JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

DOI:10.1021/jf9008197

Dietary Phloridzin Reduces Blood Glucose Levels and Reverses *Sglt1* Expression in the Small Intestine in Streptozotocin-Induced Diabetic Mice

SAEKO MASUMOTO, YUKARI AKIMOTO, HIDEAKI OIKE, AND MASUKO KOBORI*

National Food Research Institute, Tsukuba, Ibaraki 305-8642, Japan

Phloridzin is a dihydrochalcone typically contained in apples. In this study, it is shown that a diet containing 0.5% phloridzin significantly reduced the blood glucose levels in streptozotocin (STZ)-induced diabetic mice after 14 days. We detected phloridzin in the plasma of STZ-induced diabetic mice fed the phloridzin diet for 14 days, although its concentration was much lower than that of the phloridzin metabolites. A quantitative RT-PCR analysis showed a reversal of STZ induction of the sodium/glucose cotransporter gene *Sglt1* and the drug-metabolizing enzyme genes *Cyp2b10* and *Ephx1* in the small intestine of mice fed a 0.5% phloridzin diet. These mice also showed a reversal of the STZ-mediated renal induction of the glucose-regulated facilitated glucose transporter gene *Glut2.* Dietary phloridzin improved the abnormal elevations in blood glucose levels and the over-expression of *Sglt1*, *Cyp2b10*, and *Ephx1* in the small intestine of STZ-induced diabetic mice.

KEYWORDS: Phloridzin (phlorizin); diabetes; streptozotocin (STZ); sodium/glucose cotransporter 1 (SGLT1); glucose transporter 2 (GLUT2); Cyp2b10

INTRODUCTION

Epidemiological studies have shown that flavonoid intakes reduce the risk of chronic diseases, such as cardiovascular or cerebrovascular disease, cancer, and diabetes (*1−4*). Flavonoids are suggested to prevent chronic diseases through scavenging free radicals, inhibiting lipid peroxidation, and other antioxidative actions (*1−4*). Apples, which contain quercetin glycosides, procyanidins, chlorogenic acid, epicatechin, and dihydrochalcones, are major dietary sources of flavonoids (*2*, *3*, *5*, *6*) and have been reported to reduce the risk of cancer, cardiovascular disease, type 2 diabetes, and asthma (*2*, *3*, *7−10*).

Phloridzin (phloretin-2′-*o*-glucoside) is one of the dihydrochalcones typically contained in apples and processed apple foods such as ciders, juice, and purées (*5*, *11−16*) and has been suggested to contribute to the antioxidant activity of apples (*11*, *16*, *17*). Wodylo et al. reported that apple varieties contained 3−303 mg/kg of phloridzin, and among the varieties, Starkrimson contained the highest amount of phloridzin (*16*). Phloridzin was reported to be relatively stable during storage (*13*, *15*). A distinctive physiological property of phloridzin is the specific and competitive inhibition of sodium/glucose cotransporters (SGLTs) in the intestine (SGLT1) and kidney (SGLT2) (*18−20*). Subcutaneously injected phloridzin has been shown to improve hyperglycemia in rodent models of both type 1 and type 2 diabetes by inhibiting renal glucose reabsorption and promoting glucose excretion into urine (*21−23*). However, ingested phloridzin is mostly hydrolyzed to phloretin and glucose in the small intestine by lactase−phloridzin hydrolase (*24*, *25*). Although phloridzin orally administered with glucose has been reported to reduce postprandial blood glucose levels in mice (*26*), few other reports exist on the antidiabetic effect of orally administered phloridzin. Oral administration of the phloridzin derivative T-1095, which is easily absorbed into the circulation, does have antidiabetic properties (*27*).

We previously reported that STZ-induced diabetic mice fed a diet containing quercetin had reduced blood glucose levels, elevated plasma insulin levels, and decreased levels of the oxidative stress marker thiobarbituric acid reactive substances (TBARS) in the liver and pancreas (*28*). Our results suggested that dietary quercetin improved liver and pancreas functions by enabling the recovery of cell proliferation through reducing oxidative stress and inhibiting the expression of the cyclin dependent kinase inhibitor *p21*(*WAF1*/*Cip1*) (*Cdkn1a*) (*28*).

Since phloridzin has antioxidant properties and inhibits the SGLT1 and SGLT2 sugar transporters, dietary phloridzin may improve diabetic symptoms by suppressing glucose absorption in the small intestine and/or by suppressing oxidative stress in STZ-treated mice. In this article, we examined the effect of a diet containing phloridzin on diabetic symptoms and gene expressions in STZ-induced diabetic mice. We report for the first time the detection of phloridzin in the plasma of STZ-induced diabetic mice fed a phloridzin-containing diet.

MATERIALS AND METHODS

Animals and Treatments. Six-week-old male BALB/c mice were obtained from the Institute for Animal Reproduction, Charles River Japan, Inc. (Ibaraki, Japan). The mice were housed under conditions of 24 ± 1 °C, 55 ± 5% humidity, and 12 h light/dark photocycles (dark period from 0800 to 2000), with free access to water and an AIN-93G diet (Oriental Yeast Co., Tokyo, Japan), for a week prior to the experiment. A diabetic state was induced by a single intraperitoneal injection of STZ

*To whom correspondence should be addressed. Tel: +81-29-838-8041. Fax: +81-29-838-7996. E-mail: kobori@affrc.go.jp.

© 2009 American Chemical Society
Published on Web 05/04/2009

(Wako Pure Chemicals Industries, Osaka, Japan, 183 mg/kg dissolved in 0.05 M citrate buffer, pH 4.5, immediately before use). After 1 week, tail vein blood was sampled, and the blood glucose levels of the sample were measured using a blood glucose test meter: GLUCOCARD DIAMETER-α GT-1661 (ARKRAY Inc., Kyoto, Japan). The mice with nonfasting blood glucose levels of 330−590 mg/dL were divided into 3 groups of 6 mice each (diabetic control, diabetic-0.1%, or diabetic-0.5% phloridzin). One group of 6 untreated mice was included as a normal control. The normal control group and diabetic control group were fed an AIN-93G diet, and the diabetic-phloridzin groups were fed an AIN-93G diet containing 0.1% or 0.5% phloridzin (Funakoshi Ltd., Tokyo, Japan). All mice had access to food and water ad libitum during the experimental period. Body weight and blood glucose levels were monitored at weekly intervals. After 14 days, the mice were euthanized by bleeding from the abdominal aorta under anesthesia with pentobarbital, and blood, jejunum, and kidney tissues were immediately collected. Plasma was immediately separated by centrifugation at 3000 rpm for 15 min at 4 °C. Urine was collected from the bladder. The plasma, urine, and tissues were store at −80 °C until use. The animals were treated in accordance with the basic guidelines of the Ministry of Agriculture, Forestry and Fisheries for laboratory animal studies. The animal studies were approved by the Review Board of Animal Ethics of our institute (approved number H20-008).

Measurement of Plasma Insulin Levels, Urinary Glucose Levels, and Tissue Lipid Peroxidation. Plasma insulin levels were determined using a Mouse Insulin ELISA KIT AKIRIN-011 T (Shibayagi, Gunma, Japan) according to the manufacturer's instructions. Urinary glucose levels were measured using a Glucose CII Test Wako kit (Wako Pure Chemicals Industries, Osaka, Japan). Lipid peroxidation in tissues was measured by TBARS using an OXI-TEK TBARS Assay kit (ZeptoMetrix, NY, USA).

Measurement of Plasma Levels of Phloridzin and the Metabolites. Plasma samples were mixed with sulfatase type H-1 (Sigma Chemical Ltd., MO, USA) solution in 0.1 M sodium acetate buffer at pH 5.0. This enzyme preparation contained 600 units of β-glucuronidase and 20 units of sulfatase. The mixture was then incubated at 37 °C for 1 h. Released phloridzin and phloretin were extracted by adding methanol/acetic acid (100:5, v/v) to the reaction mixture, vortexing for 5 min and centrifuging for 15 min at 4 °C and 10000*g*. The supernatant was applied to an HPLC column (Ineartsill ODS-3, 3 μm, 150 mm × 2.1 mm, GL Science, Tokyo, Japan). The mobile phase was composed of isocratic 40% MeOH/0.1 M KH_2PO_4 (pH 2.0), and the flow late was 0.3 mL/min. Detection was performed using an electro chemical detector (Coulochem III, ESA, Chelmsford, MA) with the first electrode potential of +200 mV and second electrode potential of +500 mV (phloretin), and an UV detector (SPD-M20A, Shimadzu Ltd., Kyoto, Japan) set at UV 280 nm (phloridzin). The standard phloretin was purchased from Funakoshi, Ltd. (Tokyo, Japan).

RNA Isolation and cDNA Microarray Analysis. Total RNA was extracted from tissues using an RNeasy Midi Kit (Qiagen KK, Tokyo, Japan) according to the manufacturer's instructions. Double-stranded cDNA was synthesized from the total RNA of each mouse using the One-Cycle cDNA Synthesis Kit (Affymetrix Japan KK, Tokyo, Japan) with a T7-$(dT)_{24}$ primer. Biotin-labeled cRNA was then synthesized using an IVT Labeling Kit (Affymetrix Japan KK). Biotin-labeled cRNA was further purified and fragmented using the Sample Cleanup Module (Affymetrix Japan KK). Fifteen microgram aliquots of fragmented cRNA were hybridized to an array (Mouse Genome 430 2.0 array, Affymetrix Japan KK) at 45 °C for 16 h. After hybridization, the gene chips were washed and stained using a GeneChip Fluidics Station 450 (Affymetrix Japan KK), and then scanned with a GeneGhip Scanner (Affymetrix Japan KK) with GeneGhip Operation Software, version 1.3 (Affymetrix Japan KK). Three biological replicates from each representative group were selected at random for microarray analysis of the small intestine, while we determined hepatic gene expressions of all 6 mice of each group using a DNA microarray.

Data analysis was performed with a Microarray Suite and GeneSpring, version 7.3.1 (Agilent Technologies, Santa Clara, CA). Statistical analysis of differences in gene expression levels between the dosages was performed by Welch's one-way ANOVA.

Quantitative Reverse Transcription (RT)-PCR Analysis. Total RNA was extracted from the jejunum or kidneys using an RNeasy Midi Kit (Qiagen KK, Tokyo, Japan) according to the manufacturer's instructions. Quantitative RT-PCR was performed with an ABI PRISM 7000 Sequence Detection System (Applied Biosystems Japan, Ltd.) using SYBR Green Real-time PCR Master Mix (Toyobo Co. Osaka, Japan), according to the manufacturer's protocol. Sequences of primers used for quantitative RT-PCR were as follows: *Sglt1* (also known as *Slc5a1*), 5′-aagatccggaagaaggcatc-3′, and 5′-caatcagcacgaggatgaac-3′; *Sglt2* (also known as *Slc5a2*), 5′-tattggtgcagcgatcagg-3′, and 5′-cccagctttgatgtgagtcag-3′; *Glut2* (also known as *Slc2a2*), 5′-aattaccgacagcccatcct-3′, and 5′-gccagctgtctgaaaaatcg-3′; *Cyp2b10*, 5′-tggagctgttctatcagaccttt-3′, and 5′-agccagagaagagctcaaaca-3′; *Ephx1*, 5′-tcttcacggctgtctctgtg-3′, and 5′-aggcccacaggagagtcat-3′; glyceraldehyde-3-phosphate dehydrogenase (*Gapdh*), 5′-atcccagagctgaacg-3′, and 5′-gaagtcgcaggagaca-3′. The relative amount of each transcript was normalized to the amount of *Gapdh* transcript in the same cDNA.

Statistical Analysis. The significance of differences between groups was determined by ANOVA followed by two-tailed multiple *t*-tests with the Bonferroni correction. A *p*-value of less than 0.05 was considered statistically significant.

RESULTS

Dietary Phloridzin Improves the Blood Glucose Level in STZ-Induced Diabetic Mice. Over days 7−21 after STZ-injection, there was a further increase in blood glucose levels compared to the that of the untreated control group (**Figure 1**). The blood glucose levels of STZ-treated mice fed a 0.5% phloridzin diet were significantly lower ($p < 0.01$) after 14 days than the STZ-treated control mice fed a basal diet (**Figure 1** and **Table 1**). Body weights and plasma insulin levels were decreased in the STZ-treated control mice compared to those in the untreated control group ($p < 0.01$) (**Table 1**). However, neither phloridzin diet (0.1% and 0.5%) increased body weights and plasma insulin levels in STZ-treated diabetic mice (**Table 1**). Urinary glucose levels increased in the STZ-treated control mice compared to those in the untreated control mice ($p < 0.01$) (**Table 1**), but neither phloridzin diet (0.1% and 0.5%) affected urinary glucose levels in STZ-induced diabetic mice (**Table 1**).

Dietary Phloridzin Has No Effect on Oxidative Stress or Hepatic Gene Expression in STZ-Induced Diabetic Mice. While STZ-treated mice had increased levels of the oxidative stress marker TBARS in both the liver and kidney (**Table 1**), neither phloridzin diet (0.1% and 0.5%) affected the TBARS levels in the liver and kidney in these mice (**Table 1**). We next used DNA microarrays to analyze hepatic gene expression in untreated control mice and

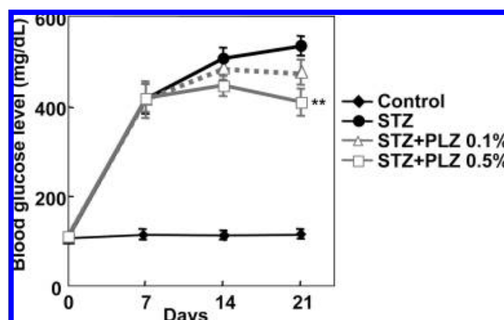

Figure 1. Effects of STZ treatment and a phloridzin diet on blood glucose levels in BALB/c mice. Mice were injected with STZ on day 0 and kept on a basal diet (AIN93G) for 7 days. The diets of 2 groups were then changed to AIN93G containing 0.1 or 0.5% phloridzin. Control, untreated mice fed a basal diet; STZ, STZ-treated mice fed a basal diet; STZ-PLZ 0.1%, STZ-treated mice fed a 0.1% phloridzin diet; STZ-PLZ 0.5%, STZ-treated mice fed a 0.5% phloridzin diet. Data are expressed as the means ± SE of 6 mice in each group. Significant difference from the STZ group, $^{**}p < 0.01$.

Table 1. Effect of STZ-Treated and Phlorizin Supplemented Diet on Weight, Blood Constituents, Urinary Glucose, and Tissue Lipid Peroxidation in BALB/c Mice[a]

	control	STZ	STZ-PLZ 0.1	STZ-PLZ 0.5
weight (g)	27.02 ± 0.88	18.58 ± 0.68 [a]	19.16 ± 0.57 [a]	20.54 ± 0.84 [a]
liver (g)	1.42 ± 0.04	1.14 ± 0.11	1.24 ± 0.08	1.39 ± 0.07
blood glucose (mg/dL)	111 ± 2	531 ± 22 [a]	470 ± 19 [a]	408 ± 19 [ab]
plasma insulin (ng/mL)	3.18 ± 0.06	0.60 ± 0.04 [a]	0.61 ± 0.05 [a]	0.73 ± 0.08 [a]
urinary glucose (mg/dL)	169.7 ± 5.1	6043.2 ± 232.5 [a]	6353.4 ± 339.0 [a]	7035.5 ± 360.1 [a]
TBARS (nmol/g liver)	13.15 ± 0.60	38.82 ± 1.91 [a]	37.70 ± 1.82 [a]	34.47 ± 1.60 [a]
TBARS (nmol/g kidney)	28.70 ± 2.29	80.77 ± 3.54 [a]	78.90 ± 3.38 [a]	78.05 ± 5.92 [a]

[a] Control, untreated mice fed a basal diet (AIN93G); STZ, STZ-treated mice fed a basal diet; STZ-PLZ 0.1, STZ-treated mice fed a 0.1% phloridzin diet; STZ-PLZ 0.5, STZ-treated mice fed a 0.5% phloridzin diet. Mice were kept on the diets ad libitum for 14 days. Values are expressed as the means ± SE of 6 mice (or 3 mice for urinary glucose levels) in each group. The letters indicate significant differences ($p < 0.01$ (two-sided)) from the two-tailed multiple *t*-test with Bonferroni correction following ANOVA.: a, compared to the control group; b, compared to the STZ group.

STZ-injected mice fed 0, 0.1, and 0.5% phloridzin diets for 14 days. Five hundred ninety-one genes were upregulated ($p < 0.05$ by one-way ANOVA), and 93 genes were down-regulated ($p < 0.05$ by one-way ANOVA) in the livers of STZ-treated diabetic mice compared to those in the controls (data not shown). These changes were unaffected by the presence of phloridzin in the diet of these mice (data not shown).

Plasma Concentrations of Phloridzin and Its Metabolites. We next determined the plasma concentrations of phloridzin and the metabolites by HPLC analysis. Phloridzin metabolites were determined as phloretin after hydrolysis. **Table 2** shows the plasma concentration of phloridzin and phloretin in STZ-treated mice fed with a 0.1% or 0.5% phloridzin diet for 14 days. Although the plasma concentration of phloretin is about 30 times higher than those of phloridzin, phloridzin itself is detected in the plasma of STZ-treated mice fed a phloridzin diet (0.1% or 0.5%) (**Table 2**).

Phloridzin Reverses the STZ-Mediated Induction of *Sglt1* in the Small Intestine. We next examined the effect of phloridzin on gene expression in the small intestine of STZ-induced diabetic mice. DNA microarray analysis failed to detect any difference in the gene expression in the small intestine between STZ-treated and untreated mice at a false discovery rate (FDR) of 0.05. However, the mean value of the *Sglt1* expression in the small intestine of STZ-induced diabetic mice was reversed by the 0.5% phloridzin diet (data not shown). We therefore determined the expression levels of *Sglt1* and the facilitative glucose transporter gene *Gult2* in the small intestines of STZ-treated or untreated mice using quantitative RT-PCR. *Sglt1* and *Gult2* were significantly elevated in the small intestine of BALB/c mice (**Figure 2**). The increase in *Sglt1*, but not that of *Gult2*, was reversed in the small intestine of STZ-treated mice by a diet containing 0.5% phloridzin (**Figure 2**).

Phloridzin Reverses the STZ-Mediated Induction of *Cyp2b10* and *Ephx1* in the Small Intestine. DNA microarray analysis showed that the change in expression levels of *Cyp2b10* in the small intestine compared to those of a control group were 6.2 ± 2.8-fold in STZ-treated mice and 3.1 ± 0.7-fold in STZ-treated mice fed a 0.1% phloridzin diet. The expression level changes of *Ephx1* were 5.8 ± 2.5-fold in STZ-treated mice and 2.9 ± 0.2-fold in STZ-treated mice fed a 0.5% phloridzin diet compared to that of a control group. We next used quantitative RT-PCR to determine the expression levels of *Cyp2b10* and *Ephx1* in the small intestine of STZ-treated and control mice. *Cyp2b10* (3.5-fold, $p < 0.01$) and *Ephx1* (3.1-fold, $p < 0.01$) were induced in the small intestine of STZ-treated BALB/c mice (**Figure 3**). While the STZ-mediated induction of *Cyp2b10* in the small intestines was reversed by both the 0.1% ($p < 0.01$) and 0.5% ($p < 0.01$) phloridzin diets (**Figure 3**), the STZ-mediated induction of *Ephx1* in the same tissue was reversed only in mice fed the 0.5% phloridzin diet ($p < 0.01$) (**Figure 3**).

Table 2. Plasma Concentrations of Phloridzin and Its Metabolites in STZ-Treated Mice Fed Phloridzin Diets[a]

	phlorizin (μmol/L)	phloretin (μmol/L)
control	n.d.	n.d.
STZ	n.d.	n.d.
STZ-PLZ 0.1%	1.00 ± 0.21	29.3 ± 9.8
STZ-PLZ 0.5%	2.81 ± 0.85	87.8 ± 13.5

[a] Control, untreated mice fed a basal diet (AIN93G); STZ, STZ-treated mice fed a basal diet; STZ-PLZ 0.1, STZ-treated mice fed a 0.1% phloridzin diet; STZ-PLZ 0.5, STZ-treated mice fed a 0.5% phloridzin diet. Mice were kept on the diets ad libitum for 14 days. Values are expressed as the means ± SE of 6 mice in each group. n.d., not detected.

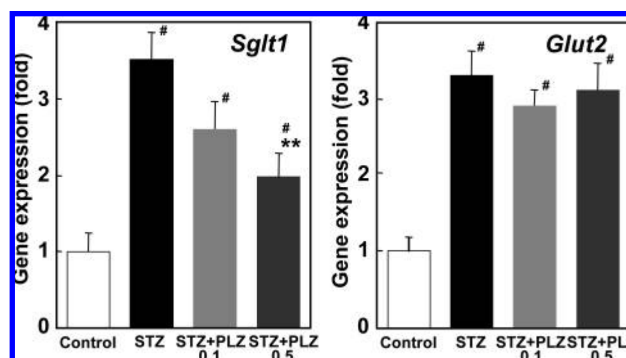

Figure 2. Effects of phloridzin diets on the expression of the sodium glucose cotransporter *Sglt1* and the facilitated glucose transporter *Glut2* in the small intestine of STZ-treated mice. Expression levels were determined by quantitative RT-PCR, normalized against *Gapdh*, and plotted relative to those of control mice. Control, untreated mice fed a basal diet; STZ, STZ-treated mice fed a basal diet; STZ-PLZ 0.1, STZ-treated mice fed a 0.1% phloridzin diet; STZ-PLZ 0.5, STZ-treated mice fed a 0.5% phloridzin diet. Data are expression as the means ± SE of 6 mice in each group. **$p < 0.01$ (two-sided), significant difference from the STZ group; #$p < 0.01$ (two-sided), significant difference from the control group.

Phloridzin Reverses the STZ-Mediated Induction of *Glut2* in Kidneys. SGLT2 is the major renal glucose transporter, while GLUT2 is found on the basolateral surface of proximal renal tubules and transport glucose and fructose (*29*). Phloridzin has been shown to inhibit renal SGLT2 (*18*). We next examined the effect of dietary phloridzin on the expression of *Sglt2* and *Glut2* in the kidneys of STZ-treated diabetic and untreated control mice. RT-PCR analysis shows that *Glut2* (5.2-fold, $p < 0.01$), but not *Sglt2*, is induced in the kidneys of STZ-treated mice (**Figure 4**) and that the induction of *Glut2* is reversed by a diet containing 0.5% phloridzin (**Figure 4**).

DISCUSSION

Subcutaneously injected phloridzin has been shown to improve hyperglycemia in STZ-induced diabetic rats as well as in other

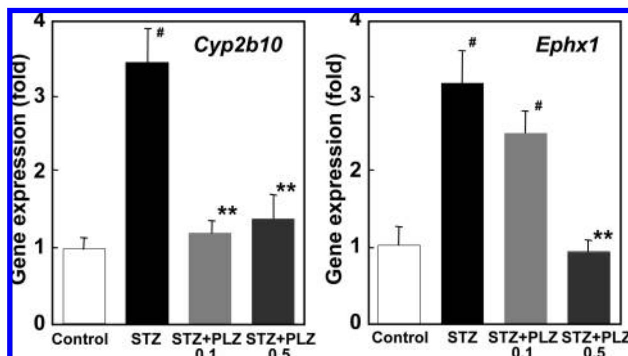

Figure 3. Effects of phloridzin diets on the expression of the drug metabolizing enzyme genes *Cyp2b10* and *Ehpx1* in the small intestine of STZ-treated mice. Expression levels were determined by quantitative RT-PCR, normalized against *Gapdh,* and plotted relative to those of control mice. Control, untreated mice fed a basal diet; STZ, STZ-treated mice fed a basal diet; STZ-PLZ 0.1, STZ-treated mice fed a 0.1% phloridzin diet; STZ-PLZ 0.5, STZ-treated mice fed a 0.5% phloridzin diet. Data are expressed as the means ± SE of 6 mice in each group. $^{**}p < 0.01$ (two-sided), significant difference from the STZ group; $^{\#}p < 0.01$ (two-sided), significant difference from the control group.

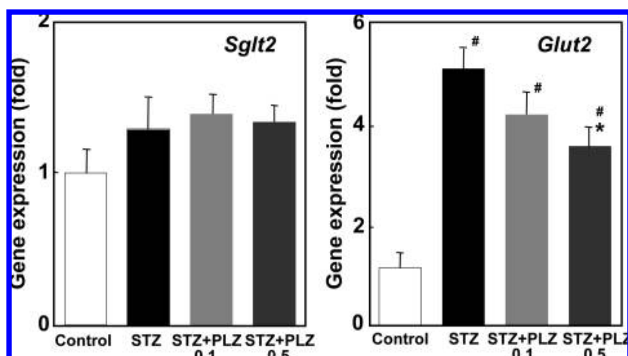

Figure 4. Effects of phloridzin diets on the expression of the *Sglt2* and *Glut2* in the kidneys of STZ-treated mice. Expression levels were determined by quantitative RT-PCR, normalized against *Gapdh*, and plotted relative to those of control mice. Control, untreated mice fed a basal diet; STZ, STZ-treated mice fed a basal diet; STZ-PLZ 0.1, STZ-treated mice fed a 0.1% phloridzin diet; STZ-PLZ 0.5, STZ-treated mice fed a 0.5% phloridzin diet. Data are expressed as the means ± SE of 6 mice in each group. $^{*}p < 0.05$ (two-sided), significant difference from the STZ group; $^{\#}p < 0.01$ (two-sided), significant difference from the control group.

type 1 and type 2 diabetic models (*21−23*). In this study, we show that dietary phloridzin improves hyperglycemia but not hypoinsulinemia and tissue lipid peroxidation in STZ-induced diabetic mice. Although it has been reported that ingested phloridzin is mostly hydrolyzed in the small intestine (*24*, *25*), we show here, to our knowledge for the first time, that phloridzin is present in the plasma of STZ-induced diabetic mice fed a 0.1 or 0.5% phloridzin diet for 14 days. Phloridzin has been reported to be transported by SGLT1 (*30*). Therefore, dietary phloridzin may be partly absorbed by SGLT1 in the small intestine of the STZ-induced diabetic mice. The plasma concentrations of phloridzin in STZ-treated mice fed a 0.5% phloridzin diet was about 3 times higher than that in the mice fed a 0.1% phloridzin diet. The plasma concentration of phloretin and its derivatives is 30 times higher than that of phloridzin in the STZ-treated mice fed phloridzin diets. Phloridzin was reported to be a competitive inhibitor of SGLT1 in the small intestine and SGLT2 in kidneys (*18−20*). Since the concentration of phloridzin was low in the plasma, phloridzin probably suppressed the glucose absorption by SGLT1 in the small intestine but not by SGLT2 in the kidneys.

In our previous study, STZ-treated diabetic mice fed an AIN93G diet containing 0.5% quercetin significantly improved hypoinsulinemia and suppressed hepatic and pancreatic lipid peroxidation (*28*). This diet also suppressed the STZ-induced change in hepatic gene expression (*28*). DNA microarray and RT-PCR analysis suggested that quercetin ameliorated liver and pancreas injury in STZ-induced diabetic mice by promoting cell proliferation following the suppression of *Cdkn1a* expression induced by oxidative stress (*28*). Phloridzin has been shown to possess antioxidant properties (*11*, *17*). However, unlike quercetin, 0.5% phloridzin did not suppress STZ-induced lipid peroxidation in the liver in the current study, indicating that the antioxidant ability of dietary phloridzin is less pronounced than that of dietary quercetin in STZ-induced diabetic mice. Moreover, the 0.5% phloridzin diet fails to improve hypoinsulinemia and has no effect on the alteration of hepatic gene expression in STZ-induced diabetic mice.

Phloridzin has been reported to inhibit SGLT1, which transports glucose from the small intestinal brush-border membrane (*18*, *31*), and a likely mechanism for the phloridzin-mediated reduction in blood glucose levels in STZ-induced diabetic mice is through its inhibition of SGLT1 in the small intestine. In order to examine global gene expression responses to a phloridzin diet, we performed DNA microarray analysis of the small intestine of STZ-treated diabetic and untreated control mice. The arrays indicated that mean values of the *Sglt1*, *Cyp2b10*, and *Ephx1* expressions were increased in STZ-treated control mice (*Sglt1*; 1.6-fold, *Cyp2b10*; 6.2-fold, *Ephx1*; 5.8-fold) compared to those in untreated control mice and decreased in STZ-treated mice fed a 0.5% phloridzin diet (*Sglt1*; 1.4-fold, *Cyp2b10*; 4.4-fold, *Ephx1*; 2.9-fold) compared to those in untreated control mice. RT-PCR analysis showed that STZ significantly induced the expressions of *Sglt1*, *Cyp2b10*, and *Ephx1* in the small intestine and that the expressions were reversed by a 0.5% phloridzin diet.

The expression of *Sglt1* in the small intestine has been reported to be increased in diabetic animals and humans (*32*, *33*). GLUT2 is a low-affinity high-capacity facilitative glucose transporter expressed on the basolateral membrane of renal tubules and intestinal basolateral membrane, and *Glut2* has been shown to be induced in STZ-treated diabetic mice (*33*). Adachi et al. reported that a diet containing the phloridzin derivative T-1095, which is absorbed into the circulation via oral administration, suppressed the STZ-mediated induction of *Sglt1* and *Glut2* in rat small intestines (*34*). Our result confirms that STZ induces the expression of *Sglt1*and *Glut2* in the small intestine. Phloridzin reverses the expression of *Sglt1* but not that of *Glut2* in the small intestine.

The drug-metabolizing enzyme *Cyp2b10* was shown to be an inducible gene in the small intestine by phenobarbital (*35*) and in the liver of STZ-induced diabetic mice (*36*). The expression of both *Cyp2b10* and *Ephx1* is significantly induced in the livers by STZ (data not shown). We have shown in this study that *Cyp2b10* and *Ephx1* are induced in the small intestine of STZ-induced diabetic mice and that phloridzin reverses these effects.

SGLT2 is the major renal glucose transporter and is inhibited by phloridzin. It has been reported that neither STZ nor the 0.1% T-1095 diet affected renal *Sglt2* expression in rats (*34*). Expression of renal *Glut2* has been shown to be increased in STZ-induced diabetic rats (*34*, *37*). The 0.1% T-1095 diet was shown to reverse the renal *Glut2* expression induced by STZ (*32*). Our results show that STZ induces *Glut2* in the kidney but has no effect on renal *Sglt2* and that a diet containing 0.5% phloridzin reverses this effect on *Glut2*. The expression of *Glut2* is regulated by glucose (*38*), and we speculate that the 0.5% phloridzin diet

reverses *Glut2* induction by reducing the blood glucose levels in STZ-treated mice.

In conclusion, we show that dietary phloridzin improves hyperglycemia but not hypoinsulinemia and tissue lipid peroxidation in STZ-induced diabetic mice. Although the concentration is much lower than that of the metabolites, we find that phloridzin is present in the plasma of STZ-treated mice fed both 0.1% and 0.5% phloridzin diets for 14 days. Dietary phloridzin reverses the abnormal overexpression of *Sglt1*, *Cyp2b10*, and *Ephx1* in the small intestine as well as that of *Glut2* in the kidney in STZ-induced diabetic mice.

ABBREVIATIONS USED

STZ, streptozptocin; SGLT, sodium/glucose cotransporter; GLUT, glucose transporter; TBARS, thiobarbituric acid reactive substances; RT-PCR, reverse transcription PCR; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; FDR, false discovery rate.

LITERATURE CITED

(1) Arts, I. C.; Hollman, P. C. Polyphenols and disease risk in epidemiologic studies. *Am. J. Clin. Nutr*. **2005**, *81* (Suppl 1), 317S–325S.
(2) Knekt, P.; Jarvinen, R.; Reunanen, A.; Maatela, J. Flavonoid intake and coronary mortality in Finland: a cohort study. *BMJ* **1996**, *312*, 478–481.
(3) Knekt, P.; Kumpulainen, J.; Jarvinen, R.; Rissanen, H.; Heliovaara, M.; Reunanen, A.; Hakulinen, T.; Aromaa, A. Flavonoid intake and risk of chronic diseases. *Am. J. Clin. Nutr*. **2002**, *76*, 560–568.
(4) Russo, G. L. Ins and outs of dietary phytochemicals in cancer chemoprevention. *Biochem. Pharmacol*. **2007**, *74*, 533–544.
(5) Boyer, J.; Liu, R. H. Apple phytochemicals and their health benefits. *Nutr. J*. **2004**, *3*, 5.
(6) Mennen, L. I.; Sapinho, D.; Ito, H.; Bertrais, S.; Galan, P.; Hercberg, S.; Scalbert, A. Urinary flavonoids and phenolic acids as biomarkers of intake for polyphenol-rich foods. *Br. J. Nutr*. **2006**, *96*, 191–198.
(7) Linseisen, J.; Rohrmann, S.; Miller, A. B.; Bueno-de-Mesquita, H. B.; Buchner, F. L.; Vineis, P.; Agudo, A.; Gram, I. T.; Janson, L.; Krogh, V.; Overvad, K.; Rasmuson, T.; Schulz, M.; Pischon, T.; Kaaks, R.; Nieters, A.; Allen, N. E.; Key, T. J.; Bingham, S.; Khaw, K. T.; Amiano, P.; Barricarte, A.; Martinez, C.; Navarro, C.; Quiros, R.; Clavel-Chapelon, F.; Boutron-Ruault, M. C.; Touvier, M.; Peeters, P. H.; Berglund, G.; Hallmans, G.; Lund, E.; Palli, D.; Panico, S.; Tumino, R.; Tjonneland, A.; Olsen, A.; Trichopoulou, A.; Trichopoulos, D.; Autier, P.; Boffetta, P.; Slimani, N.; Riboli, E. Fruit and vegetable consumption and lung cancer risk: updated information from the European Prospective Investigation into Cancer and Nutrition (EPIC). *Int. J. Cancer* **2007**, *121*, 1103–1114.
(8) Mink, P. J.; Scrafford, C. G.; Barraj, L. M.; Harnack, L.; Hong, C. P.; Nettleton, J. A.; Jacobs, D. R.Jr. Flavonoid intake and cardiovascular disease mortality: a prospective study in postmenopausal women. *Am. J. Clin. Nutr*. **2007**, *85*, 895–909.
(9) Song, Y.; Manson, J. E.; Buring, J. E.; Sesso, H. D.; Liu, S. Associations of dietary flavonoids with risk of type 2 diabetes, and markers of insulin resistance and systemic inflammation in women: a prospective study and cross-sectional analysis. *J. Am. Coll. Nutr*. **2005**, *24*, 376–384.
(10) Woods, R. K.; Walters, E. H.; Raven, J. M.; Wolfe, R.; Ireland, P. D.; Thien, F. C.; Abramson, M. J. Food and nutrient intakes and asthma risk in young adults. *Am. J. Clin. Nutr*. **2003**, *78*, 414–421.
(11) Bellion, P.; Hofmann, T.; Pool-Zobel, B. L.; Will, F.; Dietrich, H.; Knaup, B.; Richling, E.; Baum, M.; Eisenbrand, G.; Janzowski, C. Antioxidant effectiveness of phenolic apple juice extracts and their gut fermentation products in the human colon carcinoma cell line caco-2. *J. Agric. Food Chem*. **2008**, *56*, 6310–6317.
(12) Bengoechea, M. L.; Sancho, A. I.; Bartolomé, B.; Estrella, I.; Gómez-Cordovés, C.; Hernández, M. T. Phenolic composition of industrially manufactured purées and concentrates from peach and apple fruits. *J. Agric. Food Chem*. **1997**, *45*, 4071–4075.
(13) Napolitano, A.; Cascone, A.; Graziani, G.; Ferracane, R.; Scalfi, L.; Di Vaio, C.; Ritieni, A.; Fogliano, V. Influence of variety and storage on the polyphenol composition of apple flesh. *J. Agric. Food Chem*. **2004**, *52*, 6526–6531.
(14) Oleszek, W.; Lee, C. Y.; Jaworski, A. W.; Price, K. R. Identification of some phenolic compounds in apples. *J. Agric. Food Chem*. **1988**, *35*, 430–432.
(15) van der Sluis, A. A.; Dekker, M.; van Boekel, M. A. Activity and concentration of polyphenolic antioxidants in apple juice. 3. Stability during storage. *J. Agric. Food Chem*. **2005**, *53*, 1073–1080.
(16) Wojdylo, A.; Oszmianski, J.; Laskowski, P. Polyphenolic compounds and antioxidant activity of new and old apple varieties. *J. Agric. Food Chem*. **2008**, *56*, 6520–6530.
(17) Lee, K. W.; Kim, Y. J.; Kim, D. O.; Lee, H. J.; Lee, C. Y. Major phenolics in apple and their contribution to the total antioxidant capacity. *J. Agric. Food Chem*. **2003**, *51*, 6516–6520.
(18) Ehrenkranz, J. R.; Lewis, N. G.; Kahn, C. R.; Roth, J. Phlorizin: a review. *Diabetes/Metab. Res. Rev*. **2005**, *21*, 31–38.
(19) Pajor, A. M.; Randolph, K. M.; Kerner, S. A.; Smith, C. D. Inhibitor binding in the human renal low- and high-affinity Na+/glucose cotransporters. *J. Pharmacol. Exp. Ther*. **2008**, *324*, 985–991.
(20) Tyagi, N. K.; Kumar, A.; Goyal, P.; Pandey, D.; Siess, W.; Kinne, R. K. D-Glucose-recognition and phlorizin-binding sites in human sodium/D-glucose cotransporter 1 (hSGLT1): a tryptophan scanning study. *Biochemistry* **2007**, *46*, 13616–13628.
(21) Liu, I. M.; Chang, C. K.; Juang, S. W.; Kou, D. H.; Tong, Y. C.; Cheng, K. C.; Cheng, J. T. Role of hyperglycaemia in the pathogenesis of hypotension observed in type-1 diabetic rats. *Int. J. Exp. Pathol*. **2008**, *89*, 292–300.
(22) Rossetti, L.; Smith, D.; Shulman, G. I.; Papachristou, D.; DeFronzo, R. A. Correction of hyperglycemia with phlorizin normalizes tissue sensitivity to insulin in diabetic rats. *J. Clin. Invest*. **1987**, *79*, 1510–1515.
(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) Crespy, V.; Aprikian, O.; Morand, C.; Besson, C.; Manach, C.; Demigne, C.; Remesy, C. Bioavailability of phloretin and phloridzin in rats. *J. Nutr*. **2001**, *131*, 3227–3230.
(25) Nemeth, K.; Plumb, G. W.; Berrin, J. G.; Juge, N.; Jacob, R.; Naim, H. Y.; Williamson, G.; Swallow, D. M.; Kroon, P. A. Deglycosylation by small intestinal epithelial cell beta-glucosidases is a critical step in the absorption and metabolism of dietary flavonoid glycosides in humans. *Eur. J. Nutr*. **2003**, *42*, 29–42.
(26) Takii, H.; Matsumoto, K.; Kometani, T.; Okada, S.; Fushiki, T. Lowering effect of phenolic glycosides on the rise in postprandial glucose in mice. *Biosci. Biotechnol. Biochem*. **1997**, *61*, 1531–1435.
(27) Oku, A.; Ueta, K.; Arakawa, K.; Ishihara, T.; Nawano, M.; Kuronuma, Y.; Matsumoto, M.; Saito, A.; Tsujihara, K.; Anai, M.; Asano, T.; Kanai, Y.; Endou, H. T-1095, an inhibitor of renal Na+-glucose cotransporters, may provide a novel approach to treating diabetes. *Diabetes* **1999**, *48*, 1794–1800.
(28) Kobori, M.; Masumoto, S.; Akimoto, Y.; Takahashi, Y. Dietary quercetin alleviates diabetic symptoms and reduces streptozotocin-induced disturbance of hepatic gene expression in mice. *Mol. Nutr. Food Res*. 2009, in press.
(29) Wood, I. S.; Trayhurn, P. Glucose transporters (GLUT and SGLT): expanded families of sugar transport proteins. *Br. J. Nutr*. **2003**, *89*, 3–9.
(30) Walle, T.; Walle, U. K. The beta-D-glucoside and sodium-dependent glucose transporter 1 (SGLT1)-inhibitor phloridzin is transported by both SGLT1 and multidrug resistance-associated proteins 1/2. *Drug Metab. Dispos*. **2003**, *31*, 1288–1291.
(31) Drozdowski, L. A.; Thomson, A. B. Intestinal sugar transport. *World J. Gastroenterol*. **2006**, *12*, 1657–1670.
(32) Dyer, J.; Wood, I. S.; Palejwala, A.; Ellis, A.; Shirazi-Beechey, S. P. Expression of monosaccharide transporters in intestine of diabetic humans. *Am. J. Physiol. Gastrointest. Liver Physiol.* **2002**, *282*, G241–G248.

(33) Miyamoto, K.; Hase, K.; Taketani, Y.; Minami, H.; Oka, T.; Nakabou, Y.; Hagihira, H. Diabetes and glucose transporter gene expression in rat small intestine. *Biochem. Biophys. Res. Commun*. **1991**, *181*, 1110–1117.

(34) Adachi, T.; Yasuda, K.; Okamoto, Y.; Shihara, N.; Oku, A.; Ueta, K.; Kitamura, K.; Saito, A.; Iwakura, I.; Yamada, Y.; Yano, H.; Seino, Y.; Tsuda, K. T-1095, a renal Na+-glucose transporter inhibitor, improves hyperglycemia in streptozotocin-induced diabetic rats. *Metabolism* **2000**, *49*, 990–995.

(35) Zhang, Q. Y.; Dunbar, D.; Kaminsky, L. S. Characterization of mouse small intestinal cytochrome P450 expression. *Drug Metab. Dispos*. **2003**, *31*, 1346–1351.

(36) Sakuma, T.; Honma, R.; Maguchi, S.; Tamaki, H.; Nemoto, N. Different expression of hepatic and renal cytochrome P450s between the streptozotocin-induced diabetic mouse and rat. *Xenobiotica* **2001**, *31*, 223–237.

(37) Chin, E.; Zamah, A. M.; Landau, D.; Gronbcek, H.; Flyvbjerg, A.; LeRoith, D.; Bondy, C. A. Changes in facilitative glucose transporter messenger ribonucleic acid levels in the diabetic rat kidney. *Endocrinology* **1997**, *138*, 1267–1275.

(38) Waeber, G.; Thompson, N.; Haefliger, J. A.; Nicod, P. Characterization of the murine high Km glucose transporter GLUT2 gene and its transcriptional regulation by glucose in a differentiated insulin-secreting cell line. *J. Biol. Chem*. **1994**, *269*, 26912–26919.

Received for review March 10, 2009. Revised manuscript received April 15, 2009. Accepted April 15, 2009. This work was financially supported in part by a grant from the Ministry of Agriculture, Forestry and Fisheries (MAFF) Food Research Project 'Development of evaluation and management methods for supply of safe, reliable and functional food and farm produce.'