Caffeic acid and cinnamic acid ameliorate glucose metabolism via modulating glycogenesis and gluconeogenesis in insulin-resistant mouse hepatocytes

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ABSTRACT

Tumour necrosis factor-\(\alpha\) (TNF-\(\alpha\)) plays a pivotal role in cellular insulin resistance and can induce insulin resistance in mouse FL83B hepatocytes. Caffeic acid and cinnamic acid were found to improve glucose uptake in TNF-\(\alpha\)-treated insulin-resistant mouse FL83B hepatocytes. The mechanism of glucose metabolism by caffeic acid and cinnamic acid was further investigated. The result from Western blot analysis revealed that caffeic acid and cinnamic acid increased expression of glycogen synthase, whereas the expression of glycogen synthase kinase and phosphorylation of glycogen synthase at Ser641 in insulin-resistant mouse hepatocytes was decreased. Caffeic acid and cinnamic acid suppressed the expression of hepatic nuclear factor-4 in TNF-\(\alpha\)-treated mouse FL83B hepatocytes. The activity of phosphoenolpyruvate carboxykinase was also inhibited. Thus, caffeic acid and cinnamic acid ameliorated glucose metabolism by promoting glycogenesis and inhibiting gluconeogenesis in TNF-\(\alpha\)-treated insulin-resistant mouse hepatocytes.

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1. Introduction

Diabetes mellitus (DM) is a major public health problem worldwide. It is characterized by hyperglycemia resulting from deficiencies in insulin secretion and/or insulin action. Type 2 DM is the most common form of diabetes, accounting for above 90% of patients with diabetes. Insulin resistance, a key factor in the pathogenesis of obesity and Type 2 diabetes, is a pathological state in which target cells fail to respond to a normal level of circulating insulin (Kaidanovich & Eldar-Finkelman, 2002).

The primary biological effect of insulin is the maintenance of whole-body glucose homeostasis. Under normal conditions, insulin binds to insulin receptors and evokes insulin signal transduction cascades, which mediate the regulation of glucose metabolism and glucose transport as well as the promotion of glycogen synthesis (Zick, 2001). The stimulus of insulin may increase the expression of glucokinase (GK) (Saltiel & Kahn, 2001), which promotes glucose utilization and glycogen deposition in the liver (Ferrer et al., 2003). Insulin also inhibits glycogen synthase kinase-3 (GSK-3) and thus further promotes dephosphorylation and activation of glycogen synthase (GS), which is crucial for the catalysis of glycogen synthesis (Bouskila, Hirshman, Jensen, Goodyear, & Sakamoto, 2008). In addition, insulin can reduce glucose production by inhibiting hepatic nuclear factor-4 (HNF-4) and phosphoenolpyruvate carboxykinase (PEPCK) activities, thus maintaining glucose homeostasis (Saltiel & Kahn, 2001).

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The liver is an insulin-sensitive organ that is responsible for regulating energy homeostasis. Liver cells have been used as an in vitro model for evaluating or screening for antihyperglycemic agents from food ingredients (Cheng, Huang, Chang, Tsai, & Chou, 2008). Tumour necrosis factor-α (TNF-α) plays a pivotal role in the occurrence of cellular insulin resistance, which impairs insulin signal transduction (Lorenzo et al., 2008). Hepatic insulin resistance may increase hepatic glucose production and lead to hyperglycemia (Leclercq, Da-Silva-Morais, Schroyen, Van-Hul, & Geerts, 2007). TNF-α decreased the expression of GS in a mouse hepatocyte cell line, FL83B. The decrease in GS may indirectly reduce glucose uptake and may lead to insulin resistance in liver cells (Huang, Shen, & Wu, 2009).

Caffeic and cinnamic acids are two phenolic acids that exist in many fruits, vegetables, and beverages including blueberry, kiwi, cherry, plum, apple, pear, chicory, artichoke, potato, cider and coffee (Manach, Scalbert, Morand, Rémyès, & Jiménez, 2004). Recent studies have focused on the hypoglycemic effect of these two phenolic acids. Caffeic acid promotes glucose utilization for energy production as well as glycogen deposition, resulting in decreased glucose output in mice (Jung, Lee, Park, Jeon, & Choi, 2006). Recently, we reported that caffeic and cinnamic acids improve glucose uptake in TNF-α-treated insulin-resistant FL83B cells (Huang et al., 2009). Therefore, it is worthwhile to further unravel the mechanism of improved glucose utilization by these two phenolic acids.

2. Materials and methods

2.1. Chemicals and reagents

FL83B cells, a hepatocyte cell line derived from a 15 to 17 days old fetal mouse, were obtained from the Bioresource Collection and Research Center (BCRC), Hsinchu City, Taiwan. Insulin, α-glucose, caffeic acid, cinnamic acid, recombinant mouse TNF-α, and F12 Ham Kaighn’s modification (F12K) medium were purchased from Sigma (St. Louis, MO, USA). Fetal bovine serum (FBS) was from Gemini Bio-Products, Woodland, CA, USA. Bio-Rad protein assay dye was from Bio-Rad Laboratories (Hercules, CA, USA). All chemicals used in this study were analytical grade.

2.2. Cell culture

Experiments were carried out with FL83B cells, a hepatocyte cell line derived from fetal mouse. The cells were incubated in F12K medium containing 10% FBS and 1% penicillin and streptomycin (Invitrogen Corporation, Camarillo, CA, USA) in 10 cm Petri dishes at 37 °C and 5% CO₂. Experiments were performed on cells that were 80–90% confluent.

2.3. Cell preparation

FL83B cells were incubated in F12K medium without (basal) or with 30 ng/mL TNF-α for 5 h to induce insulin resistance. Then, the cells were transferred to F12K medium containing 5 mM glucose without (basal) or with insulin (5 µg/mL) and with caffeic acid (12.5 µM) or cinnamic acid (12.5 µM) for 3 h at 37 °C.

2.4. Determination of glycogen

The accumulation of glycogen in FL83B cells was determined using a glycogen assay kit (Biovision Corp., Mountain View, CA, USA). Briefly, the cells were collected, washed twice with ice-cold PBS, and homogenized in 200 µL deionized water. The homogenates were boiled for 5 min to inactivate enzymes and centrifuged at 13,000 g for 5 min to remove pellet. Fifty microlitres of supernatant of each sample were mixed with 2 µL of Hydrolysis Enzyme Mix in a 96-well plate, and the plate was incubated at room temperature for 10 min. A 50-µL aliquot of the reaction mix (46 µL Development Buffer, 2 µL Development Enzyme Mix, 2 µL OxidRed Probe) was added to each well, and the plate was incubated at room temperature for 30 min in the dark. Absorbance at 570 nm was measured using a microplate reader (Sunrise, TECAN, Salzburg, Austria). A standard glycogen curve (0, 0.4, 0.8, 1.2, 1.6, and 2.0 µg/well) was calculated by the above method.

2.5. Phosphoenolpyruvate carboxylase (PEPCK) activity assay

PEPCK activity was determined using the spectrophotometric assay developed by Greenway and Storey (2000) with a slight modification. Cells that were pretreated with TNF-α were homogenized in the extraction buffer [20 mM imidazole-HCl, pH 7.2, 100 mM NaF, 5 mM ethylenediaminetetraacetic acid (EDTA); 5 mM 2-mercaptopentoanil; and 20 µM phenylmethanesulphonyl fluoride (PMSF)] and centrifuged (13,000g, 20 min) to remove cell debris. Each 350 µL aliquot of the supernatant was mixed with 125 µL 100 mM imidazole-HCl (pH 6.6), 125 µL 0.5 mM nicotinamide adenine dinucleotide (NADH), 125 µL 1 mM MnCl₂, 50 µL 30 mM 2-mercaptopentoanil, 150 µL 1.25 mM inositol-diphosphate, 125 µL 50 mM NaHCO₃, 125 µL 2.5 U/mL malate dehydrogenase, and 150 µL 5 mM phosphoenolpyruvate in series. The final reaction volume was 1 mL. Enzyme activity was determined at 25 °C for 5 min by measuring the decrease in absorbance at 340 nm.

2.6. Western blot analysis

Cells were made insulin resistant as described above. Medium was removed from TNF-α-treated FL83B cells, and the cells were washed twice with ice-cold PBS and 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 ortho-vanadate, and 10 µg/mL antipain. Cell lysates were sonicated on ice four times for 5 s each and centrifuged (13,000g, 20 min) to recover the supernatant. The supernatant was considered the cell extract and was stored at −80 °C until use.
The protein concentration in the cell extract was determined using a Bio-Rad protein assay. Aliquots of the extract, each containing 40 μg protein, were evaluated for expression of glucokinase (GK), glycogen synthase kinase-3α (GSK-3α), glycogen synthase kinase-3β (GSK-3β), and glycogen synthase (GS) and the degree of serine phosphorylation of GS. To evaluate hepatic nuclear factor-4 (HNF-4), aliquots of the supernatant, each containing 60 μg protein, were used. The samples were subjected to 10% sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE). Separated proteins were electrotransferred to a polyvinylidene difluoride membrane. The membrane was incubated with blocking buffer (PBS containing 0.05% Tween-20 and 5% [w/v] nonfat dry milk) for 1 h, washed with PBS containing 0.05% Tween-20 (PBST) three times, and probed with anti-GK, anti-GSK-3α, anti-GSK-3β, anti-GS (1:1000 each; Cell Signaling Technology, Beverly, MA, USA), anti-GS Ser-641 (1:1000; Epitomics, Burlingame, CA, USA), or anti–HNF-4 (1:500; Abcam, Cambridge, UK) overnight at 4 °C. The intensity of the blots probed with a 1:2000 dilution of mouse monoclonal antibody to bind actin (BD Biosciences, Franklin Lakes, NJ, USA) was used as the control to ensure that a constant amount of protein was loaded into each lane of the gel. Membranes were also probed with mouse anti-actin (1:2000; BD Biosciences, Franklin Lakes, NJ, USA) as a loading control to normalize the amount of protein loaded per lane. The membrane was washed three times for 5 min each in PBST, shaken in a solution of horseradish peroxidase-conjugated anti-mouse IgG or anti-rabbit IgG secondary, washed three times for 5 min each in PBST, and incubated in enhanced chemiluminescence reagent (Millipore). Autoradiography was performed on Fuji medical X-ray film (Fuji, Tokyo, Japan).

2.7. Statistical analysis

Data obtained from six separate experiments are presented as the mean ± standard deviation. Statistical significance was analyzed by one-way ANOVA and Duncan’s new multiple range test. P-values <0.05 were considered significant.

3. Results and discussion

3.1. Effect of caffeic acid and cinnamic acid on glycogen synthesis in insulin-resistant FL83B cells

Glycogen deposition in peripheral tissues and liver is a physiological response in mammals to the increase in blood glucose concentration that occurs after a meal (Ferrer et al., 2003). Insulin induces a series of signal transduction pathways and dephosphorylates GS, which activates this enzyme and consequently promotes glycogen synthesis and reduces the blood glucose level under normal conditions (Saltiel & Kahn, 2001).

In mouse liver FL83B cells, exposure to insulin (5.0 μg/mL) for 3 h significantly increased glycogen content by 27.1%, from 1.38 ± 0.36 μg/mg protein in the normal group to 1.89 ± 0.06 μg/mg protein in the positive control (Fig. 1). Glycogen amounts in liver cells treated with caffeic and cinnamic acids were 1.93 ± 0.05 and 2.33 ± 0.13 μg/mg protein, reflecting increases of 18.1% and 42.9% as compared with the normal group (1.38 ± 0.36 μg/mg protein) (Fig. 1). Caffeic acid may activate α1-adrenoceptors and promote the phospholipase C-protein kinase C (PLC–PKC) pathway in C2C12 skeletal muscle cells (Moller, 2000). Activation of PKC may further enhance glucose uptake and glycogen synthesis (Saltiel & Kahn, 2001). α1B-Adrenoceptors are the principal type of α1-adrenoceptors in mouse liver cells (Peraldi, Hotamisligil, Buurman, White, & Spiegelman, 1996). Therefore, the effect of caffeic acid and cinnamic acid on increasing glycogen synthesis in FL83B cells may be related to the activation of α1B-adrenoceptors and the PLC–PKC pathway.

There was a 48.4% reduction in glycogen in mouse liver cells treated with TNF-α as well as insulin (1.02 ± 0.02 μg/mg protein) as compared with the positive control (1.89 ± 0.06 μg/mg protein). Glycogen amounts in TNF-α-treated insulin-resistant cells that were treated with caffeic and cinnamic acids as well as insulin were 1.39 ± 0.03 and 1.32 ± 0.04 μg/mg protein, respectively, which were 35.5% and 29.0% higher than the group treated only with TNF-α and insulin (1.02 ± 0.02 μg/mg protein; Fig. 1). TNF-α may interfere with insulin signal transduction via phosphorylation of insulin receptors (IR), tyrosyl phosphorylation of insulin receptor substrate-1 (IRS-1), and activation of phosphatidylinositol-3 kinase (PI3K), therefore influencing glucose metabolism (Cheng & Liu, 2000; Cichy et al., 1998). Recently, TNF-α has been reported to inhibit the glucose uptake ability and decrease the expression of PI3K in FL83B cells (Huang et al., 2009). Inhibition of PI3K results in interference of gene modulation, glucose uptake, and glycogen synthesis in HepG2 hepatoma cells (González-Espinosa, Romero-Avila, Mora-Rodríguez, González-Espinosa, & García-Sáinz, 2001). Our results indicate that these two phenolic acids may relieve the interference from TNF-α on glucose metabolism and improve glycogen synthesis in FL83B cells.

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![Fig. 1 – Effects of caffeic acid and cinnamic acid on glycogen amounts 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 μg/mL), caffeic acid (12.5 μM), or cinnamic acid (12.5 μM), and then incubated for an additional 3 h. Glycogen in FL83B cells was determined using a glycogen assay kit. Values are mean ± SD, n = 6. Means without a common letter differ, p < 0.05.](image-url)
3.2. Effect of caffeic acid and cinnamic acid on expression of GK in insulin-resistant FL83B cells

GK is the predominant glucose phosphorylation-related enzyme in hepatocytes and in insulin- and glucagon-secreting cells of the pancreas (Ferrer et al., 2003).Phosphorylation of glucose can be promoted by GK, which catalyzes the production of glucose-6-phosphate (G-6-P). This reaction is followed by glycolysis to create energy or enhance glycogen deposition in hepatocytes (Saltiel & Kahn, 2001). In our current study, insulin increased the expression of GK in FL83B cells by 63.3% of the basal value (Fig. 2). Caffeic acid increased the expression of GK by 20.1%, whereas cinnamic acid showed no effect (Fig. 2). Previously, Jung et al. (2006) reported that caffeic acid may enhance mRNA expression of GK in mouse liver. Caffeic acid was also reported to increase mRNA expression of GK in rat liver FAO cells (Valentova, Truong, Moncion, Waziers, & Ulrichova, 2007).

TNF-α in the presence of insulin blocked expression of GK by 44.6% as compared with the positive control (Fig. 2). TNF-α may also interfere with the action of insulin, and decrease the expression of GK in liver as a consequence (Saltiel & Kahn, 2001). Caffeic acid and cinnamic acid increased the expression of GK in TNF-α-treated insulin-resistant FL83B cells by 68.6% and 30.2%, respectively, in the presence of insulin (Fig. 2). The results from this study suggest that caffeic acid and cinnamic acid may ameliorate the impaired GK expression in insulin-resistant FL83B cells.

Hepatic GK is responsible for glucose homeostasis and is a potential target for pharmacological treatment of Type 2 diabetes (Jung et al., 2006). Mice with liver-specific knockout of GK exhibit hyperglycemia (Postic et al., 1999). The return of the expression of GK may reduce blood glucose in diabetic mice (Ferre, Pujol, Riu, Bosch, & Valera, 1996). Increasing hepatic GK may promote utilization of blood glucose for energy production or glycogen deposition in liver. GK promotes the production of G-6-P via phosphorylation of glucose, which is an essential element for activation. G-6-P changes the configuration of GS, rendering it more susceptible to dephosphorylation by protein phosphatases and increasing its activation (Villar-Palasi & Guinovart, 1997). Current results indicate that caffeic and cinnamic acids may activate GS and ameliorate glucose metabolism in insulin-resistant FL83B cells.

3.3. Effect of caffeic acid and cinnamic acid on phosphorylation of GS in insulin-resistant FL83B cells

GS is a key enzyme in catalyzing glycogen synthesis (Ferrer et al., 2003). Hepatic GS undergoes reversible phosphorylation at multiple serine residues and exists in two major forms: a
non-active phosphorylated state and an active, dephosphorylated state (Horn, Ivester, & Cunningham, 2001). In general, insulin signaling triggers several events including increases in GK expression and changes the configuration of GS for easy dephosphorylation by protein phosphatase-1 (PP-1) (Villar-Palasi & Guinovart, 1997), enhances expression of protein phosphatase-1, which phosphorylates serine residues in GS, and activation of protein kinase B (also known as Akt), which inhibits GSK activity, and consequently activates GS to promote the synthesis of glycogen (Bouskila et al., 2008). Fig. 3 shows the protein expression and serine phosphorylation of GS in TNF-α-treated insulin-resistant FL83B cells treated with caffeic and cinnamic acids. Insulin induced a 1.1-fold increase in the expression of GS and a 13.3% decrease in the phosphorylation of GS at Ser641 in FL83B cells. In addition, caffeic and cinnamic acids decreased the phosphorylation of GS at Ser641 by 52.1% and 15.7% of the basal control value, respectively (Fig. 3).

**Fig. 3 –** Effects of caffeic acid and cinnamic acid on protein expression and serine phosphorylation of glycogen synthase (GS) in TNF-α-treated insulin resistant 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 μg/mL), caffeic acid (12.5 μM), or cinnamic acid (12.5 μM), and then incubated for an additional 3 h. Values are mean ± SD, n = 6. Means without a common letter differ, p < 0.05. The relative expression was compared with actin.
In the presence of insulin, TNF-α decreased the expression of GS by 52.8% and increased the phosphorylation of GS at Ser641 by 13.9% in hepatocytes. Caffeic and cinnamic acids decreased the phosphorylation of GS at Ser641 in TNF-α-treated insulin-resistant cells by 73.6% and 20.2%, respectively, and increased the expression of GS in the presence of insulin (Fig. 3). The results from our current study revealed that caffeic and cinnamic acids ameliorated the expression of GS and increased the dephosphorylation of GS at Ser641 in TNF-α-treated insulin-resistant FL83B cells.

3.4. Effect of caffeic acid and cinnamic acid on protein expression of GSK-3 in insulin-resistant FL83B cells

Both isoforms of GSK-3, α and β, are Ser/Thr kinases and play a principal role in the regulation of glycogen synthesis. 

**Fig. 4 – Effects of caffeic acid and cinnamic acid on protein expression of glycogen synthase kinase-3α, 3β (GSK-3α, 3β) in TNF-α-treated insulin resistant 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 μg/mL), caffeic acid (12.5 μM), or cinnamic acid (12.5 μM), and then incubated for an additional 3 h. Values are mean ± SD, n = 6. Means without a common letter differ, p < 0.05. The relative expression was compared with actin.
Torchia, & Garofalo, 2000). GSK-3 may promote serine phosphorylation of GS, inactivate GS, and then impair glycogen synthesis (Shahid & Hussain, 2007). GSK-3 also plays a role in the negative regulation of IRS-1 by increasing serine/threonine phosphorylation, which suppresses the expression of PI3K and impairs glucose uptake and glycogen synthesis as a consequence (Oren˜a et al., 2000). Generally, insulin enhances the expression of PI3K, subsequently activates Akt, and promotes the phosphorylation of Ser21 and Ser9 in GSK-3α and GSK-3β, respectively, thus diminishing the activity of GSK-3 (Bouskila et al., 2008; Nikoulina et al., 2000). Lithium was reported to promote insulin signal transduction, inhibit the activation GSK-3, and diminish the phosphorylation of serine residues in GS to activate GS in 3T3L1 cells (Oren˜a et al., 2000).

Fig. 4 shows that addition of insulin led to 44.8% and 28.9% decreases in GSK-3α and GSK-3β expression, respectively, as compared with their expression in normal FL83B cells. Insulin reduces the inhibitory action of GSK-3 on GS and promotes glycogen deposition (Shahid & Hussain, 2007). In the present study, caffeic acid decreased the expression of GSK-3α by 63.9% of the basal value, whereas no significant change was observed with cinnamic acid. In addition, caffeic acid and cinnamic acid decreased GSK-3β expression by 71.4% and 30.2% in normal FL83B cells, respectively (Fig. 4).

The expression of GSK-3α in TNF-α-treated FL83B cells was 1.7-fold higher than expression in the positive control in the presence of insulin, whereas the expression of GSK-3β was not significantly changed (Fig. 4). The activity of GS in skeletal muscle cells in Type 2 diabetic patients is inhibited by high expression of GSK-3α and GSK-3β (Nikoulina et al., 2000). We found that caffeic acid reduced the expression of GSK-3α and GSK-3β by 67.1% and 56.2%, respectively, and cinnamic acid caused 52.5% and 44.2% decreases in GSK-3α and GSK-3β expression in TNF-α-treated cells, respectively, as compared with cells treated with insulin and TNF-α alone (Fig. 4). The results suggest that the two acids may inhibit GSK-3 expression and improve glycogenesis in insulin-resistant FL83B cells.

3.5. Effect of caffeic acid and cinnamic acid on glucose production in insulin-resistant FL83B cells

The liver is an insulin-sensitive organ that plays a critical role in regulating glucose utilization, mainly gluconeogenesis and glycogenesis, to maintain glucose homeostasis (Ferre et al., 1996). PEPCK, fructose-1,6-bisphosphatase (F-1,6-Pase), and glucose-6-phosphatase (G-6-Pase) are important enzymes associated with hepatic gluconeogenesis and glycolysis. Among them, PEPCK catalyses the rate-limiting step of

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hepatic gluconeogenesis (Sutherland, O’Brien, & Granner, 1996). HNF-4, a zinc finger protein and a key member of the hepatic transcription factor family of proteins (Niehof & Borlak, 2008), is responsible for regulating transcription of the gene encoding PEPCK.

Insulin may inhibit HNF-4, diminish PEPCK gene expression during insulin signaling, and inhibit hepatic gluconeogenesis (Saltiel & Kahn, 2001). Our current results showed that insulin markedly reduced HNF-4 protein expression and PEPCK activity by 29.4% and 28.1% of the basal value, respectively (Figs. 5 and 6). Caffeic and cinnamic acids suppressed HNF-4 expression by 44.4% and 34.0%, respectively (Fig. 5) and also decreased PEPCK activity in untreated FL83B cells by 47.3% and 36.4% (Fig. 6), respectively. Jung et al. (2006) previously reported that caffeic acid suppresses mRNA expression and activities of hepatic PEPCK and G-6-Pase, and hence leads to reduced glucose production in db/db mice. An in vitro study revealed that caffeic and chlorogenic acids markedly decrease gluconeogenesis in rat liver Fao cells (Valentová et al., 2007).

The occurrence of insulin resistance in the liver may promote gluconeogenesis and increase glucose output (Ferre et al., 1996). The results of our current study showed that HNF-4 expression (Fig. 5) and PEPCK activity (Fig. 6) were increased by 1.2-fold and 28.9%, respectively, in TNF-α-treated FL83B cells that were treated with insulin. Caffeic and cinnamic acids significantly suppressed HNF-4 expression by 80.9% and 64.3%, respectively, of TNF-α-treated FL83B cells in the presence of insulin (Fig. 5). The decreases in PEPCK activity in TNF-α-treated FL83B cells that were treated with caffeic and cinnamic acids in the presence of insulin were 25.5% (Fig. 6).

An increase in HNF-4 promotes the activities of PEPCK and fructose-1,6-biphosphatase to produce fructose-6-phosphate (F-6-P). F-6-P promotes the binding of GK regulation protein (GKPR) to GK, which further inhibits the action of GK on glucose phosphorylation and reduces glucose uptake and glucose utilization in hepatocytes (Ferrer et al., 2003; Saltiel & Kahn, 2001). Caffeic acid and cinnamic acid may decrease HNF-4 expression and inhibit PEPCK activity, reducing gluconeogenesis and alleviating the inhibition of GKPR on GK to improve glucose utilization in insulin-resistant FL83B cells.

The current results revealed that caffeic acid is excellent in promoting glycogenesis and inhibiting glucose production. Chang, Hsu, Liu, and Cheng (2003) reported that methoxylation or hydroxylation of cinnamic acid may play an important role in the stimulating glucose uptake to improve glucose utilization in C6 cells. Caffeic acid is the derivative of cinnamic acid which possesses two hydroxyl groups at the benzene ring. We speculate that the hydroxyl group may be related to the variation in amelioration of glucose metabolism between these two phenolic acids.

4. Conclusions

Caffeic and cinnamic acids exist widely in various fruits, vegetables, and coffee. The results from this study indicate caffeic acid and cinnamic acid are effective in improving glucose utilization in insulin-resistant mouse hepatocytes. These two phenolic acids may promote GS activity by enhancing GK expression and inhibiting GSK-3 activity, thereby ameliorating glucose utilization and glycogen synthesis in insulin-resistant hepatocytes. Furthermore, caffeic acid and cinnamic acid may also inhibit HNF-4 expression and PEPCK activity, leading to decrease glucose production. We postulate that caffeic acid and cinnamic acid may improve glucose metabolism by modulating pathways involved in promoting glycogenesis and inhibiting gluconeogenesis in insulin-resistant mouse hepatocytes.

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R E F E R E N C E S


Fig. 6 – Effects of caffeic acid and cinnamic acid on phosphoenolpyruvate carboxylase (PEPCK) activity in TNF-α-treated insulin resistant 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 μg/mL), caffeic acid (12.5 μM), or cinnamic acid (12.5 μM), and then incubated for an additional 3 h. Values are mean ± SD, n = 6. Means without a common letter differ, p < 0.05. A unit of PEPCK activity is defined as a decrease in NADH of 1 nmol/min.


