JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Contrasting Effects of Puerarin and Daidzin on Glucose Homeostasis in Mice

ELIAS MEEZAN,[†] ELISABETH M. MEEZAN,[†] KENNETH JONES,[†] RAY MOORE,[‡] STEPHEN BARNES,[†,‡,§,||] AND JEEVAN K. PRASAIN*,[†,§]

Department of Pharmacology & Toxicology, Comprehensive Cancer Center Mass Spectrometry Shared Facility, and Purdue-UAB Botanicals Center for Age-Related Diseases, and UAB Center for Nutrient−Gene Interaction in Cancer Prevention, University of Alabama at Birmingham, Birmingham, Alabama 35294

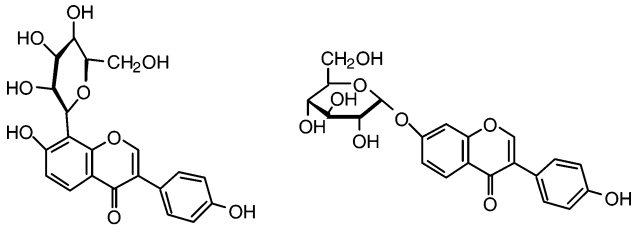
Puerarin and daidzin are the major isoflavone glucosides found in kudzu dietary supplements. In this study, we demonstrated that puerarin significantly improves glucose tolerance in C57BL/6J-ob/ob mice, an animal model of type 2 diabetes mellitus, blunting the rise in blood glucose levels after i.p. administration of glucose. In contrast, daidzin, the O-glucoside, had a significant but opposite effect, impairing glucose tolerance as compared to saline-treated controls. When they were administered i.p. with ^{14}C-glucose to C57BL/6J lean mice, puerarin inhibited glucose uptake into tissues and incorporation into glycogen, while daidzin stimulated glucose uptake, showing an opposite effect to puerarin. Puerarin also antagonized the stimulatory effect of decyl-β-D-thiomaltoside, an artificial primer of glycogen synthesis, which increases ^{14}C-glucose uptake and incorporation into glycogen in mouse liver and heart. A liquid chromatography−tandem mass spectrometry procedure was used to investigate the metabolism and bioavailability of puerarin and daidzin. The blood puerarin concentration−time curve by i.p. and oral administration indicated that puerarin was four times more bioavailable via i.p. injection than via the oral route of administration. This may account for the increased hypoglycemic effect seen in the i.p. glucose tolerance test vs that seen orally. Our results suggest that puerarin is rapidly absorbed from the intestine without metabolism, while daidzin is hydrolyzed to the aglycone daidzein. The opposing effects of puerarin and daidzin on glucose homeostasis may have implications for the activity of dietary supplements that contain both of these isoflavonoids.

KEYWORDS: Glucose tolerance test; puerarin; daidzin, kudzu; metabolism; LC-MS-MS

INTRODUCTION

Diabetes mellitus is a disease characterized by chronic hyperglycemia that can lead to several complications related to cardiovascular disease, renal failure, blindness, and neurological disorders. Despite significant advances in treatment and prevention of diabetic complications, the disease is progressive and is a leading cause of death in the United States. In recent years, there has been a growing interest in hypoglycemic agents from natural products, especially those derived from plants, because plant sources with ethnomedicinal backgrounds are considered to be less toxic, with fewer side effects than synthetic medicines (*1*−*3*).

Radix Pueraria (the root of the kudzu *Pueraria lobota*) is one of the most popular traditional Chinese medicines. A number of companies are now marketing kudzu dietary supplements (*4*). Isoflavonoids of kudzu root include puerarin, daidzin, daidzein, formononetin, and their C- and O-glycosides (*4*, *5*). They have been associated with antioxidant, antidipsotropic, and other pharmacological effects (*6*−*8*). Puerarin (daidzein 8-C-glucoside) and daidzin (daidzein 7-O-glucoside) (**Figure 1**) are the major isoflavones of kudzu root and have attracted considerable attention, because they have a variety of interesting activities (*9*−*12*). A previous report has indicated that puerarin is antihyperglycemic in streptozotocin (STZ)-induced diabetic



Puerarin; mol. wt. 416 Daidzin; mol. wt. 416

Figure 1. Chemical structures of puerarin and daidzin. Each value is the mean ± SEM.

* To whom correspondence should be addressed. Tel: 205-996-2612. Fax: 205-934-6944. E-mail. jprasain@uab.edu.
[†] Department of Pharmacology & Toxicology.
[‡] Comprehensive Cancer Center Mass Spectrometry Shared Facility.
[§] Purdue-UAB Botanicals Center for Age-Related Disease.
[||] UAB Center for Nutrient−Gene Interaction in Cancer Prevention.

10.1021/jf058105e CCC: $30.25
Published on Web 10/04/2005

rats (*13*). This observation warrants further investigation of the possible effects of puerarin on glucose uptake and disposal as an explanation for its hypoglycemic activity. Isoflavones have many effects similar to those of estrogen and have become popular among postmenopausal women as an alternative for hormone replacement therapy (*14*, *15*). Nutritional intervention studies performed in animals and humans suggest that the ingestion of soy protein associated with isoflavones such as genistin and daidzin (O-glucosides) and flaxseed rich in lignans improves glucose control and insulin resistance (*16*). Soy phytochemicals have been shown to have antidiabetic and hypolipidemic effects (*17*).

In diabetics, hyperglycemia is most pronounced following a meal due to the absorption of glucose from the gastrointestinal (GI) tract. Inhibiting glucose uptake in the intestines and/or promoting glucose disposal in the tissues may be beneficial for diabetic patients to control the blood glucose level in the postprandial state. On the basis of our previous studies that puerarin is rapidly absorbed intact, whereas daidzin is hydrolyzed in the intestine to release the aglycone by the action of intestinal glucosidases/hydrolases, we hypothesized that puerarin may be transported across the intestinal wall intact by the involvement of the sodium-dependent glucose transporter (SGLT1) (*18*). This may have a role in the suppressive effect of puerarin on glucose uptake in the intestines. It was important to determine whether these isoflavones (C- and O-glucosides) have a differential effect on glucose uptake and metabolism.

In the present study, we investigated the effect of puerarin and daidzin on glucose uptake and metabolism in male obese mice (C57Bl/6J-ob/ob) and their lean controls (C57Bl/6J-+/+) and correlated these effects with the metabolism and blood concentration−time profile of these isoflavone glucosides.

MATERIALS AND METHODS

Materials. Puerarin, phlorizin, and apigenin were purchased from Sigma Chemical Co. (St. Louis, MO). Decyl-β-D-thiomaltoside was obtained from Anatrace, Inc. (Maumee, OH). Standards of daidzin and daidzein were purchased from LC Laboratories (Woburn, MA). All solvents and reagents were purchased from Fisher (Norcross, GA) and were of high-performance liquid chromatography grade. D-Glucose [^{14}C(U)], specific activity 245 mCi/mmol, 0.1 mCi/mL, 73.6 μg/mL, was obtained from Moravek Biochemicals, Inc. (Brea, CA).

Animals. Male obese mice (C57BL/6J-ob/ob) and their lean controls (C57Bl/6J-+/+) were purchased from the Jackson Laboratory (Bar Harbor, ME) at 4−5 weeks of age. They were housed under standard animal care conditions with free access to food and water.

^{14}C-Glucose Uptake and Incorporation. Puerarin (75 mg/kg), daidzin (75 mg/kg), phlorizin (75 mg/kg), or decyl-β-D-thiomaltoside (75 mg/kg) were administered i.p. to C57BL/6J-+/+ lean mice (weight = 28.5 ± 2 g) with U-^{14}C-glucose (10 μCi) in saline containing ethanol (1 g/kg) to improve solubility of the compounds. Mice given saline/ethanol alone served as controls. The animals were sacrificed after 2 h by cervical dislocation under isoflurane anesthesia. The liver, heart, diaphragm, leg skeletal muscles, brain, and kidneys were removed and homogenized in 5 volumes (liver) or 2 mL (other tissues) of ice cold 4 mol/L guanidine hydrochloride using 3 × 15 s bursts of a polytron homogenizer at 60−80% maximal output with cooling on ice. Two 100 μL aliquots of the tissue extracts were counted to determine uptake of ^{14}C-radiolabel into the tissues. Plasma was obtained from blood, and two 20 μL aliquots were counted to determine ^{14}C-glucose levels. The red blood cell pellet was extracted in 2 mL of 4 mol/L guanidine hydrochloride, and aliquots were counted as for the tissue to determine radiolabel uptake. For determination of ^{14}C-glucose incorporation into glycogen, two 0.5 mL aliquots of each tissue extract were made 1 mol/L in NaOH and heated for 1 h at 60 °C. The glycogen was precipitated overnight at −20 °C with two volumes of absolute ethanol, and the pellets sedimented and washed three times with 66% ethanol before solubilization and determination of radiolabel incorporation in a scintillation counter. Uptake and incorporation of ^{14}C-radiolabel are given as dpm/g wet weight of tissue.

Glucose Tolerance Tests. C57Bl/6J-ob/ob mice (weight = 49.4 ± 1 g) were fasted overnight. Puerarin (75 mg/kg), daidzin (75 mg/kg), or phlorizin (75 mg/kg) was administered i.p. or by oral gavage in saline containing 2 g/kg glucose, and blood samples were taken from the tail cut under isoflurane anesthesia at 15, 30, 45, 60, 90, 120, 180, and 240 min for the measurement of glucose using a Glucometer Elite meter and strips (Bayer, Elkhart, IN). The glucose meter measured the electrical potential produced when glucose in the blood sample reacted with glucose oxidase and potassium ferricyanide on the strip. This produced a current that was proportional to the amount of glucose in the sample. Results are given as blood glucose at each time point divided by blood glucose at time = 0 before administration of the compounds and glucose bolus to correct for variability in the baseline blood glucose values among the animals. Mice given glucose in saline alone served as controls.

Statistical Analysis of ^{14}C-Glucose and Glucose Tolerance Data. All data are expressed as means ± SEM and were analyzed for significance by analysis of variance (ANOVA) using a significance level of 0.05 with a complete statistical program, Statview 5, which evaluates all possible pair wise comparisons between control and experimental groups with a multiple *t*-test statistic (Fisher's PLSD).

Liquid Chromatography−Mass Spectrometry. LC-MS/MS analyses were performed using a system consisting of a model SIL-HT refrigerated Shimadzu autosampler (Shimadzu Scientific Instruments, Inc., Columbia, MD) and an API 4000 Q TRAP (Applied Biosystems/MDS Sciex, Concord, Ontario, Canada). Chromatography was carried out on a 100 mm × 2.1 mm i.d. Waters X-Terra C_{18} column with Waters X-Terra guard column (10 mm × 2.1 mm) preequilibrated with 10 mmol/L ammonium acetate (NH_4OAc). The mobile phase consisted of a gradient of 10−70% acetonitrile in 10 mmol/L NH_4OAc over 6 min with a flow rate of 0.2 mL/min. Multiple reaction monitoring (MRM) was used to perform mass spectrometric quantification of puerarin, and daidzein. The column effluent was introduced into the mass spectrometer using electrospray ionization in the negative mode. The LC-MS/MS system was controlled by BioAnalyst 1.4 software. The analysis was carried out in the negative ion mode with a declustering potential of −110 V and an ion spray voltage of −4500 V. The temperature of the turbo gas was 450 °C. Nitrogen was used as the collision gas. The MRM analysis was conducted by monitoring the precursor ion to product ion transitions from *m*/*z* 415/267 (puerarin), 253/223 (daidzein), and 269/149 (apigenin).

Sample Preparation and Analysis. We followed the published method with slight modification (*18*, *19*). Blood samples collected at 0, 60, 120, and 240 min were analyzed by the MRM method. To 10 μL of blood sample, 10 μL of internal standard (apigenin) and 40 μL of methanol in 1% acetic acid were added. Samples were mixed on a vortex and then centrifuged (8000*g* 10 min) in Eppendorf tubes. The supernatant was transferred to an autosampler vial, and a 7 μL aliquot was injected onto the LC-MS/MS system for analysis.

For analysis in liver samples, a portion of the liver was homogenized in 5 volumes of ice cold 60% methanol using 3 × 15 s bursts of a polytron homogenizer at 60−80% maximal output with cooling on ice to extract the isoflavonoids. The methanol extract was centrifuged at 3000*g* for 10 min at 4 °C, and the supernatant was concentrated by a speed-vac and then analyzed by MRM method.

Standard curves were generated following the same procedure used for sample preparation. Specificity was established by the lack of interference peaks at the retention time for the internal standard and puerarin. Linearity was tested at seven levels of concentrations covering a range from 0.01 to 10 μmol/L. The calibration curves were established by linear least-squares regression ($1/x^2$ weighting) from peak area ratios (analyte/internal standard) vs nominal concentrations. Puerarin and daidzein had linear response curves with correlation coefficients greater than 0.98. The area under the curve (AUC) values were calculated by the trapezoidal method using a computer program developed by Dr. Edward Acosta, UAB.

Table 1. ^{14}C-Glucose Uptake and Incorporation into Glycogen[a]

	dpm/g wet weight					
	saline		puerarin		daidzin	
tissue	uptake	glycogen	uptake	glycogen	uptake	glycogen
liver	292395 ± 43873	18891 ± 2862	203150 ± 40586[b]	16597 ± 2985[b]	429059 ± 3907[c]	26105 ± 2749
heart	248662 ± 23407	16725 ± 2080	104695 ± 27685[d]	3824 ± 1350[d]	240805 ± 16488	14697 ± 3917
brain	380971 ± 47615	9535 ± 675	175036 ± 35348[d]	4894 ± 1127	567535 ± 90395	12279 ± 2290
kidney	257216 ± 21294	16314 ± 1187	147231 ± 27788[d]	9907 ± 2684	332446 ± 29282	18463 ± 438
diaphragm	389942 ± 37959	72965 ± 13256	282612 ± 69505	38533 ± 17504	612323 ± 157856	114473 ± 54336
muscle	207212 ± 39680	16617 ± 3509	135499 ± 29569	16400 ± 7779	180721 ± 19304	14090 ± 1655
RBC	222076 ± 33673	34433 ± 6456	78655 ± 15143[d]	12565 ± 3641	299810 ± 34176	50452 ± 13111
plasma	272619 ± 60436		96063 ± 16759[d]		348513 ± 17473	

[a] Each value is the mean ± SEM ($n = 4$ mice). [b] Significantly different from daidzin, $p < 0.05$. [c] Significantly different from saline control, $p < 0.05$. [d] Significantly different from saline control and daidzin, $p < 0.05$.

RESULTS

Effects of Puerarin and Daidzin on ^{14}C-Glucose Uptake and Incorporation into Glycogen. Puerarin or daidzin was administered to C57BL/6J-+/+ lean mice i.p. at a dose of 75 mg/kg with U-^{14}C-glucose, and the uptake of the radiolabel by the tissues and its incorporation into glycogen were compared to saline-treated controls. Mice given puerarin showed a greater than 2-fold reduction in ^{14}C-glucose in plasma at 2 h after administration as compared to saline controls, while those given daidzin had 3-fold higher plasma ^{14}C-glucose levels than the puerarin-treated animals (**Table 1**). The lower ^{14}C-glucose levels in the plasma of puerarin-treated animals were reflected in a lower uptake of ^{14}C in all tissues examined, with the most pronounced decreases observed in heart, brain, kidney, and red blood cells. In contrast, the higher ^{14}C-glucose levels in the plasma of daidzin-treated animals were accompanied by greater uptake of ^{14}C in almost all tissues, with the exception of heart and leg skeletal muscle, when compared with saline controls. ^{14}C-Glucose incorporation into glycogen reflected the same trend as the plasma and tissue levels, being lower in puerarin-treated and higher in daidzin-treated animals than in the saline controls. The most pronounced differences in ^{14}C-glucose incorporation into glycogen between the puerarin and the daidzin-treated animals were seen in liver, heart, brain, and red blood cells, while no difference was seen in leg skeletal muscle. Additional support for an inhibitory effect by puerarin on ^{14}C-glucose uptake and incorporation in liver and heart of C57BL/6J-+/+ lean mice was obtained by examining the effects of this agent on animals, which had also been given decyl-β-D-thiomaltoside (DecbSM), a compound that we have previously shown stimulates both ^{14}C-glucose uptake and incorporation into glycogen in lean and ob/ob mice by acting as an artificial primer of glycogen synthesis and by stimulating glucose uptake into tissues (*20*, *21*). Mice given puerarin (75 mg/kg) showed a greater than 30−60% reduction in ^{14}C-glucose uptake in liver and heart at 2 h after i.p. administration as compared to saline-treated controls, in contrast to the almost 3-fold stimulation of ^{14}C-glucose uptake in these tissues seen with DecbSM (75 mg/kg) (**Figure 2**). When puerarin was administered i.p. with DecbSM, both at 75 mg/kg, there was a significant inhibition of the stimulatory effect of the thiomaltoside indicating that puerarin blocks this effect and suggesting that the increased glucose uptake into liver and heart produced by the maltoside may reflect in part the stimulation of glucose transporters in these organs, which is antagonized by puerarin (**Figure 2**). Puerarin also reduced the increased ^{14}C-glucose incorporation into glycogen in both liver and heart induced by DecbSM, an effect that is probably a result of inhibition of glucose transport into the tissues rather than a direct effect of puerarin on glycogen synthesis, since even in the presence of puerarin, DecbSM still produced a 1.7- and 2.8-fold stimulation of ^{14}C-glucose incorporation into glycogen in liver and heart, respectively. Therefore, puerarin antagonized the effects of DecbSM, a compound that is known to increase glucose uptake into liver and heart and its incorporation into glycogen in both C57BL/6J lean and obese mice.

Effects of Puerarin, Daidzin, and Phlorizin on i.p. and Oral Glucose Tolerance in C57BL/6J-ob/ob Mice. Administration of a single 75 mg/kg dose of puerarin with a 2 g/kg bolus of glucose i.p. to C57BL/6J-ob/ob mice resulted in a highly significant blunting of the rise in blood glucose seen in animals given glucose in saline alone (**Figure 3**). In contrast, mice given 75 mg/kg of daidzin with a 2 g/kg bolus of glucose i.p. showed increased blood glucose levels as compared to saline controls. Phlorizin (75 mg/kg), a known inhibitor of the SGLT1, also improved glucose tolerance in the ob/ob mice, but its effect was significantly less pronounced than that of puerarin at the early time points. Therefore, i.p. puerarin improved the disposal of a glucose load in C57BL/6J-ob/ob mice, while i.p. daidzin impaired glucose disposal as compared with untreated animals.

When the effects of oral puerarin and daidzin on the disposition of an oral 2 g/kg bolus of glucose were compared, a similar pattern of effects to that seen in the i.p. glucose tolerance tests was observed, but the effects were much less pronounced (**Figure 4**). Oral puerarin blunted the rise in blood glucose primarily at the earliest times after glucose administration (15 and 30 min), while oral daidzin produced equal or slightly elevated blood glucose levels to those seen in animals given saline alone. Phlorizin showed no significant effect on oral glucose tolerance as compared to saline controls (**Figure 4**).

Metabolism and Bioavailability of Puerarin and Daidzin. We performed a preliminary investigation on metabolism and relative bioavailability of puerarin and daidzin, after i.p. vs oral administration (75 mg/kg). A sensitive analytical method had to be developed in order to analyze puerarin and daidzein (a hydrolyzed metabolite of daidzin) in small blood samples (10 μL). We developed a LC-MS/MS-based MRM method using an API 4000 QTRAP machine. The MRM method for quantification was demonstrated to be very sensitive and specific, and a linear response was obtained over a range of 0.01−10 μmol/L. The resulting chromatograms of mice blood samples collected at 0 and 60 min after i.p. administration of puerarin and of a standard (0.01 μmol/L) demonstrated the specificity and sensitivity of the MRM method (**Figure 5**). The mean blood puerarin concentration−time curves for i.p. and oral administra-

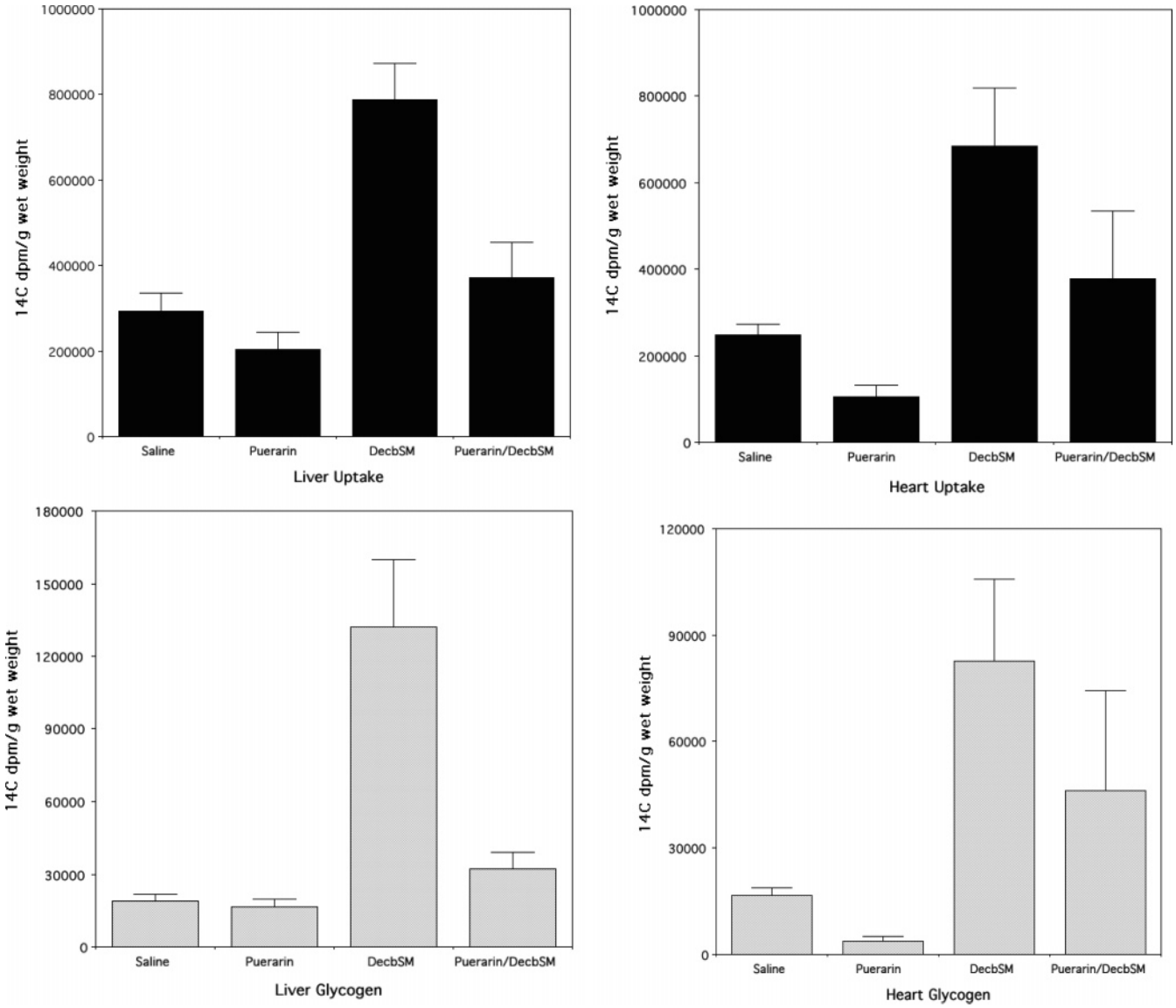


Figure 2. Antagonism by puerarin of the stimulatory effects of DecbSM on ^{14}C-glucose uptake into liver and heart and its incorporation into glycogen in C57BL/6J-+/+ mice. Each bar represents the mean ± SEM of four mice.

tion are shown in **Figure 6**. It appears that puerarin is absorbed rapidly as its peak blood concentration occurs at 60 min in both cases. Within the time range of 0−240 min, puerarin administered i.p. yielded AUC values four times those obtained with the same dose administered orally (**Figure 7**). Thus, i.p. administration markedly enhances the bioavailability of puerarin. Because the therapeutic efficacy of a drug is generally related to its bioavailability, the higher concentrations of puerarin in mice given the compound i.p. as compared to oral administration may be responsible for its greater inhibition of glucose uptake in mice.

We also analyzed the methanolic extracts of liver tissue samples obtained from C57BL/6J-+/+ lean mice treated with puerarin or daidzin i.p. by the LC-MS/MS method. Only intact puerarin was detected in the liver sample (**Figure 8**). In contrast, in daidzin-treated samples, daidzein eluting at 6.7 min together with conjugates at 5.23 and 5.73 min were detected. This and our previous result (*18*) in rats demonstrated that puerarin is rapidly absorbed intact from the intestine without metabolism, while daidzin is hydrolyzed to the aglycone daidzein.

DISCUSSION

This study reports for the first time the contrasting effects (inhibitory and stimulatory) of puerarin and daidzin, respectively, on glucose uptake in mice. Flavonoids and polyphenols have been reported to both stimulate and inhibit glucose transport in a variety of experimental systems in vitro and in vivo. A common finding is the ability of these compounds to affect the activity of the SGLT1 in the small intestine. SGLTs mediate the uptake of glucose at the small intestinal brush border membrane and its reabsorption from the glomerular filtrate at the renal tubular brush border (*22*). The prototypical compound, which inhibits both intestinal glucose uptake and renal glucose reabsorption, is phlorizin, and these activities appear to be the basis of its antidiabetic actions (*23*). Several other plant-derived compounds have also been reported to inhibit SGLT1 and/or to have hypoglycemic activity. These include green tea polyphenols (*24*), quercetin glucosides (*25*−*27*), and the citrus bioflavonoids hesperidin and naringin (*28*). Flavonoids have also been reported to have effects on glucose transport in other tissues. Strobel et al. reported that myricetin, quercetin, and

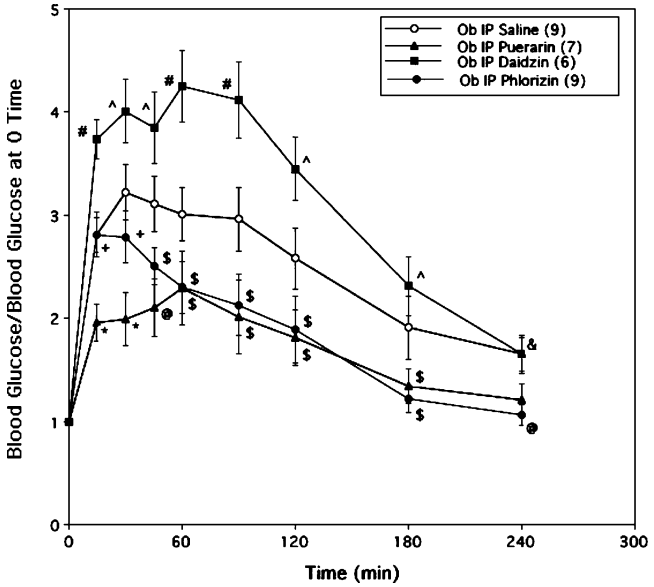


Figure 3. Effects of i.p. puerarin, daidzin, and phlorizin on i.p. glucose tolerance in C57BL/6J-ob/ob mice. Values are given as means ± SEM. *, Significantly different from saline, daidzin, and phlorizin; #, significantly different from saline, puerarin, and phlorizin; +, significantly different from puerarin and daidzin; ∧, significantly different from puerarin and phlorizin; @, significantly different from saline and daidzin; $, significantly different from daidzin; and &, significantly different from phlorizin, $p < 0.05$.

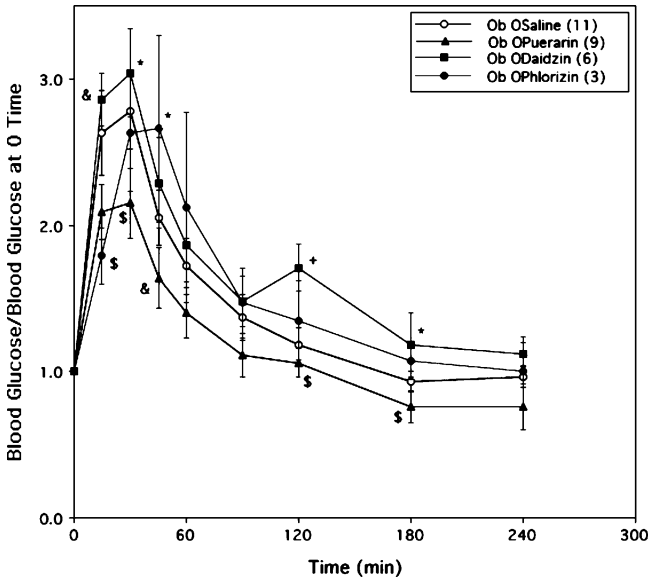


Figure 4. Effects of oral puerarin, daidzin, and phlorizin on oral glucose tolerance in C57BL/6J-ob/ob mice. Values are given as means ± SEM. $, Significantly different from daidzin; &, significantly different from phlorizin; *, significantly different from puerarin; and +, significantly different from saline and puerarin, $p < 0.05$.

catechin-gallate inhibit glucose uptake in isolated rat adipocytes (*29*). On the other hand, myricetin has been reported to have stimulatory effects on glucose transport in rat adipocytes and to enhance insulin-stimulated lipogenesis (*30*). This discrepancy may be due to the use of different experimental conditions (*31*). The inhibition by flavonoids of the Glut1 glucose transporter in human erythrocytes has also been reported (*32*). Therefore, these compounds can interact with more than one type of glucose transporter in small intestine, kidney, and other tissues.

Puerarin has previously been reported to decrease the plasma glucose concentration in a dose-dependent manner and to

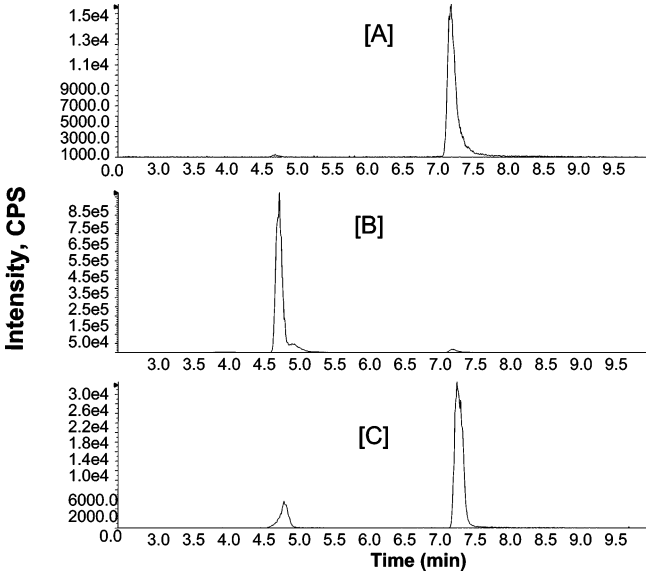


Figure 5. Representative MRM chromatograms for puerarin (*m*/*z* 415/267) (**A**) blank mice blood, (**B**) a blood sample 60 min after a single i.p. adminstration of puerarin, and (**C**) puerarin spiked standard (0.01 μmol/L).

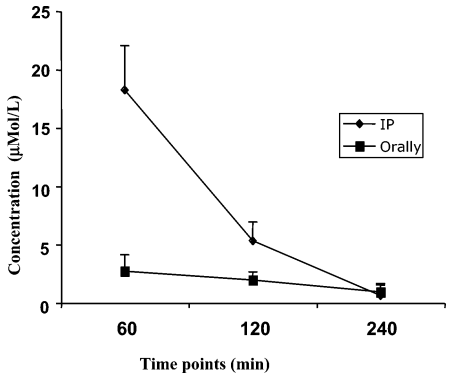


Figure 6. Mean blood concentration–time profile of puerarin after a single i.p. or oral administration to mice. Data are means ± SEM. Experimental conditions are described in the Materials and Methods.

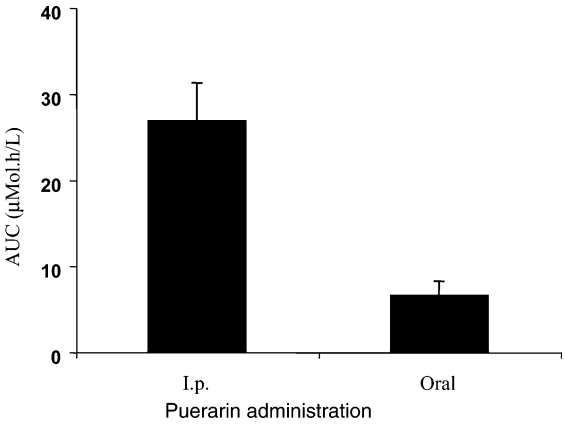


Figure 7. Mean puerarin AUC_{0-4h} after a single i.p. vs oral administration to mice. Data are means ± SEM.

improve glucose tolerance in an i.v. glucose tolerance test in STZ diabetic rats (*13*). In these experiments, puerarin was administered by intravenous injection, thus precluding any direct effects of the flavonoid on glucose uptake from the GI tract. It also increased the utilization of glucose in the isolated soleus muscle of STZ diabetic rats. These findings warranted further

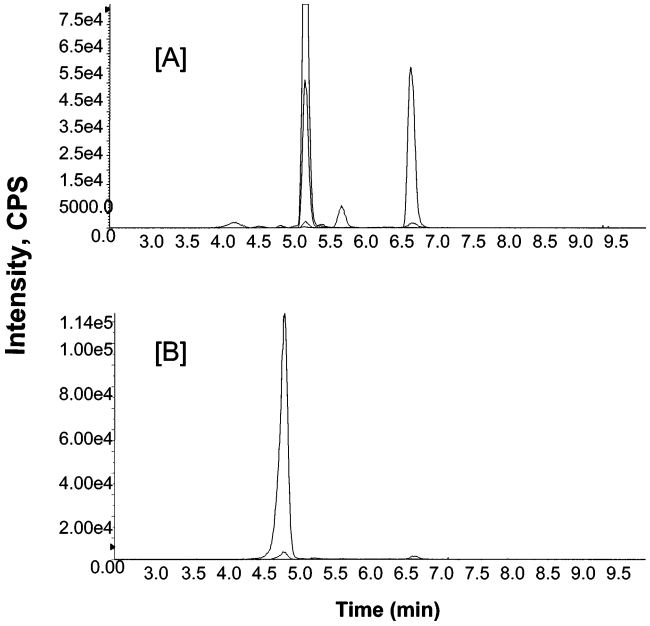


Figure 8. Representative MRM chromatograms of methanolic extracts of mice liver treated with (**A**) daidzin and (**B**) puerarin.

investigation concerning the possible effects of puerarin on glucose uptake and disposal in an animal model of type 2 diabetes mellitus. In this study, we demonstrated that puerarin significantly improved glucose tolerance in C57BL/6J-ob/ob mice. While both administrations (oral and i.p.) of puerarin decreased blood glucose levels in glucose tolerance tests, oral administration of puerarin was not as effective as i.p. injection. This differential activity based on route of administration may be, at least in part, due to the much higher bioavailability of puerarin in blood when given i.p. However, it is also possible that when given i.p. puerarin may have a direct inhibitory effect on glucose transporters in peritoneal mesothelial cells, which form the barrier to glucose absorption across the peritoneum. Glucose uptake by differentiated peritoneal mesothelial cells is mediated by both the SGLT1 and the facilitative glucose transporters GLUT1 and GLUT3 (*33*). If puerarin is a more effective inhibitor of peritoneal glucose transport than it is of intestinal glucose transport, this could account for its greater effectiveness in improving glucose tolerance when given by the i.p. route than when given orally.

An inhibitory effect of puerarin on SGLT1 and facilitative glucose transporters could also explain its differential effects on the uptake and incorporation into glycogen of ^{14}C-glucose by several tissues and its antagonism of the effects of decyl-β-D-thiomaltoside, a compound known to stimulate glucose uptake and glycogen synthesis in both lean and obese mice (*20*, *21*). The most marked inhibition of ^{14}C-glucose uptake and incorporation into glycogen was seen in heart, brain, kidney, and red blood cells. It is of interest that human cardiomyocytes express high levels of the Na^+-glucose cotransporter SGLT1, second only to those found in the small intestine (*34*). SGLT1 has also recently been localized in the blood−brain barrier and may function in the transport of glucose into the brain (*35*). The SGLT inhibitor phlorizin and analogues of phlorizin exert their hypoglycemic effects by inhibiting Na^+-glucose cotransporters in both the intestine and the kidney, but phlorizin did not inhibit glycogen synthesis in skeletal muscle or liver (*23*, *36*). Similarly, in the present experiments, puerarin had no effect on ^{14}C-glucose incorporation into glycogen in skeletal muscle or liver but exerted a pronounced inhibition in heart with lesser effects in brain, kidney, and red blood cells. Our results, therefore, are consistent with a phlorizin-like effect of puerarin on inhibiting Na^+-glucose cotransporters and facilitative glucose transporters in intestine, kidney, heart, and other tissues, thus resulting in decreased glucose uptake into the circulation and reduced tissue uptake of glucose and its incorporation into glycogen. The differential effect of puerarin seen in different tissues may be attributed to their varying sensitivity to inhibition of glucose uptake by this agent.

In contrast to phlorizin, which is susceptible to hydrolysis by glucosidase, puerarin is absorbed and is present in the circulation as the intact glycoside. In this regard, it is of interest that attempts to synthesize C-glucosides of phlorizin, which like puerarin would be stable to glycosidases, produced compounds that were much poorer inhibitors of SGLT than phlorizin itself (*37*). This finding was interpreted as indicating the importance of the glycosidic oxygen linkage to the inhibitory activity of phlorizin on SGLT. However, in our study, daidzin, an O-glycoside structurally analogous to puerarin, stimulated glucose uptake in both oral and i.p. glucose tolerance tests. The stimulatory effect on glucose absorption was also evident from our studies on ^{14}C-glucose uptake and incorporation into glycogen. Therefore, in vivo, daidzin was not an inhibitor of glucose uptake into the circulation or into tissues. However, in in vitro studies, a soy phytochemical extract containing genistin and daidzin has been reported to have inhibitory effects on glucose uptake into rabbit intestinal brush border membrane (*38*).

Hyperglycemia in diabetics is marked in the postprandial state, due to intestinal absorption of the glucose generated from a meal, which occurs across the epithelial enterocytic cells of the small intestine, involving glucose transporters such as the SGLT-1. Like puerarin, the glucoside phlorizin, a well-known inhibitor of SGLT-1, inhibited the increase in blood glucose levels in the i.p. glucose tolerance test, but its effect was less pronounced than puerarin. It is possible that puerarin may be transported across the intestinal wall intact by the involvement of the SGLT1 and competes with glucose for transport (*18*, *39*).

At this point, the molecular mechanism of these contrasting effects of puerarin and daidzin on glucose uptake and incorporation into glycogen in vivo is unclear. It should be noted that the observed effects of daidzin on glucose tolerance and metabolism cannot be explained by the release of glucose from this compound as the maximum amount of glucose, which could be obtained from daidzin by its total hydrolysis in the GI tract is less than 5% of the glucose bolus administered to the mice. While our data and the analogy with phlorizin favor a primary action of puerarin as an inhibitor of glucose uptake into the circulation and tissues mediated by Na^+-glucose cotransporters and facilitated glucose transporters, puerarin may affect glucose homeostasis by other mechanisms as well. In STZ diabetic rats, recent results indicate that puerarin may reduce plasma glucose by activating α(1)-adreno-receptors in the adrenal gland to enhance the secretion of β-endorphin (*40*). Soy isoflavones have also been reported to exert antidiabetic effects through the PPAR pathways in obese Zucker rats (*17*). The relevance of these findings to our studies in a mouse model of type 2 diabetes is unclear but illustrates that puerarin exerts multiple biochemical and pharmacological effects in vivo, which contribute to its antidiabetic and hypoglycemic activity.

LITERATURE CITED

(1) Perez, R. M.; Zavala, M. A.; Perez, S.; Perez, C. Antidiabetic effect of compounds isolated from plants. *Phytomedicine* **1998**, *5*, 55−75.

(2) Grover, J. K.; Yadav, S.; Vats, V. Medicinal plants of India with antidiabetic potential. *J. Ethnopharmacol.* **2002**, *81*, 81−100.
(3) Li, W. L.; Zheng, H. C.; Bukuru, J.; De Kimpe, N. Natural medicines used in the traditional Chinese medical system for therapy of diabetes mellitus. *J. Ethnopharmacol.* **2004**, *92*, 1−21.
(4) Prasain, J. K.; Jones, K.; Kirk, M.; Wilson, L.; Smith-Johnson, M.; Weaver, C.; Barnes, S. Profiling and quantification of isoflavonoids in kudzu dietary supplements by high-performance liquid chromatography and electrospray ionization tandem mass spectrometry. *J. Agric. Food Chem.* **2003**, *51*, 4213−4218.
(5) Keung, W. M.; Vallee, B. L. Kudzu root: An ancient Chinese source of modern antidipsotropic agents. *Phytochemistry* **1998**, *47*, 499−506.
(6) Lee, J. S. Supplementation of Pueraria radix water extract on changes of antioxidant enzymes and lipid profile in ethanol-treated rats. *Clin. Chim. Acta*. **2004**, *347*, 121−128.
(7) Chueh, F. S.; Chang, C. P.; Chio, C. C.; Lin, M. T. Puerarin acts through brain serotonergic mechanisms to induce thermal effects. *J. Pharmacol. Sci*. **2004**, *96*, 420−427.
(8) Overstreet, D. H.; Keung, W. M.; Rezvani, A. H.; Massi, M.; Lee, D. Y. Herbal remedies for alcoholism: promises and possible pitfalls. *Alcohol.: Clin. Exp. Res.* **2003**, *27*, 177−185.
(9) Zhu, J. H.; Wang, X. X.; Chen, J. Z.; Shang, Y. P.; Zhu, J. H.; Guo, X. G.; Sun, J. Effects of puerarin on number and activity of endothelial progenitor cells from peripheral blood. *Acta Pharmacol. Sin*. **2004**, *25*, 1045−1051.
(10) Xu, X. H.; Zhao, T. Q. Effects of puerarin on D-galactose-induced memory deficits in mice. *Acta Pharmacol. Sin*. **2002**, *23*, 587−590.
(11) Zhang, G. Q.; Hao, X. M.; Zhou, P. A.; Wu, C. H.; Dai, D. Z. Puerarin blocks transient outward K+ current and delayed rectifier K+ current in mice hippocampal CA1 neurons. *Acta Pharmacol. Sin.* **2001**, *22*, 253−256.
(12) Gao, G. Y.; Li, D. J.; Keung, W. M. Synthesis of daidzin analogues as potential agents for alcohol abuse. *Bioorg. Med. Chem*. **2003**, *11*, 4069−4081.
(13) Hsu, F. L.; Liu, I. M.; Kuo, D. H.; Chen, W. C.; Su , H. C.; Cheng, J. T. Antihyperglycemic effect of puerarin in streptozotocin-induced diabetic rats. *J. Nat. Prod.* **2003**, *66*, 788−792.
(14) Cheng, S. Y.; Shaw, N. S.; Tsai, K. S.; Chen, C. Y. The hypoglycemic effects of soy isoflavones on postmenopausal women. *J. Womens Health (Larchmt)* **2004**, *13*, 1080−1086.
(15) Petri Nahas, E.; Nahas Neto, J.; De Luca, L.; Traiman, P.; Pontes, A.; Dalben, I. Benefits of soy germ isoflavones in postmenopausal women with contraindication for conventional hormone replacement therapy. *Maturitas* **2004**, *48*, 372−380.
(16) Bhathena, S. J.; Velasquez, M. T. Beneficial role of dietary phytoestrogens in obesity and diabetes. *Am. J. Clin. Nutr*. **2002**, *76*, 1191−1201.
(17) Mezei, O.; Banz, W. J.; Steger, R. W.; Peluso, M. R.; Winters, T. A.; Shay, N. Soy isoflavones exert antidiabetic and hypolipidemic effects through the PPAR pathways in obese Zucker rats and murine RAW 264.7 cells. *J. Nutr.* **2003**, *133*, 1238−1243.
(18) Prasain, J. K.; Jones, K.; Brissie, N.; Moore, R.; Wyss, J. M.; Barnes, S. Identification of puerarin and its metabolites in rats by liquid chromatography-tandem mass spectrometry. *J. Agric. Food Chem.* **2004**, *52*, 3708−3712.
(19) Bateman, K. P.; Castonguay, G.; Xu, L.; Rowland, S.; Nicoll-Griffith, D. A.; Kelly, N.; Chan, C. C. Reduction of animal usage by serial bleeding of mice for pharmacokinetic studies: Application of robotic sample preparation and fast liquid chromatography−mass spectrometry. *J. Chromatogr., B: Biomed. Sci. Appl*. **2001**, *754*, 245−251.
(20) Meezan, E.; Meezan, E.; Meezan, J.; Manzella, S.; Roden, L. Alkylglycosides as artificial primers for glycogen biosynthesis. *Cell. Mol. Biol.* **1997**, *43*, 369−381.
(21) Meezan, E.; Meezan, E. Decyl-beta-D-thiomaltoside is an artificial glycogen primer with hypoglycemic activity. *Diabetes* **1999**, *48* (Suppl. 1), A280.
(22) Scheepers, A.; Joost, H. G.; Schurmann, A. The glucose transporter families SGLT and GLUT: Molecular basis for normal and aberrant function. *J. Parenter. Enteral Nutr*. **2004**, *28*, 364−371.
(23) Zhao, H.; Yakar, S.; Gavrilova, O.; Sun, H.; Zhang, Y.; Kim, H.; Setser, J.; Jou, W.; LeRoith, D. Phloridzin improves hyperglycemia but not hepatic insulin resistance in a transgenic mouse model of type 2 diabetes. *Diabetes* **2004**, *53*, 2901−2909.
(24) Kobayashi, Y.; Suzuki, M.; Satsu, H.; Arai, S.; Hara, Y.; Suzuki, K.; Miyamoto, Y.; Shimizu, M. Green tea polyphenols inhibit the sodium-dependent glucose transporter of intestinal epithelial cells by a competitive mechanism. *J. Agric. Food Chem*. **2000**, *48*, 5618−5623.
(25) Ader, P.; Black, M.; Pietzsch, S.; Wolfrom, S. Interaction of quercetin glucosides with the intestinal sodium/glucose cotransporter (SGLT-1). *Cancer Lett*. **2001**, *162*, 175−180.
(26) Vessal, M.; Hemmati, M.; Vasei, M. Antidiabetic effects of quercetin in streptozotocin-induced diabetic rats. *Comp. Biochem. Physiol., Part C: Pharmacol., Toxicol., Endocrinol.* **2003**, *135*, 357−364.
(27) Cermak, R.; Landgraf, S.; Wolffram, S. Quercetin glucosides inhibit glucose uptake into brush border-membrane vesicles of porcine jejunum. *Br. J. Nutr*. **2004**, *91*, 849−855.
(28) Jung, V. J.; Lee, M.-K.; Jeong, K.-S.; Choi, M.-S. The hypoglycemic effects of hesperidin and naringin are partly mediated by hepatic glucose-regulating enzymes in C57BL/KSJ-db/db mice. *J. Nutr*. **2004**, *134*, 2499−2503.
(29) Strobel, P.; Allard, C.; Perez-Acle, T.; Calderon, R.; Aldunate, R.; Leighton, F. Myricetin, quercetin and catechin-gallate inhibit glucose uptake in isolated rat adipocytes. *Biochem. J*. **2004**.
(30) Ong, K. C.; Khoo, H. E. Insulinomimetic effects of myricetin on lipogenesis and glucose transport in rat adipocytes but not glucose transport translocation. *Biochem. Pharmacol*. **1996**, *51*, 423−429.
(31) Jorge, A. P.; Horst, H.; de Sousa, E.; Pizzolatti, M. G.; Silva, F. R. Insulinomimetic effects of kaempferitrin on glycaemia and on 14C-glucose uptake in rat soleus muscle. *Chem.-Biol. Interact.* **2004**, *149*, 89−96.
(32) Martin, H.-J.; Kornmann, F.; Fuhrmann, G. F. The inhibitory effects of flavonoids and antiestrogens on the Glut 1 glucose transporter in human erythrocytes. *Chem-Biol. Interact.* **2003**, *146*, 225−235.
(33) Schroppel, B.; Fischereder, M.; Wiese, P.; Segerer, S.; Huber, S.; Kretzler, M.; Heiss, P.; Sitter, T.; Schlondorff, D. Expression of glucose transporters in human peritoneal mesothelial cells. *Kid. Int*. **1998**, *53*, 1278−1287.
(34) Zhou, L.; Cryan, E. V.; D'Andrea, M. R.; Belkowski, S.; Conway, B. R.; Demarest, J. T. Human cardiomyocytes express high level of Na^+/glucose cotransporter 1 (SGLTI). *J. Cell. Biochem*. **2003**, *90*, 339−346.
(35) Elfeber, K.; Kohler, A.; Lutzenberg, M.; Osswald, C.; Galla, H. J.; Witte, O. W.; Koepsell, H. Localization of the Na^+-D-glucose cotransporter SGLT1 in the blood-brain barrier. *Histochem. Cell. Biol.* **2004**, *121*, 201−207.
(36) Arakawa, K.; Ishihara, T.; Oku, A.; Nawano, M.; Veta, K.; Kitamura, K.; Matsumoto, M.; Saito, A. Improved diabetic syndrome in C57Bl/KsJ-db/db mice by oral administration of the Na^+-glucose cotransporter inhibitor T-1095. *Br. J. Pharmacol.* **2001**, *132*, 578−586.
(37) Link, J. T.; Sorensen, B. K. A method for preparing C-glycosides related to phlorizin. *Tetrahedron Lett*. **2000**, *41*, 9213−9217.
(38) Vedavanam, K.; Srijayanta, S.; O'Reilly, J.; Raman, A.; Wiseman, H. Antioxidant action and potential antidiabetic properties of an isoflavonoid-containing soyabean phytochemical extract (SPE). *Phytother. Res.* **1999**, *13*, 601−608.
(39) Wolffram, S.; Block, M.; Ader, P. Quercetin-3-glucoside is transported by the glucose carrier SGLT1 across the brush border membrane of the rat small intestine. *J. Nutr*. **2002**, *132*, 630−635.

(40) Chen, W. C.; Hayakawa, S.; Yamamoto, T.; Su, H. Cl.; Liu, I. M.; Cheng, J. T. Mediation of beta-endorphin by the isoflavone puerarin to lower plasma glucose in streptozotocin-induced diabetic rats. *Planta Med.* **2004**, *70*, 113.

Received for review May 19, 2005. Accepted August 10, 2005. Studies on isoflavones are supported by a grant-in-aid from the National Center for Complementary and Alternative Medicine-sponsored Purdue-UAB Botanicals Center for Age-Related Diseases (P50 AT-00477, Connie Weaver, PI). Studies on glucose uptake and metabolism were supported in part by a grant-in-aid from the American Heart Association, Southeast Affiliate (E.M., PI). Operation of the UAB Comprehensive Cancer Center Mass Spectrometry Shared Facility was supported in part by a NCI Core Research Support Grant to the UAB Comprehensive Cancer Center (P30 CA-13148). The mass spectrometers used in our studies were purchased by funds from NIH/NCRR Shared Instrumentation Grants (S10 RR-06487 and RR-19231).

JF058105E