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Anti-hyperglycemic effects of plum in a rat model of obesity and type 2 diabetes, Wistar fatty rat

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ABSTRACT

Dried plums, considered a healthy food in the West and used as medicine in India, contain phenolic compounds with protective actions against age-related diseases. Effects of oral plum ekisu (concentrated juice) on lipid and glucose tolerance were assessed in insulin-resistant obese Wistar fatty rats. Plum ingestion decreased blood glucose ($P < 0.05$) and plasma triglyceride concentrations ($P < 0.01$) compared with controls. Plum treatment for 2 weeks reduced areas under the curve (AUCs) for glucose and insulin during a glucose tolerance test. In db/db mice, plum decreased these AUCs, and also blood glucose during an insulin tolerance test. Plum treatment significantly increased plasma adiponectin concentrations and PPARγ mRNA expression in adipose tissue from Wistar fatty rats. Plum thus may increase insulin sensitivity in these rats via adiponectin-related mechanisms.

The Asian plum and food products derived from it have been consumed regionally since ancient times. Currently, the plum is eaten throughout the world because of its possible health benefits. High concentrations of phenolic compounds contained in this fruit are believed to offer protection against various age-related diseases. Phenolic flavonoids possess antioxidative properties opposing low-density lipoprotein cholesterol (LDLc), lipid peroxidation (16), and also have been reported to scavenge free radicals (1, 22). In humans, dietary supplementation with nutrients rich in polyphenols, such as black or green tea (25), olive oil (3, 29), licorice root extract (8, 28), and red wine (9) was associated with increased resistance of plasma LDLc to oxidation and with an increase in plasma antioxidant capacity.

Type 2 diabetes is a leading cause of death in the developed world. Recently, flavonoids were reported to significantly reduce blood glucose in mice with streptozotocin-induced hyperglycemia. Intake of flavonoid was associated with reduced risk of type 2 diabetes (13). However, the Asian plum has undergone little investigation in this context.

The Wistar fatty (WF) rat was developed by crossbreeding obese Zucker (13 M strain, *fa/fa*) rats and Wistar Kyoto rats. WF (*fa/fa*) rats develop obesity and related metabolic characteristics, such as hyperinsulinemia and hyperlipemia, similarly to the obese Zucker rat. WF rats also show hypertension. Thus, WF rats are a good model of common human metabolic disorders. Male WF rats show marked hyperglycemia, glucosuria, and polyuria as early as 8 weeks of age. The present study was undertaken to assess the effect of the Asian plum on blood glucose, including underlying mechanisms, in obese di-

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abetic WF rats.

MATERIALS AND METHODS

Animals. Wistar (*fa/−*) rats were originally obtained from the breeding colony at the Biological Institute of Takeda Pharmaceutical Co. in June 1989. db/db (*db/db*) mice were obtained from CLEA JAPAN Inc. (Tokyo, Japan). All were maintained at the Laboratory Animal Facility, Yokohama City University School of Medicine. WF (*fa/fa*) rats and age-matched Wistar lean (WL) (*fa*/+ or +/+) rats (individually marked) were used. All had free access to water and standard rat chow pellets and were housed under controlled temperature (22 ± 1°C) and humidity (50% to 60%) with a photoperiod from 7 A.M. to 7 P.M.

Reagents. Plum ekisu, the fruit juice concentrate of Asian plum, was obtained from Minabegawa Yakuba (Wakayama, Japan). The detailed composition of plum-ekisu has been published (19).

Biochemical Measurements. Blood samples were obtained from the tail vein after overnight fasting. Samples were centrifuged, divided into aliquots, frozen, and later assayed for total cholesterol and triglyceride. Blood glucose concentrations were measured by a glucose oxidase method with a Beckman glucose analyzer. Plasma insulin levels were measured by ELISA (Morinaga, Yokohama, Japan) with rat insulin standards. Plasma adiponectin levels were also measured by ELISA (Otsuka Pharmaceutical Co., Tokyo, Japan) as described by the manufacturer.

Oral glucose tolerance test (OGTT). After overnight fasting, WF rats and db/db mice underwent an oral glucose tolerance test (OGTT) with a 1-g/kg body weight glucose feeding by gavage. Blood was drawn from a cut at the tip of the tail at 0, 30, 60, 90, and 120 min after the glucose feeding. Plasma glucose was immediately determined as above.

Intraperitoneal insulin tolerance test (ITT). Food was removed 2 h before the db/db mice were highly anesthetized with halothane, between 9 : 00 and 10 : 00 A.M. Insulin (0.075 units/kg) was administered by intraperitoneal injection, and blood samples were obtained from the orbital sinus at the time points indicated. Plasma glucose was immediately determined as above.

RNA Preparation and Northern Blot Analysis. Total cellular RNA was extracted from liver and adipose tissues of WF rats by a single-step method (4). Total RNA (10 μg) was size-separated by electrophoresis on a 1% agarose −1% formaldehyde gel and transferred to a Hybond-N^+ membrane (Amersham, Arlington Heights, IL) by a capillary transfer method in 20 × SSC (1 × SSC: 150 mmol/L NaCl and 15 mmol/L sodium citrate, pH 7.0). The membrane was cross-linked by UV transillumination. Rat peroxisome proliferator-activated receptor (PPAR)-α, PPAR-γ, or sterol regulatory element-binding protein 1c (SREBP-1c) cDNA were labeled with [α-^{32}P]dATP by a Prime-It Random Primer Labeling kit (Stratagene, La Jolla, CA) and used as a probe after heat denaturation. Prehybridization (2 h) and hybridization (overnight) were carried out at 65°C in 1 mol/L NaCl, 10% dextran, 1% SDS, and 0.1 mg/mL heat-denatured salmon sperm DNA (Trevigen Inc., Gaithersburg, MD). The membrane was washed with 2 × SSC for 10 min at room temperature and then washed with 0.2 × SSC, 0.1% SDS at 65°C for 30 min. The membrane was exposed to Kodak (Rochester, NY) XAR-5 film at −70°C.

Statistical analysis. Data are shown as means ± SE. Comparison between groups was performed by Student's t test. Repeated measures ANOVA was performed to compare glucose curves in OGTT and ITT. A difference at $P < 0.05$ was considered statistically significant.

RESULTS

To determine whether plum-ekisu affects body weight or food consumption, plum was mixed with water and given to WF rats. Figure 1 shows food consumption and body weight in WL and WF rats during the course of the study. WF rats consumed more chow at all time points than WL rats. Neither 0.25% nor 1% concentrations of plum-ekisu affected food consumption in WL rats. While 0.25% plum-ekisu caused no significant change in food consumption or weight in WF rats, 1% plum-ekisu had significantly decreased food consumption and weight in WF rats at 2 weeks of treatment compared with controls given water. To avoid a confounding effect of weight loss on blood glucose, we chose 0.25% plum-ekisu for further experiments.

Table 1 shows weights of rats and serum concentrations of total cholesterol (TC) and triglyceride (TG). Serum TC and TG were significantly higher in WF than WL rats throughout the treatment period

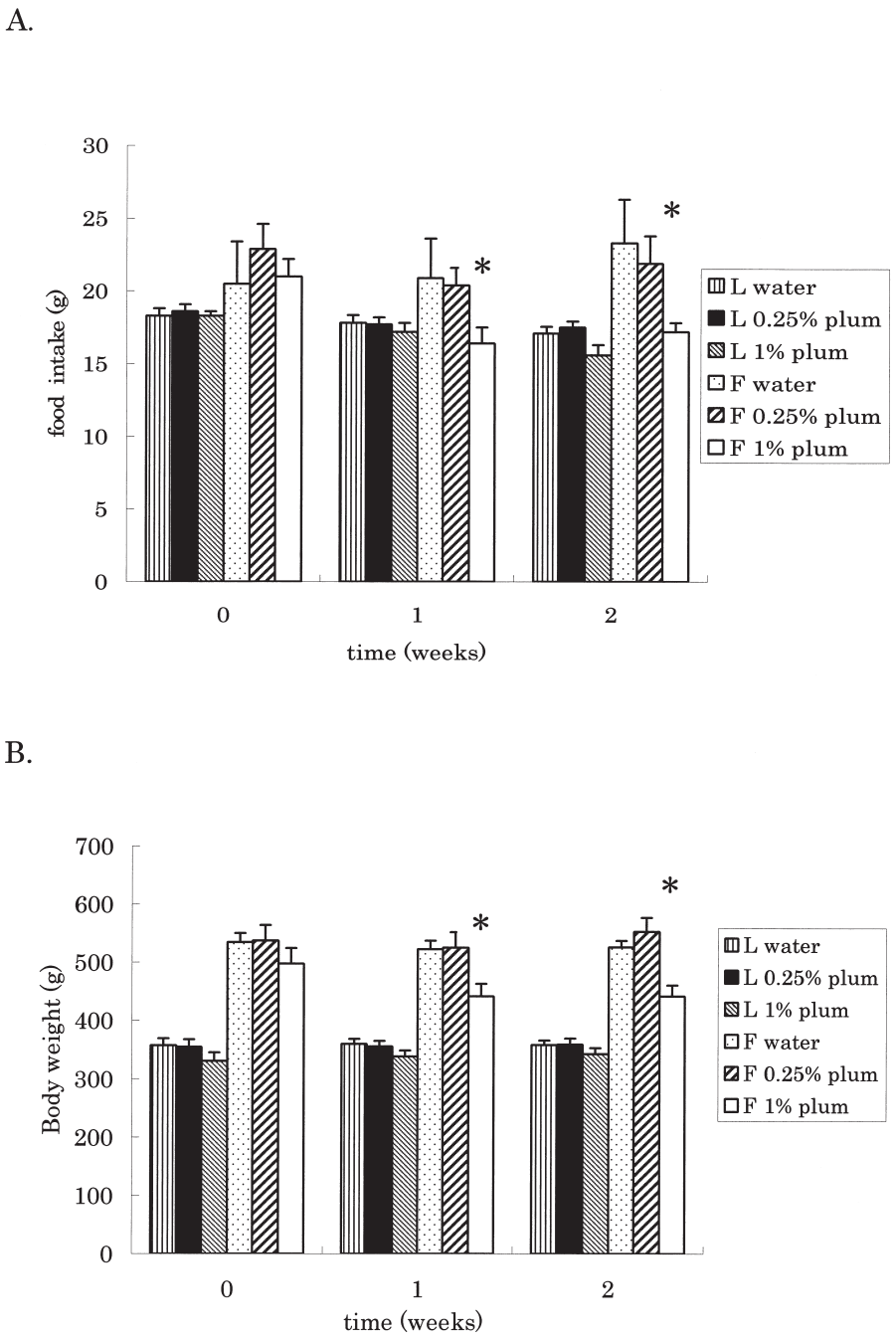

Fig. 1 Effect of different concentrations of plum-ekisu on food intake and body weight in Wistar lean and fatty rats. L, Wistar lean rats; F, Wistar fatty rats. $^{*}P < 0.05$ vs. water-treated Wistar fatty rats.

($P < 0.01$). In water-treated WF rats, TC had increased significantly after 2 weeks (from 3.7 ± 0.06 to 4.4 ± 0.06 mmol/L). No such increase was observed in plum-ekisu treated WF rats (from 4.0 ± 0.1 to 3.8 ± 0.1 mmol/L); the TC increase was suppressed significantly by treatment with plum-ekisu ($P < 0.05$). Serum TG also increased significantly in water-treated WF rats after 2 weeks, but the increase in TG was not suppressed significantly by treatment with plum-ekisu.

Oral administration of plum-ekisu to WF rats significantly reduced plasma glucose concentrations during the OGTT, and the integrated area under the plasma glucose curve (AUCs) was reduced by 24%

Table 1 *Effect of 0.25% plum-ekisu for 2 weeks on lipid profile*

	WL rat (*-/fa or ?*)		WF rat (*fa/fa*)	
	before	after	before	after
Body weight (g)				
Water	370 ± 21	371 ± 23	535 ± 31[a]	526 ± 28[a]
plum-ekisu	357 ± 18	359 ± 22	537 ± 54[a]	525 ± 28[a]
Total cholesterol (mmol/L)				
Water	2.5 ± 0.06	2.4 ± 0.06	3.7 ± 0.06[a]	4.4 ± 0.06[a]
plum-ekisu	2.3 ± 0.1	2.4 ± 0.11	4.0 ± 0.1[a]	3.8 ± 0.1[a,b]
Triglyceride (mmol/L)				
Water	0.7 ± 0.02	0.7 ± 0.01	4.7 ± 0.12[a]	7.2 ± 0.2[a]
plum-ekisu	0.8 ± 0.02	0.5 ± 0.02	3.6 ± 0.09[a]	3.6 ± 0.09[a]

Statistical significance: [a]$P < 0.01$ vs. WL rats, [b]$P < 0.05$ vs. water-treated WF rats.
WL: Wistar lean rat, WF: Wistar fatty rat

in plum-consuming WF rats compared with water-treated controls (Fig. 2A). Reduction of plasma glucose concentration in plum-treated WF rats was accompanied by a significant reduction in plasma insulin concentration at 60 min after glucose challenge ($P < 0.001$), compared with water-treated controls, with significance still evident at 120 min (Fig. 2B). The glucose-insulin index was significantly lower in plum-treated than water-treated WF rats, reflecting improved insulin sensitivity.

When we additionally examined the effect of plum-ekisu on glucose tolerance in diabetic db/db mice, AUCs for plum-treated mice also were significantly smaller than for water-treated mice (Fig. 3A). To evaluate more directly whether insulin resistance was decreased by treatment with plum, an intraperitoneal ITT was carried out in db/db mice. Plum-treated db/db mice exhibited significantly lower blood glucose during the ITT than water-treated db/db mice (Fig. 3B).

Plasma adiponectin concentrations are decreased in obese and type 2 diabetic human subjects with insulin resistance, and insufficient adiponectin has been implicated in insulin resistance (30). To investigate the mechanisms by which plum treatment lowered excessive blood glucose, we measured plasma adiponectin concentrations in WF rats before and after plum-ekisu administration. Treatment of WF rats with plum-ekisu increased plasma adiponectin concentrations significantly beyond those in water-treated animals (Fig. 4).

To investigate the mechanisms by which plum treatment decreased serum TG and increased plasma adiponectin concentrations, we assessed PPARα and SREBP-1c mRNA expression in the liver and PPARγ expression in adipose tissue. As shown in Fig. 5, treatment of WF rats with plum-ekisu significantly increased PPARα mRNA expression in the liver compared with expression in vehicle-treated animals. Hepatic SREBP-1c expression showed no significant difference between groups. In adipose tissue, PPARγ expression in WF rats was increased significantly by plum-ekisu treatment.

DISCUSSION

We designed this study to determine whether and how plum-ekisu affects blood glucose in WF rats and db/db mice. Major findings in this study were that plum-ekisu attenuated the increase of TG in WF rats, improved glucose tolerance and insulin sensitivity, and increased plasma adiponectin concentration.

To determine appropriate doses of plum in WF rats, we first examined the effects of different plum concentrations in water on body weight and food consumption. As shown in Figure 1, a high concentration of plum-ekisu (1%) reduced both body weight and food consumption in WF rats, while the same doses of plum-ekisu caused no significant differences in WL rats. However, 0.25% plum-ekisu treated WF rats showed no reduction in weight or oral intake compared with water-treated controls; accordingly, high doses of plum-ekisu may suppress appetite. Body weight loss caused by reduction of food intake is known to improve glucose tolerance. To avoid an effect of altered food consumption on glucose tolerance, we selected 0.25% plum-ekisu for subsequent experiments.

As reflected by a reduction in the glucose-insulin index base on glucose and insulin responses during an OGTT, whole-body insulin sensitivity was significantly enhanced in WF rats by administration of plum-ekisu. These findings were confirmed by the

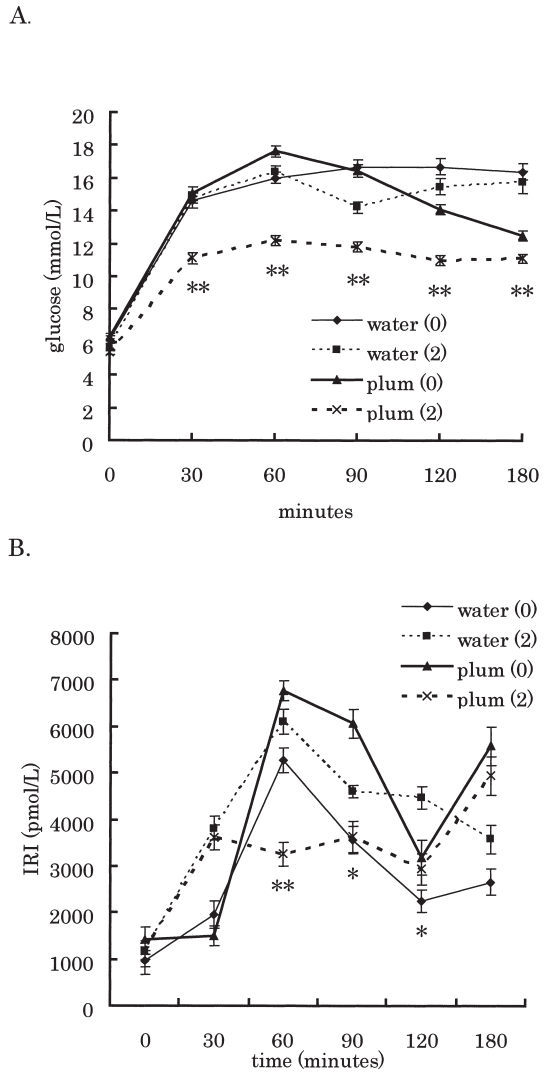

Fig. 2 Effect of plum-ekisu on oral glucose tolerance in Wistar fatty rats. Glucose (A) and insulin concentrations (B) after an oral glucose challenge. Values are means ± SE for eight animals per group. 0, before treatment; 2, treatment for 2 weeks. $^{*}P < 0.05$, $^{**}P < 0.001$ vs. water-treated Wistar fatty rats. IRI: immunoreactive insulin

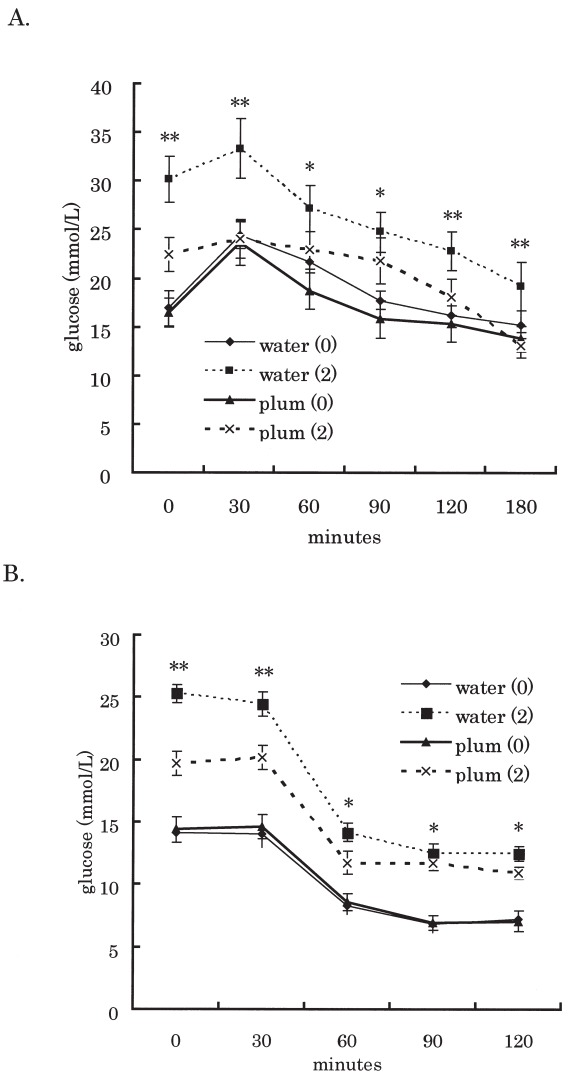

Fig. 3 Effect of plum-ekisu on oral glucose tolerance and insulin tolerance test in db/db mice. Glucose concentrations are shown during a glucose tolerance test (A) and an insulin tolerance test (B). Values are means ± SE for eight animals per group. $^{*}P < 0.05$, $^{**}P < 0.001$ vs. water-treated db/db mice.

results of OGTT and ITT in db/db mice (Fig. 3). Molecular mechanisms underlying the anti-diabetic effect of plum-ekisu were not examined in the present study. Also whether this effect involves glucose uptake in skeletal muscle or in adipose tissues is not clear. In humans, insulin resistance and dyslipidemia are associated most closely with increased visceral adipose tissue mass. An insulin sensitizer, pioglitazone, was reported to decrease insulin resistance by the adipose tissue remodeling in Zucker (*fa/fa*) rats (6). Thus, studying the effect of plum-ekisu on adipose tissue physiology in WF rats should be informative. In addition, the effect of plum-ekisu on TG content in muscle and adipose tissue should be determined since TG content was reported to affect insulin sensitivity (21).

As hyperlipidemia is closely related to insulin resistance, the observed reduction in excessive TG by plum-ekisu might be mediated by improved insulin sensitivity. This explanation would be compatible with the observation that thiazolidines, which are insulin sensitizers, decrease hyperlipidemia associated with obesity. In addition, plum-ekisu also increased PPARα mRNA expression. Activation of PPARα in-

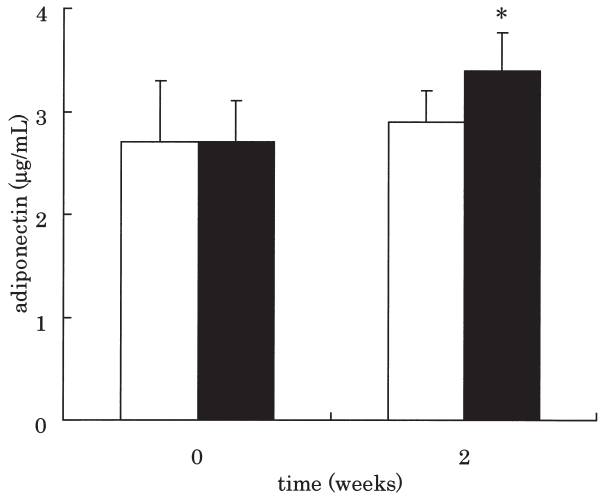

Fig. 4 Effect of plum-ekisu on plasma adiponectin concentrations in Wistar fatty rats. Open bar, water-treated group; filled bar, plum-ekisu-treated group. Values are means ± SE for eight animals per group. $^*P < 0.05$ vs. water-treated Wistar fatty rats.

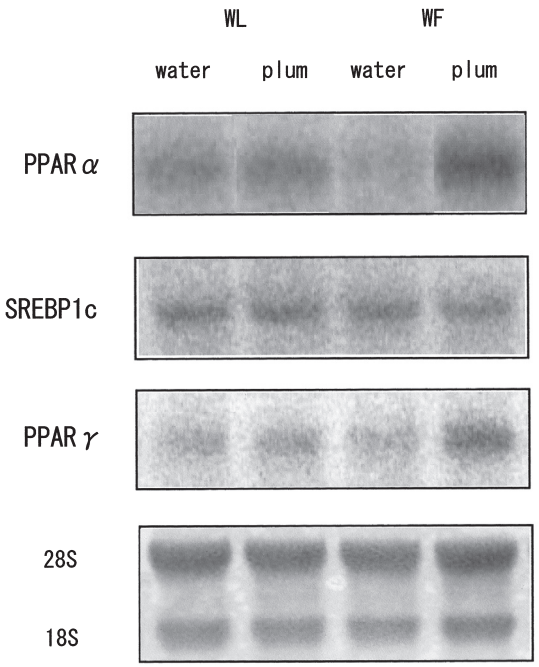

Fig. 5 Effect of plum-ekisu on PPARα, SREBP-1c and PPARγ mRNA expression in Wistar lean and fatty rats. Total RNA was extracted, and Northern blotting analysis was performed with use of each indicated probe. WL, Wistar lean rats; WF, Wistar fatty rats. Filters were stained with methylene blue to check relative loading of total RNA. Results are representative of three individual experiments.

creases hydrolysis of plasma TG by inducing lipoprotein lipase and also reduces apoC-III expression, both resulting in a decrease in serum TG concentrations (23). The hypotriglyceridemic action of plum-ekisu therefore involved improved insulin sensitivity and increased expression of PPARα.

TC also was reduced by treatment with plum-ekisu. Plums contain phenolic compounds including phenolic acid derivatives, flavonoids, and coumarins (14). Phenolics contribute to the health-promoting value of various fruits and vegetables. One potential health benefit of phenolics in fruits and vegetables is antioxidant activity that protects LDLc from oxidation, an effect that may decrease risk of various age-related diseases. Major components of dried plums include chlorogenic acid isomers, which have high antioxidant activities and inhibit LDLc oxidation (7, 17, 20). Among minor components of plums, nine flavonol glycosides have been identified, with rutin predominanting (10, 26). Other phenolics, including ethyl cinnamate and coumarins have been detected in dried plums (18, 27). Recently, Chuda *et al*. reported that a concrete of the juice of the Asian plum, bainiku-ekisu, improved human blood fluidity (5). A bioactive substance in the concentrate has been identified as mumefral, which is produced during processing of plum-ekisu. In addition to the effect of plum-ekisu on blood fluidity, it may have a direct effect on the vasculature, acting to oppose cardiovascular diseases such as hypertension and/or atherosclerosis.

Circulating adiponectin concentrations have been shown to correlate negatively with body mass index (2) and with plasma glucose, TG, and insulin concentrations (11). Studies in obese nonhuman primates have suggested involvement of adiponectin in regulation of metabolism (12). In an obese Pima Indian population, low whole-body insulin sensitivity was independently associated with reduction in circulating adiponectin concentrations (30). In our WF rats, plum-ekisu increased plasma adiponectin concentrations. Taken together, these observations suggest that improvement of insulin sensitivity with plum-ekisu may be mediated partly through increased adiponectin.

We next investigated mechanisms underlying elevation of adiponectin following plum-ekisu treatment. As PPARγ and SREBP-1c have been reported to regulate adiponectin gene expression (15, 24), we assessed gene expression for both PPARγ in adipose tissue and for SREBP-1c in the liver. We found that PPARγ but not SREBP-1c mRNA expression was enhanced by plum-ekisu treatment. PPARγ has been shown to increase adiponectin gene expression in adipocytes, resulting in increased circulating adipo-

nectin (15). These results suggest that plum-ekisu may increase circulating adiponectin via induction of PPARγ in adipose tissue. Establishing whether elevation of PPARγ following plum-ekisu treatment is a cause or a consequence of improved insulin sensitivity will require further study.

In conclusion, we found that in WF rats administered a dose of plum-ekisu not affecting body weight, plum-ekisu reduced blood glucose in a glucose tolerance test, as well as serum concentrations of TG and TC. Plum treatment also increased plasma adiponectin in these animals. The antidiabetic effect of plum-ekisu on obese WF rats may be mediated in part by adiponectin.

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