We previously found that passion fruit (Passiflora edulis) seeds contained a high amount of piceatannol (3,5,3',4'-trans-tetrahydroxystilbene), a natural analog of resveratrol (3,5,4'-trans-trihydroxystilbene). Resveratrol has been proposed as a potential anti-metabolic disorder compound, by its activation of sirtuin and AMP-activated protein kinase. Many reports show that resveratrol ameliorates diet-induced obesity and insulin resistance. However, it is not known whether piceatannol also affects diet-induced obesity. We explored the effect of piceatannol on high fat diet-fed mice. The results showed that piceatannol did not affect high fat diet-induced body weight gain or visceral fat gain in mice. However, piceatannol did reduce fasting blood glucose levels. Furthermore, to explore the potential of passion fruit seed extract containing piceatannol as a functional food, passion fruit seed extract was administered in a genetic diabetic mouse model (db/db mice). Single administration of passion fruit seed extract, as well as piceatannol reduced the blood glucose levels of these db/db mice. These results suggest that piceatannol and passion fruit seed extract may have potential application in the prevention of diabetes.

Key words piceatannol; passion fruit seed; antidiabetic activity; high fat diet; db/db
lights on at 7:00 a.m. After 14-d acclimatization to the laboratory conditions, 12-week-old mice (n=48) were divided into six groups according to their average body weight. They had free access to their respective food (normal diet [ND; AIN-93G; Oriental Yeast Co., Japan] or HFD [HDF32; Clea Japan Inc.]) and water. ND and HFD control groups (n=7–8) were administered 5 mL/kg control solution [0.5% CMC (Nacalai Tesque, Japan)] once a day for 5 weeks. Treatment groups in the HFD-fed mice (n=8 each) were administered each dose (1, 3, 10, or 30 mg/kg body weight) of piceatannol solution (8 mL/kg in 0.5% CMC) once a day for 5 weeks. Body weight and food intake were measured throughout the experiment. One
day before sacrifice, the mice were deprived of food. Blood was collected under ether anesthesia in tubes, the clots were allowed to retract for 30 min at room temperature, and clear serum was obtained by centrifugation. Serum glucose was measured using UV methods with hexokinase. Serum leptin levels were measured using an enzyme-linked immunoassay kit (Moriyag Institute of Biological Science, Japan). All animal experiments were performed in strict compliance with the Guidelines for Proper Conduct of Animal Experiments of the Science Council of Japan. The experimental protocols and procedures were approved by the Animal Experimental Committee of Morinaga & Co., Ltd. (165-1-001-01).

**Effect of PFSE and Piceatannol on Blood Glucose Levels in Diabetic Mice** A type II diabetic animal model (db/db mice) was used in this study. db/db mice develop obesity, elevated blood glucose levels, and increased or normal blood insulin concentration due to a genetic mutation in the leptin receptor gene. Nine-week-old male BKS.Cg-Dock7m+/-Leprdb/J mice were obtained from Charles River Japan. Animals were maintained under standard conditions (temperature 21.9–24.5°C, humidity 39–49%, 12:12-h light–dark cycle; lights on at 7.00 a.m.) They had free access to food (D08111509; Research Diet, Inc., Japan) and water. After 14-d acclimatization to the laboratory conditions, 11-week-old mice (n=32) were divided into four groups according to their average body weight and blood glucose levels. Mice of the control group (n=8) were administered 5mL/kg control solution (0.5% CMC (Wako Pure Chemical Industries, Ltd., Osaka, Japan)). Mice of the piceatannol group (n=8) were administered 50mg/kg piceatannol in 0.5% CMC. Mice of the PFSE group (n=8) were administered 5mL/kg PFSE (10mg/kg or 50mg/kg piceatannol in 0.5% CMC). After oral administration, the mice were deprived of food and blood was collected at 0, 1, 2, 3, and 4h. Blood samples were analyzed using a self-monitoring blood glucose instrument (FreeStyle Freedom, Nipro, Japan). All animal experiments were conducted in accordance with guidelines established by the Animal Care and Use Committee of the testing facility and were approved by this committee.

**Statistical Analysis** All data are expressed as mean±standard error of the mean (S.E.M.). Statistical analysis was performed using SPSS software (version 22). Statistical differences between the ND group and control HFD group were determined using the Student’s t-test. One-way ANOVA was performed to compare HFD groups receiving different piceatannol doses, and Dunnett’s test was performed for post-hoc comparisons. Statistical differences between the control db/db group and piceatannol group were determined using the one-sided Student’s t-test. One-way ANOVA was also performed to compare db/db groups receiving different PFSE doses, and Dunnett’s one-sided test was performed for post-hoc comparisons. A p-value of <0.05 or <0.01 was considered statistically significant.

**RESULTS**

**Effect of Piceatannol in HFD-Fed Mice** To explore the effect of piceatannol on metabolism, an HFD mouse model was used. Mice were fed an ND or HFD for 5 weeks. Although the food intake was significantly less in the HFD group, body weight, subcutaneous fat, visceral fat, blood glucose, and blood leptin of HFD-fed mice were significantly higher than those of ND-fed mice due to the higher energy content of the diet (Fig. 1). Gavage administration of piceatannol (1, 3, 10, or 30mg/kg body weight) once a day during HFD lording did not change body weight, subcutaneous fat, visceral fat, food intake, or blood leptin levels. Visceral fat was measured as the sum of epididymal, retroperitoneal, and mesenteric fat, and administration of piceatannol did not affect any of the fat types (data not shown). Only administration of piceatannol at a dose of 10mg/kg body weight significantly suppressed fasting blood glucose levels in HFD-fed mice compared to the control HFD-fed mice (p=0.020).

**Glucose-Lowering Effect of PFSE in db/db Mice** To explore the possibility of PFSE as a functional food, PFSE and piceatannol were administered to 11-week-old db/db mice. As shown in Fig. 2, oral administration of 0.5% CMC (control) increased blood glucose levels at 1h, which recovered at 2h, and then decreased over time. Single oral administration of piceatannol (50mg/kg body weight) reduced blood glucose levels 1h after administration in db/db mice compared to the control CMC-administered mice (p=0.040). Oral administration of PFSE (10mg/kg or 50mg/kg piceatannol) also reduced blood glucose levels 1h after administration in db/db mice compared to the control CMC-administered mice (p=0.215, 0.034, respectively), and this effect was maintained for up to 3h.

**DISCUSSION**

In this study, we examined the effect of piceatannol, an analog of resveratrol, on HFD-induced obesity. Administration of piceatannol lowered blood glucose levels, but did not affect body weight. Administration of PFSE with high levels of piceatannol as well as piceatannol reduced blood glucose levels in db/db mice. These results indicate that PFSE containing piceatannol shows potential for the treatment or prevention of diabetes mellitus.
The structure of piceatannol is very similar to that of resveratrol. Resveratrol has been reported to reduce the body weight of HFD-fed mice via AMPK activation, sirtuin activation, adipogenesis inhibition, or improvement of microbiota dysbiosis. Piceatannol is relatively less well studied, although one report showed that piceatannol inhibits adipogenesis of 3T3-L1 preadipocytes. Therefore, piceatannol is expected to also reduce the body weight of HFD-fed mice; however, there has been no in vivo study conducted to confirm this. In the present study, we showed that oral administration of piceatannol once a day did not change the body weight of HFD-fed mice. Administration of piceatannol did not affect the fat composition of HFD-fed mice. Taken together, these results suggest that in vivo administration of piceatannol did not reduce the body weight gain of HFD-fed animal.

In our study, administration of 10 mg/kg piceatannol lowered blood glucose levels of HFD-fed mice. Administration of 30 mg/kg piceatannol tended to lower blood glucose levels, though its effect was not statistically significant. The effects of resveratrol were observed at optimal doses. Cho et al. reported that supplementation of resveratrol at a low dose (0.005%) significantly suppressed body weight gain; however, the higher dose of resveratrol (0.02%) was not effective. Therefore, piceatannol may also have an optimal dose; however, this would warrant further investigation.

Although we were not able to determine the precise mechanism of the glucose-lowering effects in the present study, the fact that piceatannol showed a glucose-lowering effect in fasted condition indicates that its effects are not dependent on inhibition of glucose absorption. Piceatannol was suggested to lower blood glucose in db/db mice through AMPK activation, and our data do not conflict with this potential mechanism. Further study will be needed to reveal the precise mechanism underlying the glucose-lowering effect of piceatannol.

Passion fruit is consumed fresh or processed for juice worldwide. The seeds are often eaten together with the pulp in the natural state. The major polyphenol of PFSE is piceatannol, and the content of piceatannol in passion fruit seeds is greater than that found in other plants such as grape. It is reported that polyphenols-rich natural products may offer unique treatment modalities for various aspects of type 2 diabetes. The particular effect will depend on the structure of the compound, and stilbenes such as resveratrol and piceatannol are polyphenols that show the most promise for beneficial health effects. We previously investigated the absorption and metabolism of piceatannol in rats, and the area under the time–concentration curve for intact piceatannol was two-times higher than that of intact resveratrol. After administration of piceatannol, isorhapontigenin, an O-methyl piceatannol metabolite, was detected in the intact form in rat plasma. However, the specific effects of isorhapontigenin remain unknown. The second-most abundant polyphenol of PFSE is scirpusin B, which is a dimer of piceatannol. Scirpusin B is reported to repress increases in blood glucose after oral administration of glycogen through inhibition of α-amylase. Inhibition of α-glucosidase is a major mechanism of reducing blood glucose by foods. Isonaphtigenin and scirpusin B may contribute the antidiabetic effect of PFSE. PFSE containing high levels of piceatannol is expected to emerge as a new attractive anti-diabetic functional food. Further clinical study is warranted to clarify the potential of piceatannol and PFSE in the treatment and prevention of diabetes.

Conflict of Interest The authors declare no conflict of interest.

REFERENCES


