic syndrome'' that an inducible state of cardiovascular diseases. And it is regarded that increase of visceral fat is one of the most crucial risk factors during the development of the metabolic syndrome [15—17]. Therefore, we suggest that these Aloe vera gel-derived phytosterols might be beneficial for the prevention of metabolic syndrome by suppressing visceral fat accumulation.

Several studies concerning the compounds possessing similar chemical structures with Lo or Cy have been also reported. For examples, 5-campestenone (24-methylcholest-5-en-3-on), an

Table 1 Body weight and food intake of male Zucker diabetic fatty (ZDF) rats and their lean littermates

	Lean	Control	Lophenol	Cycloartanol
Body weight (g)				
0 week	280.4 ± 12.8	371.9 ± 16.7***	372.6 ± 17.8***	371.6 ± 13.2***
2 weeks	328.8 ± 17.5	447.1 ± 27.6***	441.6 ± 21.9***	447.4 ± 22.6***
4 weeks	378.4 ± 16.7	488.4 ± 29.6***	479.7 ± 24.7***	490.3 ± 28.9***
Food intake (g/day)				
0 week	16.0 ± 0.3	23.3 ± 1.3*	21.6 ± 2.3*	22.0 ± 2.8*
2 weeks	15.2 ± 2.7	20.5 ± 1.6*	22.1 ± 0.4*	22.8 ± 0.6*
4 weeks	16.4 ± 1.7	21.9 ± 1.6*	20.8 ± 2.0*	22.9 ± 1.2*

Values are means ± S.D. ($n = 5$). Significantly different from lean group at *$p < 0.05$ and ***$p < 0.001$, respectively.

oxidized derivative of campesterol is chemically synthesized and its effects (0.3 and 0.6% dietary exposure) were studied both in db/db mice and ZDF rats [18,19]. Konno et al. showed that 5-campestenone has no effect of on blood glucose and insulin level, but decreased plasma HbA1c, T-Cho, TG and FFA, and improved the glucose tolerance in ZDF rats [19]. In this report, treatment with 5-campestenone increased insulin response on OGTT and reduced visceral fat mass in ZDF rats. Oryzanol is a group of ferulic acid esters of plant sterols and triterpenes, reported cholesterol lowing activity [20,21]. Rong et al. demonstrated that oryzanol (0.5 and 1% feeding) reduced plasma T-Cho level by reducing cholesterol absorption in hypercholesterolemic diet feeding hamsters [22]. Therefore, we expected the cholesterol-lowering effect of phytosterols isolated from Aloe vera gel also, but the administration of Lo and Cy did not affect serum T-Cho level in ZDF rats. We speculated the reason that the dosages of Lo and Cy in our experiment were very low (25 μg/(kg day)) compared with dietary feeding protocols. However, our data indicated that Lo and Cy has a potential of lipid-lowering, containing the reducing effects of TG and FFA by the quite low dose.

In the recent report, it was exhibited that 5-campestenone exerted pleiotropic effects through the activation of peroxisome-proliferator-activated receptor-α (PPAR-α) and the reduction of sterol element binding protein-1 (SREBP-1) (data not shown). It is known that PPAR-α regulates β-oxidation, and SREBP-1 controls fatty acid synthesis [23,24]. In our preliminary experiments, we analyzed the mRNA level of SREBP-1c in the liver by quantitative RT-PCR, and found that the expression of SREBP-1c was markedly reduced in the livers of Lo- and Cy-treated ZDF rats (data not shown). This finding suggested that the reduction of SREBP-1c might partially contribute to the anti-diabetic and lipid-lowering effects of phytosterols isolated from Aloe vera gel. Additionally, we confirmed that Lo activated both PPAR-α and PPAR-γ, and Cy activated PPAR-γ transcription using a luciferase reporter assay [25]. Therefore, it is considered that Lo and Cy work as PPARs ligand, and would be partially contribute to the effects on both glucose and lipid metabolism.

To clarify the detail mechanism of Lo and Cy, further investigation would be required to evaluate the effects of phytosterols derived from Aloe vera gel on the enzyme groups and their regulators (transcription factors) concerned in metabolism of both glucose and lipid, i.e. glycolysis, gluconeogenesis, glycogenolysis, lipolysis, lipogenesis, etc.

In conclusion, we demonstrated that Lo and Cy reduced blood glucose, serum FFA, and TG levels, and visceral fat accumulation in ZDF rats. From our results, we predict that the administration of Lo and Cy could prevent visceral fat obesity and improve hyperglycemia and hyperlipidemia. Furthermore, phytosterols derived from Aloe vera gel might be beneficial for the prevention of metabolic syndrome.

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