JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

J. Agric. Food Chem. 2009, 57, 7687–7692 7687
DOI:10.1021/jf901376x

Effects of Caffeic Acid and Cinnamic Acid on Glucose Uptake in Insulin-Resistant Mouse Hepatocytes

Da-Wei Huang,[†] Szu-Chuan Shen,[§] and James Swi-Bea Wu*,[†]

[†]Graduate Institute of Food Science and Technology, College of Bioresources and Agriculture, National Taiwan University, Taipei 106, Taiwan, and [§]Department of Medical Nutrition, College of Medicine, I-Shou University, Yanchao Township, Kaohsiung 840, Taiwan

Tumor necrosis factor-α was used to induce insulin resistance of mouse liver FL83B cells. Two phenolic acids, caffeic acid and cinnamic acid, were then added separately to investigate their effects on glucose uptake of the insulin-resistant cells. The results suggest that these two phenolic acids may promote insulin receptor tyrosyl phosphorylation, up-regulate the expression of insulin signal associated proteins, including insulin receptor, phosphatidylinositol-3 kinase, glycogen synthase, and glucose transporter-2, increase the uptake of glucose, and alleviate insulin resistance in cells as a consequence.

KEYWORDS: Diabetes mellitus; caffeic acid; cinnamic acid; glucose uptake; insulin resistance

INTRODUCTION

Diabetes mellitus (DM) is a chronic disease associated with carbohydrate metabolism (*1*) and is caused by a deficiency in insulin secretion or by ineffectiveness in insulin action (*2*). About 95% of the diabetic incidence belongs to type 2 diabetes, of which the main cause of hyperglycemia is the ineffectiveness in insulin action or the inability to induce a normal response to insulin in the cells (*3*−*6*).

In insulin signal transduction, insulin binds to its transmembrane receptor and activates the insulin signaling cascade, which mediates the metabolic and growth-promoting functions, such as glucose transport, stimulation of glycogen and protein synthesis, and initiation of specific gene transcription (*7*). The proinflammatory cytokines, including TNF-α, interleukin-1 (IL-1), interleukin-6 (IL-6), and interferon-γ (INF-γ), are involved in the induction of insulin resistance (*8*, *9*). High levels of circulating TNF-α may be associated with insulin-resistant state in animals (*10*, *11*). The administration of exogenous TNF-α to cells and animals may induce insulin resistance (*12*, *13*). The literature indicates that TNF-α plays a pivotal role in the pathogenesis of insulin resistance by the impairment of insulin signal transduction (*8*, *14*−*16*). The possible mechanisms for TNF-α to impair insulin signal transduction involve the down-regulation of insulin receptor (IR) and insulin receptor substrate-1 (IRS-1) expressions, the inhibition of tyrosyl phosphorylation of IR and IRS-1, the increase in serine/threonine phosphorylation of IRS-1, the decrease in the activities of insulin receptor kinase and protein tyrosine phosphatases (PTPs), and the inhibition of insulin-stimulated glucose transporter (*12*, *15*).

Phenolic compounds are widely distributed in the plant kingdom. They may prevent the occurrence of atherosclerosis, cardiovascular disease, and cancer (*17*, *18*). In a previous study, the phenolic compound quercetin was identified as the possible major active compound for the antihyperglycemic effect of guava leaf extract (*19*). Among the other phenolic compounds, caffeic acid and cinnamic acid are two phenolic acids that are commonly present in many fruits, vegetables, and coffee (*18*, *20*, *21*). The reported pharmacologic properties of caffeic acid include antioxidative, anticancer, and antimutagenic activities (*17*, *18*). Cinnamic acid exhibits hepatoprotective, antimalarial, and antioxidative activities (*21*). Anti-hyperglycemic activity has become a focus in recent studies on these two phenolic acids. Jung et al. and Adisakwattana et al. reported that caffeic acid and cinnamic acid are antihyperglycemic and proposed the stimulation of insulin secretion from pancreatic β-cells to be the mechanism (*18*, *21*). However, the antihyperglycemic effect may also result from the alleviation of insulin resistance of target cells. The present study was therefore aimed to investigate the involvement of insulin resistance alleviation in the mechanisms of antihyperglycemia of caffeic acid and cinnamic acid.

Two normal cell lines, C_2C_{12} muscle cells and 3T3-L1 adipose cells, are commonly used as in vitro models for investigating the effect of antidiabetic agents (*22*, *23*). The differentiation of these cell lines for the acquisition of insulin sensitivity needs to be completed first, which increases the complexity in the execution of experiments. FL83B cells from mouse liver are a better alternative because they need no differentiation to gain insulin sensitivity (*24*). In the present study, FL83B cells were treated with TNF-α to induce insulin resistance for evaluating the antihyperglycemic effects of caffeic acid and cinnamic acid. The uptake of radioactive glucose analogue 2-[1-^{14}C]deoxy-D-glucose (2-DG) in insulin-resistant FL83B cells was monitored. The expressions of insulin signal associated proteins in the treated FL83B cells were analyzed to elucidate the mechanism of antihyperglycemia for these two phenolic acids.

MATERIALS AND METHODS

Chemicals and Reagents. The FL83B cell line from the liver of a 15−17-day-old fetal mouse was supplied by Bioresource Collection and

*Corresponding author (telephone 886-233664117; fax 886-223620849; e-mail jsbwu@ntu.edu.tw).

Published on Web 08/17/2009

Research Center (BCRC), Hsinchu City, Taiwan. Insulin, D-glucose, caffeic acid, cinnamic acid, recombinant mouse TNF-α, and F12 Ham Kaighn's modification (F12K) medium were purchased from Sigma Co. (St. Louis, MO). Fetal bovine serum (FBS) was from Gemini Bio-Products (Woodland, CA). The radioactive 2-DG was from Perkin-Elmer Life and Analytical Science (Boston, MA). The Bio-Rad protein assay dye was from Bio-Rad Laboratories (Hercules, CA). All of the chemicals used in this study were of analytical grade.

Cell Culture. FL83B cells were cultured in F12K medium containing 10% FBS and 1% penicillin and streptomycin mix (Invitrogen Corp., Camarillo, CA) in 10 cm Petri dishes at 37 °C in a 5% CO_2 environment.

Induction of Insulin Resistance by TNF-α. The FL83B cells were seeded in 10 cm dishes and incubated at 37 °C for 48 h to reach 80% confluence; serum-free F12K medium containing 10, 30, or 50 ng/mL recombinant mouse TNF-α was added, and incubation was continued for 5 h for the induction of insulin resistance.

Glucose Uptake. The assay for glucose uptake followed the method reported by Cheng et al. (*19*). Briefly, the insulin-resistant FL83 cells were detached from the dish by trypsin treatment and then suspended in Hank's balanced salt solution (HBSS; containing 5 mM glucose and 5.0 μg/mL insulin). Each 20 μL aliquot of the cell suspension was amended with 40 μL of HBSS and 20 μL of 2-DG (0.25 μCi mL^{-1}) and then incubated at 37 °C for 10 min. The reaction was terminated by cooling on ice, followed by centrifugation (8000*g*, 3 min). The cell pellet was dissolved in 50 μL of 1 N NaOH and then set aside for 90 min before neutralization with 50 μL of 1 N HCl. After the lytic reaction, 3 mL of aqueous counting scintillant (ASC; Amersham, Arlington Heights, IL) was added. The radioactivity was then determined using a β-counter (model LS5000CE, Beckman, CA). Specific 2-DG uptake was expressed as counts per mimute per milligram of protein. Insulin at 5.0 μg/mL was used in the preparation of the positive control.

Effect of Caffeic Acid and Cinnamic Acid on Glucose Uptake. After incubation in serum-free F12K medium with or without TNF-α (30 ng/mL) at 37 °C for 5 h, FL83B cells were suspended in HBSS containing 5 mM glucose and 5.0 μg/mL insulin. Each 20 μL aliquot of the cell suspension was incubated with the mixture of 20 μL of 12.5 μM caffeic acid or cinnamic acid and 20 μL of HBSS at 37 °C for 30 min with constant shaking. The uptake of 2-DG in the cells was determined according to the above-described procedure.

Western Blot Analysis. After preincubation in serum-free F12K medium with or without 30 ng/mL TNF-α at 37 °C for 5 h, FL83B cells were transferred to another serum-free F12K medium with or without 5.0 μg/mL insulin, 12.5 μM caffeic acid, or cinnamic acid for 3 h. The medium was removed, and the cells were washed twice with ice-cold PBS. The cells were then lysed in ice-cold lysis buffer containing 20 mM Tris-HCl (pH 7.4), 1% Triton X-100, 0.1% SDS, 2 mM EDTA, 10 mM NaF, 1 mM PMSF, 500 μM sodium *o*-vanadate, and 10 μg/mL of aprotinin. Cell lysates were sonicated with ice cooling, four times each 5 s, and then centrifuged (10000*g*, 20 min) to recover the supernatant. The supernatant was taken as the cell extract and stored at −80 °C for further use.

The protein concentration in the cell extract was determined using a Bio-Rad protein assay. Aliquots of the extract each containing 80 μg of protein were taken as the samples for evaluating the expression and the degree of phosphorylation of IR, and the expression of the p85 regulatory subunit of phosphatidylinositol 3-kinase (PI3K) as well. For the evaluation of the glucose transporter-2 (GLUT-2) and glycogen synthase (GS), aliquots of supernatant each containing 40 μg of protein were used. The samples were subjected to 10% sodium dodecyl sulfate−polyacrylamide gel electrophoresis (SDS-PAGE). The protein spots were electrotransferred to a polyvinylidene difluoride membrane. The membrane was incubated with block buffer (phosphate-buffered saline (PBS) solution containing 0.05% Tween-20 and 5% w/v nonfat dry milk) for 1 h, washed with PBS containing 0.05% Tween-20 (PBS-T) three times, and then probed with 1:1000 diluted solution of anti-IR antibody (Cell Signaling Technology, Beverly, MA), 1:1500 diluted solution of antiphosphotyrosine IR, anti-PI3-kinase p85 subunit, anti-GS antibody (Cell Signaling Technology), or anti-GLUT-2 (Millipore Corp., Billerica, MA) overnight at 4 °C. The intensity of the blots probed with 1:1500 diluted solution of mouse monoclonal antibody to bind actin (BD Biosciences, Franklin Lakes, NJ) was used as the control to ensure that a constant amount of protein was loaded into each lane of the gel. The membrane was washed three times each for 5 min in PBS-T, shaken in a solution of HRP-linked anti-mouse IgG or anti-rabbit IgG secondary antibody, washed three more times each for 5 min in PBS-T, and then exposed to the enhanced chemiluminesence (ECL) reagent (Millipore) following the manufacturer's instructions. Autoradiography was performed on Fuji medical X-ray film (Fuji, Tokyo, Japan).

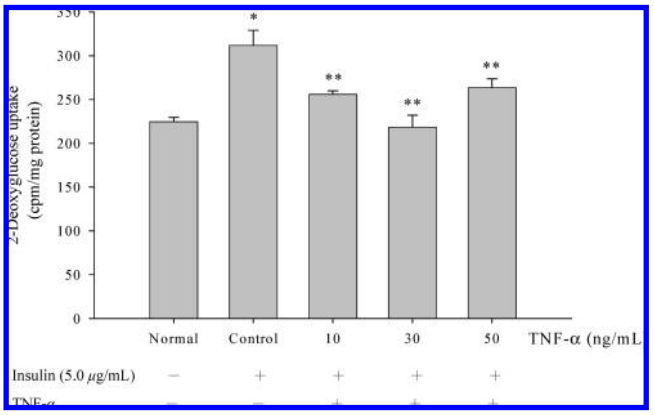

Figure 1. Effect of TNF-α on inhibition of glucose uptake in mouse liver FL83B cells. Normal, cells incubated with serum-free F12K medium alone; control, cells incubated with serum-free F12K medium containing 5.0 μg/mL insulin. ∗, significantly different ($p < 0.01$) from normal; ∗∗, significantly different ($p < 0.01$) from control.

Statistical Analysis. The data were analyzed by one-way ANOVA and Duncan's new multiple-range test. *P* values of < 0.01 were considered to be significant.

RESULTS AND DISCUSSION

Reduction of Glucose Uptake by TNF-α. Cytokines are involved in the development of insulin resistance (*8*−*16*). The negative effect of TNF-α on insulin action was mediated by inhibition on the insulin signaling pathway (*12*−*14*). The ability of TNF-α to induce insulin resistance in FL83B cells has been demonstrated previously (*24*). **Figure 1** shows the results in the present study on treating mouse liver FL83B cells with insulin and TNF-α. Insulin (5.0 μg/mL) significantly increased glucose uptake in the liver cells, from 224.6 ± 4.9 cpm/mg of protein in the normal group to 311.8 ± 17.2 cpm/mg of protein in the positive control, or a 38.8% increment. The enhancement in glucose uptake of the cells by insulin is thus proved. **Figure 1** also shows that TNF-α significantly reduced the glucose uptake. For example, treating the cells with 30 ng/mL TNF-α followed by 5.0 μg/mL insulin reduced glucose uptake from 311.8 ± 17.2 cpm/mg of protein (the positive control) to 218.4 ± 13.8 cpm/mg of protein, or a 30.0% decrement. We observed that TNF-α impairs insulin sensitivity to induce insulin resistance and to reduce glucose uptake in FL83B cells. These results reconfirmed that cytokine TNF-α is a mediator of insulin resistance. Incubation with 30 ng/mL TNF-α is therefore proved to be a proper treatment for preparing insulin-resistant FL83B cells.

Effect of Caffeic Acid and Cinnamic Acid on Glucose Uptake of Insulin-Resistant FL83B Cells. Phenolic acids are secondary plant metabolites having a hydroxycinnamic or hydroxybenzoic structure. A few phenolic acids have been reported to be antihyperglycemic (*25*−*27*). Among them, isoferulic acid was found to reduce the plasma glucose concentration in streptozotocin (STZ)-induced diabetic rats via the suppression of hepatic gluconeogenesis (*25*). Ferulic acid has been reported to effectively suppress blood glucose level in diabetic mice (*26*), to reduce the toxicity of STZ, to promote the proliferation of

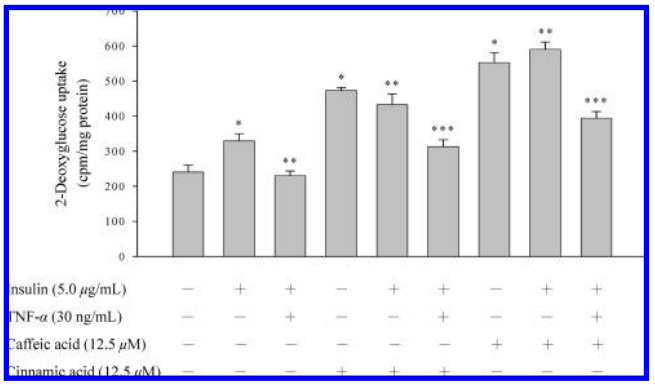

Figure 2. Effect of caffeic acid and cinnamic acid on TNF-α-induced inhibition of glucose uptake in FL83B cells. FL83B cells were incubated in serum-free F12K medium, with or without added TNF-α (30 ng/mL), at 37 °C for 5 h, and then further incubated in HBSS with or without insulin (5.0 μg/mL), caffeic acid (12.5 μM), or cinnamic aicd (12.5 μM) for 30 min. The basal level of glucose uptake was evaluated by incubating the cells with HBSS. ∗, significantly different ($p < 0.01$) from normal; ∗∗, significantly different ($p < 0.01$) from insulin-treated group; ∗∗∗, significantly different ($p < 0.01$) from TNF-α and insulin treated group.

pancreatic β-cells, and to restore the insulin secretion ability (*27*). The phenolic acids used in the present study, caffeic acid and cinnamic acid, may be beneficial for the treatment of diabetes mellitus. For example, caffeic acid was proved to be effective in the reduction of glucose level in C57BL/KsJ-db/db mice (*21*) and in the promotion of glucose uptake in C_2C_{12} cells (*28*). Cinnamic acid and its derivatives were found to increase insulin secretion from pancreatic β-cells and to decrease the plasma glucose level in rats (*18*). The above-mentioned literature indicates that the antihyperglycemic effects of caffeic acid and cinnamic acid result from the promotion of insulin secretion, overlooking the improvement in insulin sensitivity as the other possibility.

The present study used a 2-DG uptake test on insulin-resistant FL83B cells to evaluate the effect of these two phenolic acids on the improvement of insulin sensitivity. Glucose uptake in mouse liver FL83B cells treated with caffeic acid (552.6 ± 28.6 cpm/mg of protein) and cinnamic acid (472.8 ± 8.9 cpm/mg of protein) is 2.3 and 2.0 times of the basal uptake value (240.6 ± 20.2 cpm/mg of protein), respectively. Some other batches of FL83B cells were treated with TNF-α and insulin, followed with caffeic acid or cinnamic acid. The values of glucose uptake in the cells treated with caffeic acid and cinnamic acid are 394.0 ± 19.2 and 313.0 ± 19.6 cpm/mg of protein, respectively, which are 71.0 and 35.9% higher than the value in the cells without phenolic acid treatment (230.4 ± 13.1 cpm/mg of protein) (**Figure 2**). The results reveal that caffeic acid and cinnamic acid significantly increased glucose uptake in normal and TNF-α-induced insulin-resistant FL83B cells. On the basis of these results, we propose that caffeic acid and cinnamic acid help insulin-resistant FL83B cells regain insulin sensitivity, restore the glucose uptake, and promote glucose utilization as a consequence.

We also evaluated the effect of other phenolic acids, including coumaric acid, ferulic acid, and gallic acid, on glucose uptake (data not shown) and found that these acids are less effective than caffeic acid and cinnamic acid. Gallic acid as a hydroxybenzoic acid is less potent than those acids with a hydroxycinnamic structure, including caffeic acid, coumaric acid, and ferulic acid, on glucose uptake. Caffeic acid as a derivative of cinnamic acid containing two hydroxy groups in the structural formula is more potent than those containing single hydroxy group (coumaric acid and ferulic acid). Furthermore, caffeic acid is more potent than cinnamic acid, which has no hydroxyl group in the structure, on glucose uptake (**Figure 2**). The above findings suggest that the hydroxy groups on the cinnamic moiety enhance glucose uptake. There are many other naturally occurring functional phenolic acids, for example, chlorogenic acid, quinic acid, shikimic acid, and tartaric acid, not yet tested. A comprehensive investigation into these acids could be very rewarding.

Effect of Caffeic Acid and Cinnamic Acid on Insulin Signaling in Insulin-Resistant FL83B Cells. *Expression and Tyrosyl Phosphorylaion of IR.* The expression and the tyrosyl phosphorylaion of IR in FL83B cells were not significantly increased by caffeic acid and cinnamic acid as compared with the basal expression or the expression in normal condition (**Figure 3**). Caffeic acid and cinnamic acid significantly increased glucose uptake in normal FL83B cells, indicating that these two phenolic acids promote glucose uptake in normal cells via a pathway involving no increase in the expression and tyrosyl phosphorylation of IR.

In the insulin signaling cascade, insulin binds to IR and triggers tyrosyl phosphorylation, which regulates the homeostasis of glucose (*5*). The addition of 5.0 μg/mL insulin resulted in 15.5 and 34.2% increments in the protein expression and tyrosyl phosphorylation of IR as compared with the basal control. TNF-α (30 ng/mL) reduced IR expression and tyrosyl phosphorylation by 24.6 and 52.8%, respectively, as compared with the positive control. Caffeic acid caused a significant 15.5% increase in IR expression of TNF-α-induced insulin-resistant FL83B cells, whereas cinnamic acid caused no significant changes. Treatments with caffeic acid and cinnamic acid increased the expression of IR tyrosyl phosphorylaion in TNF-α-induced insulin-resistant FL83B cells by 78.7 and 49.8%, respectively (**Figure 3**). These two phenolic acids were thus shown to improve the tyrosyl phosphorylaion of IR, which may restore insulin signaling in insulin-resistant FL83B cells.

IR is a heterotetrameric membrane glycoprotein consisting of two α-subunits and two β-subunits. Insulin binds to the α-subunit and causes a configurational shift that moves the two α-subunits closer to each other and induces autophosphorylation on β-subunits (*5*). The insulin-bound IR causes insulin signaling by phosphorylation on tyrosine residues of several cellular substrates, including insulin receptor substrate (IRS) proteins 1, 2, 3, and 4 (*7*, *29*). TNF-α treatment was reported to decrease the protein expression of IR and to inhibit IR tyrosyl phosphorylation in insulin-stimulated 3T3-L1 and myeloid 32D cells (*8*). Caffeic acid and cinnamic acid are able to ameliorate the protein expressions of IR and IR tyrosyl phosphorylation in TNF-α-induced insulin-resistant FL83B cells. These two phenolic acids may promote the downstream activation of insulin signals as well.

Expression of PI3K. In insulin-stimulated action, IR undergoes tyrosyl phosphorylation and catalyzes the IRS family to initiate a group of signaling pathways, including the activation of PI3K and Ras/MAP kinase cascade (*30*). PI3K plays a critical role in insulin signaling. It induces the phosphorylation of phosphoinositides to produce phosphatidylinositol-3,4,5-phosphates, which are associated with glucose transporter translocation and glycogen synthesis (*5*, *31*).

In the present study, insulin increased the expression of PI3K in normal FL83B cells by 36.8% of the basal value, whereas TNF-α decreased the expression by 24.8% of the positive control. Caffeic acid increased the expression of PI3K by 76.2% of the basal value, whereas cinnamic acid caused no changes. Caffeic acid and cinnamic acid increased PI3K expression in TNF-α-induced insulin-resistant FL83B cells by 10.1 and 67.1% of the negative control, respectively (**Figure 3**). Restated, treatment with caffeic acid or cinnamic acid increased the expression of PI3K in

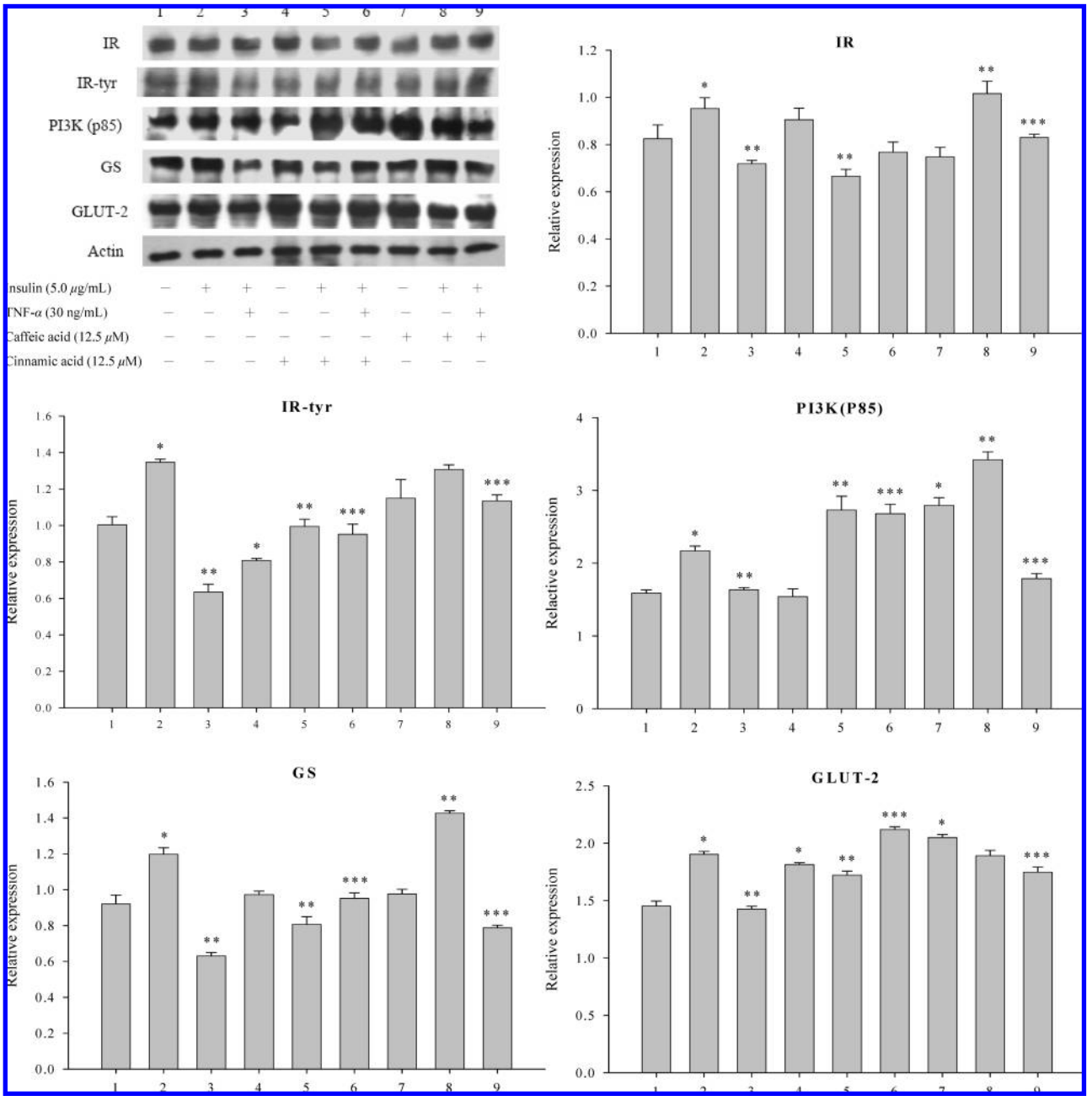

Figure 3. Effect of caffeic acid and cinnamic acid on TNF-α-induced inhibition of insulin signals in FL83B cells. FL83B cells were incubated in serum-free F12K medium, with or without added TNF-α (30 ng/mL), incubated at 37 °C for 5 h, transferred to another serum-free F12K medium containing 5 mM glucose, with or without insulin (5.0 μg/mL), caffeic aicd (12.5 μM), or cinnamic aicd (12.5 μM), and then incubated for an additional 3 h. The basal level of glucose uptake was evaluated by incubating cells with the serum-free F12K medium. The relative expressions of IR, IR-tyr, PI3K (p85), GS, and GLUT-2 in each treatment group were calculated using actin as the standard. ∗, significantly different ($p < 0.01$) from normal; ∗∗, significantly different ($p < 0.01$) from insulin-treated group; ∗∗∗, significantly different ($p < 0.01$) from TNF-α and insulin treated group.

TNF-α-induced insulin-resistant FL83B cells. Therefore, we postulate that the two phenolic acids improve IR expression and tyrosyl phosphorylation, up-regulate PI3K expression, and activate downstream signal molecules.

Expression of GS and GLUT-2. Caffeic acid and cinnamic acid caused no significant changes in the expression of GS in normal FL83B cells. Insulin increased GS protein expression in normal FL83B cells by 30.1% of the basal value. TNF-α impaired GS expression in the positive control by 47.4%. Caffeic acid and cinnamic acid increased GS expression in TNF-α-induced insulin-resistant cells by 24.9 and 51.0%, respectively (**Figure 3**). GS has been recognized as a major enzyme to catalyze glycogen synthesis (*32*). Glycogen synthesis was regulated by insulin signaling (*7*). Insulin stimulates the utilization and storage of glucose in the forms of lipid and glycogen (*31*). The FL83B cell may store glycogen and actively synthesize cholesterol (*33*). The present study showed that caffeic acid and cinnamic acid may increase the expression of GS to promote glycogen synthesis in TNF-α-induced insulin-resistant FL83B cells.

At least 12 glucose transporters (GLUTs) that mediate glucose uptake in various cells have been reported (*5*). GLUT-2 is found at high levels in liver, small intestine, kidney, and pancreatic β-cells (*34*). In the present study, the FL83B cells of each treatment were incubated in F12K medium containing glucose. Glucose-sensitive GLUT-2 may play a role in mediating both glucose influx and efflux in hepatocytes (*35*). Insulin increased the

expression of GLUT-2 in normal cells by 31.0% of the basal value. Caffeic acid and cinnamic acid increased GLUT-2 protein expression by 41.1 and 24.9% of the basal value, respectively. TNF-α treatment decreased GLUT-2 expression by 25.1% of the positive control. Caffeic acid and cinnamic acid increased the expression of GLUT-2 in TNF-α-induced insulin-resistant FL83B cells by 22.6 and 48.6%, respectively (**Figure 3**). On the basis of the above-described findings in **Figure 3**, we propose that the signal molecules activated by caffeic acid and cinnamic acid further improve the expression of GS in TNF-α-induced insulin-resistant FL83B cells, activate glycogen synthesis, promote the expression of GLUT-2, increase glucose uptake, and alleviate insulin resistance as the consequence.

The average phenolic acid intake among adults is 222 mg/day. The intake of caffeic acid, the predominant phenolic acid in food, is 206 mg/day. The major source of caffeic acid is coffee, which supplies approximately 190 mg/day/adult (*36*, *37*). The absorption rate for caffeic acid was reported to be 5.9% (*38*). The concentration of caffeic acid in blood as estimated by the equation

$$\text{concentration in blood} = \text{ingestion quantity} \times \text{absorption rate} / \text{quantity of blood in a 60 kg adult}$$

is 2.4 μg/mL, which is higher than the concentration (12.5 μM or 2.3 μg/mL) used in the present study. Therefore, we speculate that the ingestion of caffeic acid from coffee may be helpful in the alleviation of insulin resistance.

In conclusion, we evaluated the effect of caffeic acid and cinnamic acid on glucose uptake in TNF-α-induced insulin-resistant FL83B hepatocytes. We found that caffeic acid and cinnamic acid may alleviate insulin resistance by improving insulin signaling and enhancing glucose uptake in insulin-resistant cells. We propose that alleviation in insulin resistance is involved in the antihyperglycemic effect of these phenolic acids.

ABBREVIATIONS USED

DM, diabetes mellitus; TNF-α, tumor necrosis factor-alpha; IR, insulin receptor; IRS-1, insulin receptor substrate-1; PTPs, protein tyrosine phosphatases; 2-DG, 2-[1-^{14}C]deoxy-D-glucose; BCRC, Bioresource Collection and Research Center; F12K, F12 Ham Kaighn's modification medium; FBS, fetal bovine serum; PI3-kinase, phosphatidylinositol 3-kinase; GLUT-2, glucose trnasporter-2; GS, glycogen synthase; SDS-PAGE, sodium dodecyl sulfate−polyacrylamide gel electrophoresis; PBS-T, PBS containing 0.05% Tween-20; IL-1, intereukin-1; IL-6, intereukin-6; INF-γ, interferon-γ; STZ, streptozotocin; GLUTs, glucose transporters.

LITERATURE CITED

(1) Sharma, S.; Chaturvedi, M.; Edwin, E.; Shukla, S.; Sagrawat, H. Evaluation of the phytochemicals and antidiabetic activity of *Ficus bengalensis*. *Int. J. Diabetes Dev. Countries* **2007**, *27*, 56–59.

(2) Nagappa, A. N.; Thakurdesai, P. A.; Venkat Raob, N.; Singh, J. Antidiabetic activity of *Terminalia catappa* Linn fruits. *J. Ethnopharmacol.* **2003**, *88*, 45–50.

(3) Alice, Y. Y.; Cheng, I.; George, F. Oral antihyperglycemic therapy for type 2 diabetes mellitus. *CMAJ* **2005**, *172*, 213–226.

(4) Defronzo, R. A.; Gunnarsson, R.; Björkman, O.; Olsson, M.; Wahren, J. Effects of insulin on peripheral and splanchnic glucose metabolism in noninsulin-dependent (type II) diabetes mellitus. *J. Clin. Invest.* **1985**, *76*, 149–155.

(5) Björnholm, M.; Zierath, J. R. Insulin signal transduction in human skeletal muscle: identifying the defects in type II diabetes. *Biochem. Soc. Trans.* **2005**, *33*, 354–357.

(6) Ratchada, P.; Hugo, A. B.; David, S. The development of insulin resistance in type 2 diabetes: insights from knockout studies. *Sci. Progress* **2008**, *91*, 285–316.

(7) Zick, Y. Insulin resistance: a phosphorylation-based uncoupling of insulin signaling. *Trends Cell. Biol.* **2001**, *11*, 437–441.

(8) Peraldi, P.; Hotamisligil, G. S.; Buurman, W. A.; Whitei, M. F.; Spiegelman, B. M. Tumor necrosis factor (TNF)-α inhibits insulin signaling through stimulation of the p55 TNF receptor and activation of sphingomyelinase. *J. Biol. Chem.* **1996**, *271*, 13018–13022.

(9) Senn, J. J.; Klover, P. J.; Nowak, I. A.; Mooney, R. A. Interleukin-6 induces cellular insulin resistance in hepatocytes. *Diabetes* **2002**, *51*, 3391–3399.

(10) Copeland, G. P.; Leinster, S. J.; Davis, J. C.; Hipkin, L. J. Insulin resistance in patient with colorectal cancer. *Br. J. Surg.* **1987**, *74*, 1031–1035.

(11) Hotamisligil, G. S.; Shargill, N. S.; Spiegelman, B. M. Adipose expression of tumor necrosis factor-α: direct role in obesity-linked insulin resistance. *Science* **1993**, *259*, 87–91.

(12) Moller, D. E. Potential role of TNF-α in the pathogenesis of insulin resistance and type 2 diabetes. *Trends Endocrinol. Metab.* **2000**, *6*, 212–217.

(13) Kroder, G.; Bossenmaier, B.; Kellerer, M.; Capp, E.; Stoyanov, B.; Mühlhöfer, A.; Berti, L.; Horikoshi, H.; Ullrich, A.; Häring, H. Tumor necrosis factor-α and hyperglycemia-induced insulin resistance. *J. Clin. Invest.* **1996**, *97*, 1471–1477.

(14) Hotamisligil, G. S.; Arner, P.; Caro, J. F.; Atkinson, R. L.; Spiegelman, B. M. Increased adipose tissue expression of tumor necrosis factor-alpha in human obesity and insulin resistance. *J. Clin. Invest.* **1995**, *95*, 2409–2415.

(15) Cheung, A. T.; Wang, J.; Ree, D.; Kolls, J. K.; Bryer-Ash, M. Tumor necrosis factor-α induces hepatic insulin resistance in obese zucker (*fa*/*fa*) rats via interaction of leukocyte antigen-related tyrosine phosphatase with focal adhesion kinase. *Diabetes* **2000**, *49*, 810–819.

(16) Iwata, M.; Haruta, T.; Usui, I.; Takata, Y.; Takano, A.; Uno, T.; Kawahara, J.; Ueno, E.; Sasaoka, T.; Ishibashi, O.; Kobayashi, M. Pioglitazone ameliorates tumor necrosis factor-α-induced insulin resistance by a mechanism independent of adipogenic activity of peroxisome proliferator-activated receptor-γ. *Diabetes* **2001**, *50*, 1083–1092.

(17) Scalbert, A.; Manach, C.; Morand, C.; Rémésy, C.; Jiménez, L. Dietary polyphenols and the prevention of diseases. *Crit. Rev. Food Sci. Nutr.* **2005**, *45*, 287–306.

(18) Adisakwattana, S.; Moonsan, P.; Yibchok-Anun, S. Insulin-releasing properties of a series of cinnamic acid derivatives in vitro and in vivo. *J. Agric. Food Chem.* **2008**, *56*, 7838–7844.

(19) Cheng, F. C.; Shen, S. C.; Wu, J. S. B. Effect of guava (*Psidium guajava* L.) leaf extract on glucose uptake in rat hepatocytes. *J. Food Sci.* **2009**, *74*, H132–H138.

(20) Garrait, G.; Jarrige, J. F.; Blanquet, S.; Beyssac, E.; Cardot, J. M.; Alric, M. Gastrointestinal absorption and urinary excretion of *trans*-cinnamic and *p*-coumaric acids in rats. *J. Agric. Food Chem.* **2006**, *54*, 2944–2950.

(21) Jung, U. J.; Lee, M. K.; Park, Y. B.; Jeon, S. M.; Choi, M. S. Antihyperglycemic and antioxidant properties of caffeic acid in *db*/*db* mice. *J. Pharmacol. Exp. Ther.* **2006**, *318*, 476–483.

(22) Tzeng, T. F.; Liu, I. M.; Cheng, J. T. Activation of opioid μ-receptors by loperamide to improve interleukin-6-induced inhibition of insulin signals in myoblast C_2C_{12} cells. *Diabetologia* **2005**, *48*, 1386–1392.

(23) Yu, Z. W.; Buren, J.; Enerback, S.; Nilsson, E.; Samuelsson, L.; Eriksson, J. W. Insulin can enhance GLUT4 gene expression in 3T3-F442A cells and this effect is mimicked by vanadate but counteracted by cAMP and high glucose-potential implications for insulin resistance. *Biochim. Biophys. Acta* **2001**, *1535*, 174–85.

(24) Cheng, H. L.; Huang, H. K.; Chang, C. I.; Tsai, C. P.; Chou, C. H. A cell-based screening identifies compounds from the stem of *Momordica charantia* that overcome insulin resistance and activate AMP-activated protein kinase. *J. Agric. Food Chem.* **2008**, *56*, 5835–6843.

(25) Liu, I. M.; Hsu, F. L.; Chen, C. F.; Cheng, J. T. Antihyperglycemic action of isoferulic acid in streptozotocin-induced diabetic rats. *Br. J. Pharmacol.* **2000**, *129*, 631–636.

(26) Ohnishi, M.; Matuo, T.; Tsuno, T.; Hosoda, A.; Nomura, E.; Taniguchi, H.; Sasaki, H.; Morishita, H. Antioxidant activity and hypoglycemic effect of ferulic acid in STZ-induced diabetic mice and KK-Ay mice. *Biofactors* **2004**, *21*, 315–319.

(27) Balasubashini, M. S.; Rukkumani, R.; Menon, V. P. Protective effects of ferulic acid on hyperlipidemic diabetic rats. *Acta Diabetol.* **2003**, *40*, 118–122.

(28) Cheng, J. T.; Liu, I. M. Stimulatory effect of caffeic acid on α1A-adrenoceptors to increase glucose uptake into cultured C_2C_{12} cells. *Naunyn. Schmiedebergs. Arch. Pharmacol.* **2000**, *362*, 122–127.

(29) White, M. F. Insulin signaling in health and disease. *Science* **2003**, *302*, 1710–1711.

(30) Saltiel, A. R.; Kanh, C. R. Insulin signalling and the regulation of glucose and lipid metabolism. *Nature* **2001**, *414*, 799–806.

(31) Cantley, L. C. The phosphoinositide 3-kinase pathway. *Science* **2002**, *296*, 1655–1657.

(32) Ferrer, J. C.; Favre, C.; Gomis, R. R.; Fernandez-Novell, J. M.; Garica-Rocha, M.; Iglesia, N.; Cid, E.; Guinovart, J. J. Control of glycogen deposition. *FEBS Lett.* **2003**, *546*, 127–132.

(33) Breslow, J. L.; Sloan, H. R.; Ferrans, V. J.; Anderson, J. L.; Levy, R. I. Characterization of the mouse liver cell line FL83B. *Exp. Cell Res.* **1973**, *78*, 441–453.

(34) Thorens, B.; Cheng, Z. Q.; Brown, D.; Lodish, H. F. Liver glucose transporter: a basolateral protein in hepatocytes and intestine and kidney cells. *Am. J. Physiol.* **1990**, *259*, C279–C285.

(35) James, D. E. The mammalian facilitative glucose transporter family. *Int. Union Physiol. Sci.* **1995**, *10*, 67–71.

(36) Manach, C.; Scalbert, A.; Morand, C.; Rémésy, C.; Jiménez, L. Polyphenols: food sources and bioavailability. *Am. J. Clin. Nutr.* **2004**, *79*, 727–747.

(37) Radtke, J.; Linseisen, J.; Wolfram, G. Phenolic acid intake of adults in a Bavarian subgroup of the national food consumption survey. *Z. Ernaehrungswiss.* **1998**, *37*, 190–197.

(38) Rechner, A. R.; Spencer, J. P. E.; Kuhnle, G.; Hahn, U.; Rice-Evans, C. A. Novel biomarkers of the metabolism of caffeic acid. *Free Radical Biol. Med.* **2001**, *30*, 1213–1222.

Received April 27, 2009. Revised manuscript received July 22, 2009. Accepted July 27, 2009.