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# Resveratrol Upregulates Nrf2 Expression To Attenuate Methylglyoxal-Induced Insulin Resistance in Hep G2 Cells

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**ABSTRACT:** Oxidative stress can result in insulin resistance, a primary cause of type-2 diabetes. Methylglyoxal (MG), a highly reactive dicarbonyl metabolite generated during glucose metabolism, has also been confirmed to cause pancreatic injury and induce inflammation, thereby resulting in insulin resistance. Recently, resveratrol has been reported to exert antioxidant properties, protecting cells from the generation of reactive oxygen species (ROS). The aim of this study was to evaluate resveratrol activation of nuclear factor erythroid 2-related factor 2 (Nrf2) to attenuate MG-induced insulin resistance in Hep G2 cells. Therefore, the molecular signaling events affecting resveratrol-mediated heme oxygenase-1 (HO-1) and glyoxalase expression levels were further investigated in this study. Our findings indicated that resveratrol activated the extracellular signal-regulated kinase (ERK) pathway but not the p38 or c-Jun N-terminal kinase (JNK) pathways, subsequently leading to Nrf2 nuclear translocation and elevation of HO-1 and glyoxalase expression levels. Moreover, resveratrol significantly elevated glucose uptake and protected against MG-induced insulin resistance in Hep G2 cells. In contrast, depletion of Nrf2 by small interfering RNA (si-RNA) resulted in the abrogation of HO-1 and glyoxalase expression in the MG-treated resveratrol group in Hep G2 cells. Administration of an appropriate chemopreventive agent, such as resveratrol, may be an alternative strategy for protecting against MG-induced diabetes.

**KEYWORDS:** *Methylglyoxal, insulin resistance, resveratrol, nuclear factor erythroid 2-related factor 2, heme oxygenase-1, glyoxalase*

## INTRODUCTION

Hyperglycemia is associated with protein glycation; advanced glycation end products (AGEs) are generated by non-enzymatic reactions between carbohydrates and proteins.[1] These AGEs have a propensity to generate free radicals and undergo auto-oxidation to generate other reactive intermediates, thereby resulting in the development of diabetes.[2] Methylglyoxal (MG), a highly reactive dicarbonyl metabolite generated during glucose metabolism,[3] is a major precursor of AGEs, which are involved in the pathogenesis of diabetes and inflammation.[4,5] Studies suggest that MG-induced reactive oxygen species (ROS) formation and apoptotic biochemical changes in Hep G2 cells.[6] ROS may interfere with insulin signaling, contributing to insulin resistance.[7] MG could inhibit phosphorylation of the insulin receptor substrate and the activation of the phosphatidylinositol 3-kinase (PI3K)/protein kinase B (PKB) pathway.[8] Another hypothesis suggests that excess MG reduces the activation of the AMP kinase (AMPK) that would have increased and prolonged liver insulin resistance.[9] AGEs and MG have been shown to generate large quantities of pro-inflammatory cytokines,[10−12] related to the modulation of pro-inflammatory molecules by oxidative stress.

Nuclear factor erythroid 2-related factor 2 (Nrf2) is an essential component of antioxidant responsive element (ARE)-mediated induction, including the regulation of antioxidant enzymes, such as heme oxygenase-1 (HO-1).[13,14] Moreover, Nrf2 has been reported to induce glyoxalase-1 expression.[15] Glyoxalase-1 catalyzes the conversion of MG to lactic acid, thereby reducing AGE levels.[16] Under physiological conditions, glyoxalase degrades MG into D-lactate, which is involved in Nrf2 activation.[17] Various antioxidants, such as quercetin and phenolic acid, have been investigated for their ability to attenuate oxidative damage by activating Nrf2.[18,19] Furthermore, it is also known that the antioxidant silymarin can inhibit the generation of AGEs, thereby ameliorating the symptoms of diabetes.[20]

Several lines of evidence have suggested that resveratrol inhibits oxidative stress and inflammation through Nrf2 activation,[21,22] thereby minimizing the symptoms of diabetes.[23] In addition, recent studies have reported that MG causes pancreatic damage and kidney injury.[24,25] Several antioxidants have been suggested to attenuate tissue damage, the symptoms of diabetes, and MG-induced inflammation, including silymarin,[20] rutin,[26] and *N*-acetyl cysteine (NAC).[27] Resveratrol also protects mouse blastocysts against MG-induced apoptosis during embryonic development.[28] However, few studies have evaluated the inhibitions of MG-induced insulin resistance and oxidative stress and the promotion of MG metabolism.

Therefore, the aim of this study is to investigate resveratrol-based protection against MG-induced oxidative stress and insulin resistance in Hep G2 cells and confirm whether this effect depends upon Nrf2 activation.

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## MATERIALS AND METHODS

**Reagents.** NAC, rosiglitazone, glucose, 2′,7′-dichlorofluorescein diacetate (DCFH-DA), and MG were purchased from Sigma-Aldrich (St. Louis, MO). The rat anti-Nrf2 antibody was purchased from Bioss, Inc. (Woburn, MA). Anti-Keap-1 antibody, antiglyoxalase antibody, and Nrf2 siRNA were purchased from Santa Cruz Biotechnology, Inc. (Burlingame, CA). Anti-Nrf2, anti-PTP1B, anti-p-Nrf2, anti-IR, anti-p-IR, anti-Akt, anti-p-Akt, anti-HO-1, antilamin B, anti-p-p38, anti-p-JNK, anti-p-ERK, anti-GLUT2, and anti-GAPDH antibodies were purchased from Epitomics, Inc. (Burlingame, CA). Fetal bovine serum (FBS) was purchased from Hyclone (Logan, UT). Dulbecco's modified Eagle's medium (DMEM), L-glutamine, sodium pyruvate, and antibiotics (penicillin/streptomycin) were purchased from Gibco (Grand Island, NY). 2-[*N*-(7-Nitrobenz-2-oxa-1,3-diazol-4-yl)amino]-2-deoxy-D-glucose (2-NBDG) was purchased from Invitrogen (Carlsbad, CA).

**Cell Culture.** Hep G2 hepatoma cells were obtained from the Bioresource Collection and Research Center (Hsinchu, Taiwan). Hep G2 cells were grown under standard cell culture conditions (humidified atmosphere, 5% $CO_2$, and 37 °C) in DMEM containing 10% FBS, 100 units/mL penicillin, 100 μg/mL streptomycin, and 0.25 μg/mL amphotericin.

**Insulin Resistance Induction and Glucose Uptake.** Glucose uptake in Hep G2 cells was assessed using the fluorescent glucose analogue, 2-NBDG. Hep G2 cells were seeded in 10 cm dishes at a density of $5 \times 10^5$/well and grown until 80% confluence was achieved. Cells were treated with MG (500 μM) and resveratrol (50 μM), rosiglitazone (50 μM), or NAC (500 μM) for 24 h, and in turn, insulin (500 nM, final concentration) and 2-NBDG (160 μM, final concentration) were added and incubated for 30 min. Subsequently, the glucose uptake activity of Hep G2 cells was determined with a FACS flow cytometer (Becton Dickinson Immunocytometry Systems, San Jose, CA) and analyzed using CellQuest software. The background of 2-NBDG fluorescence was deduced.

**Membrane and Cytosolic Extraction.** Extraction for plasma membrane and cytosolic extracts was carried out with an isolation kit (BioVision, Mountain View, CA).

**ROS Measurement.** The level of oxidative stress was monitored by the measurement of ROS.[29] Collected cells were suspended in 500 μL of phosphate-buffered saline (PBS), mixed with 10 μM (final concentration) DCFH-DA, and incubated for 30 min at 37 °C. The cells were washed 3 times with PBS to remove excess DCFH-DA. The cell pellet was mixed with 500 μL of PBS, and the ROS level was assayed by FACS (Becton Dickinson Immunocytometry Systems, San Jose, CA).

**Nuclear Extraction of Hep G2.** Nuclear protein extraction from Hep G2 cells was performed according to the kit protocol supplied by the manufacturer (Fermentas Life Sciences, Burlington, Ontario, Canada).

**Nrf2 Knockdown in Hep G2 Cells by Specific siRNA.** For the Nrf2 knockdown assay, Nrf2 interference of Hep G2 cells was performed with lipofectamine RNAiMAX transfection reagent (Invitrogen, Carlsbad, CA).[30]

**Western Blot.** Proteins separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) were electrophoretically transferred to polyvinylidene difluoride (PVDF) membranes. Blots were first incubated in PBS containing 5% nonfat dry milk for 2 h (to block non-specific binding sites), followed by incubation in PBS containing a dilution of primary antibodies at 4 °C overnight. After an initial washing to remove the primary antibody, the membrane was washed 3 times, each for 5 min, in PBS Tween-20 (PBST), shaken in a solution of horseradish peroxidase (HRP)-linked anti-rabbit IgG secondary antibody, and washed 3 additional times for 5 min each in PBST. The expressions of proteins were detected by enhanced chemiluminescent (ECL) reagent (Millipore, Billerica, MA).

**Statistical Analysis.** Data were expressed as the mean ± standard deviation (SD). Statistical significance was determined by one-way analysis of variance (ANOVA) using the general linear model procedure of SPSS, version 17.0 (SPSS Institute, Inc., Chicago, IL), followed by ANOVA with Duncan's test.

## RESULTS

**Effects of Resveratrol on Glucose Uptake of MG-Treated Hep G2 Cells.** The finding demonstrated that MG (500 μM) treatment significantly decreased the glucose uptake (2-NBDG) activity compared to that of the control group (Figure 1). However, resveratrol (50 μM) treatment markedly

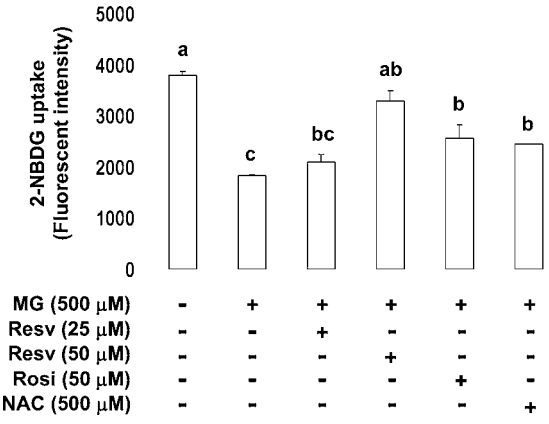


**Figure 1.** Effect of resveratrol on glucose uptake capacity of Hep G2 cells treated with MG for 24 h. Results are shown as the mean ± SD ($n$ = 3). Significant differences were indicated with different letters (a–c; $p < 0.05$). Resv, resveratrol; Rosi, rosiglitazone; and NAC, *N*-acetyl cysteine.

recovered glucose uptake of MG-treated Hep G2 cells compared to the antioxidant NAC (500 μM) and the PPARγ agonist rosiglitazone (50 μM). This effect contributed to resveratrol-mediated promotion of glucose transporter 2 (GLUT2) translocation to membranes of Hep G2 cells from the cytosol. MG also reduced expression levels of cytosolic GLUT2 in Hep G2 cells compared to that of the control group, while resveratrol treatment protected against MG suppression of GLUT2 expression (Figure 2A).

**Effects of Resveratrol on the Insulin Signaling Pathway.** We investigated the effects of resveratrol on insulin receptor (IR) and Akt phosphorylation of Hep G2 cells treated with insulin (500 nM) for 30 min. The findings demonstrated that MG administration markedly suppressed IR and Akt phosphorylation, suggesting that MG treatment caused insulin resistance in Hep G2 cells. However, we found that resveratrol treatment (50 μM) promoted a significant increase in the ratio of phosphorylated/non-phosphorylated substrates (i.e., p-IR/IR and p-Akt/Akt) in MG-treated Hep G2 cells (Figure 2).

**Inhibitory Effects of Endoplasmic Reticulum (ER) Stress and Oxidative Stress in MG-Treated Hep G2 Cells by Resveratrol.** The study has reported that MG could result in oxidative stress, and the ROS level was correlated with ER stress.[31,32] Protein tyrosine phosphatase 1B (PTP1B) can be induced by ER stress, thereby dephosphorylating the tyrosine residues of insulin receptor substrate (IRS), suggesting that PTP1B displayed an important role in the development of insulin resistance.[29] We found that resveratrol markedly attenuated oxidative stress caused by MG treatment in Hep G2 cells compared to rosiglitazone and NAC (Figure 3A).

Nrf2 has been reported to induce glyoxalase-1 and antioxidant enzyme expressions. Glyoxalase-1 catalyzes the

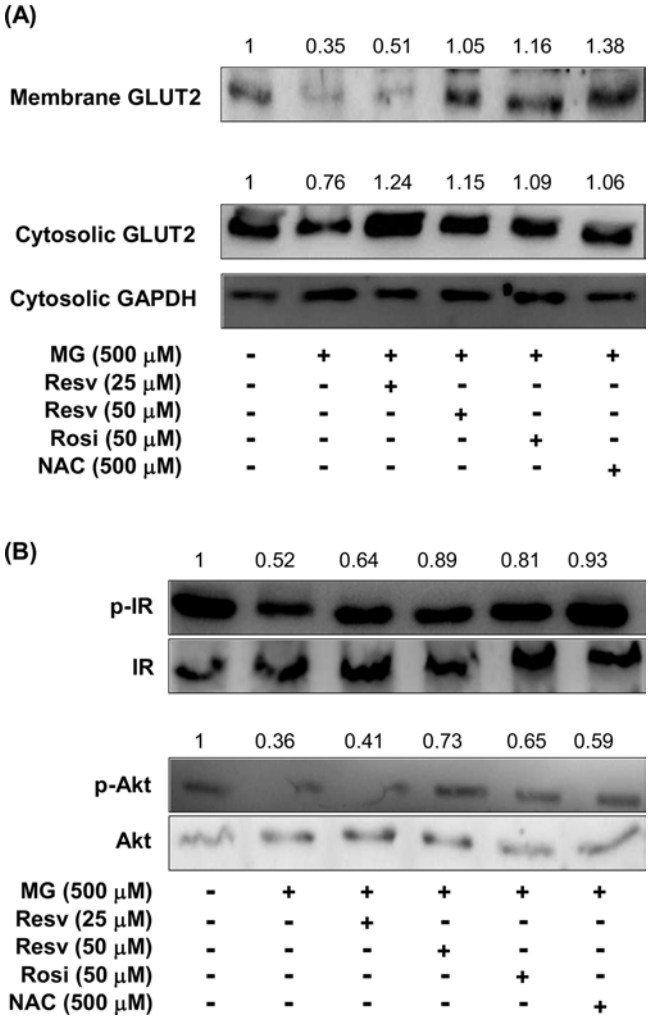


**Figure 2.** Effects of resveratrol on (A) GLUT2 translocation, (B) activation of insulin signal molecules, such as IR and Akt, phosphorylation in MG-treated Hep G2 cells. Resv, resveratrol; Rosi, rosiglitazone; and NAC, *N*-acetyl cysteine.

conversion of MG to lactic acid, thereby reducing the generation of AGEs.[16] Resveratrol has been demonstrated to inhibit oxidative stress through Nrf2 activation.[21,22] For Nrf2 activation, several potential mechanisms of Nrf2 phosphorylation by mitogen-activated protein kinase (MAPK) have been reported.[33,34] We found that resveratrol (50 $\mu$M) markedly elevated Nrf2 phosphorylation (at Ser 40) to improve protection against oxidative stress, thereby reducing PTP1B expression (Figure 3B), and promoted greater glyoxalase and HO-1 expressions in MG-treated Hep G2 cells compared to those in cells treated with rosiglitazone (50 $\mu$M) or NAC (500 $\mu$M) for 24 h (Figure 3C).

**Resveratrol Induces Nrf2 Nuclear Translocation and Activation.** We also found that resveratrol treatment for 9 h resulted in Nrf2 activation in a time-dependent manner by promoting Nrf2 translocation to the nucleolus of Hep G2 cells from the cytosol (Figure 4). Therefore, HO-1 and glyoxalase expressions levels were elevated in Hep G2 cells treated with resveratrol for 9 h (Figure 5).

**Mechanism for Resveratrol-Activated Nrf2.** Resveratrol treatment resulted in the elevation of Nrf2 phosphorylation; therefore, we investigated its effect on several MAPK activities, including p-p38, p-c-Jun N-terminal kinase (p-JNK), and p-

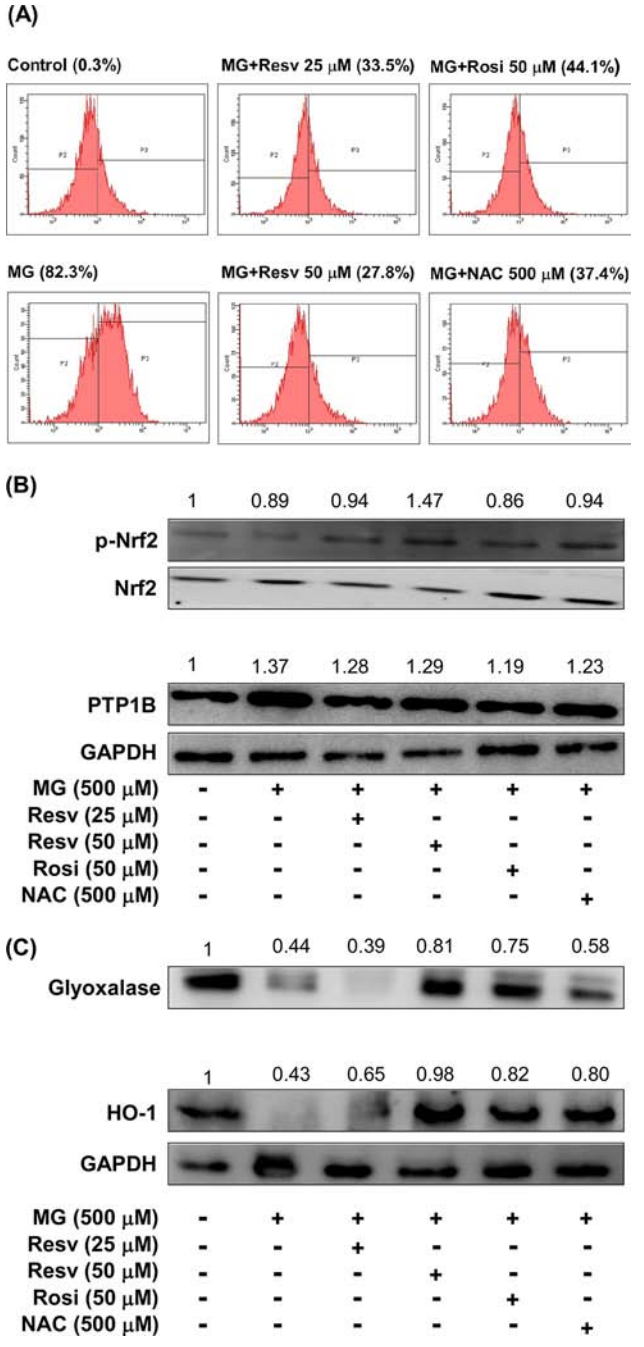


**Figure 3.** Effects of resveratrol on (A) oxidative stress, (B) Nrf2 phosphorylation and PTP1B expression, and (C) glyoxalase and HO-1 expression in MG-treated Hep G2 cells. Resv, resveratrol; Rosi, rosiglitazone; and NAC, *N*-acetyl cysteine.

extracellular signal-regulated kinase (p-ERK). The findings demonstrated that resveratrol treatment for 6 h significantly increased ERK phosphorylation in Hep G2 cells, while p38 and JNK were not phosphorylated by resveratrol treatment (Figure 6). These results suggested that resveratrol treatment improved insulin sensitivity in a Nrf2-dependent manner to inhibit ER and oxidative stress.

**Nrf2 Knockdown.** Although resveratrol has been reported to exert antioxidative effects to improve insulin sensitivity,[23] this antioxidative activity may not be the result of Nrf2 activation. To confirm the anti-inflammatory effect of Nrf2 activation mediated by resveratrol, we investigated the

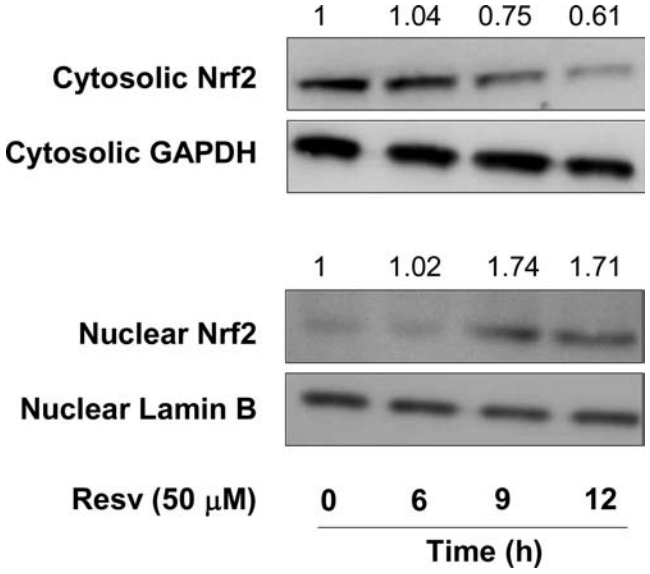


**Figure 4.** Activation of Nrf2 by resveratrol treatment for various times in Hep G2 cells. Resv = resveratrol.

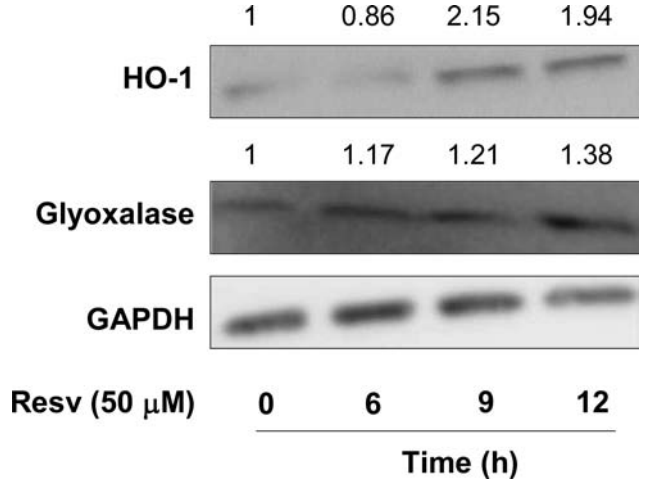


**Figure 5.** Effect of resveratrol on HO-1 and glyoxalase expressions in Hep G2 cells. Resv = resveratrol.

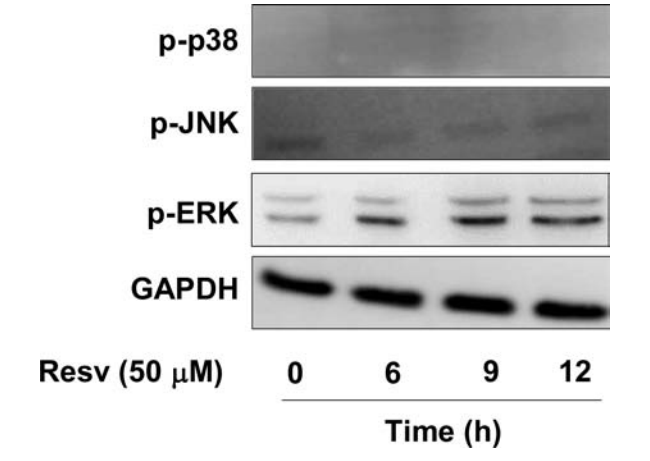


**Figure 6.** Pathway for Nrf2 activation by resveratrol treatment for various times in Hep G2 cells.

improvement of insulin sensitivity and inhibition of ROS in MG-treated Nrf2 knockdown of Hep G2 cells. We found that a 72 h treatment with specific siRNA (50 nM) effectively abrogated Nrf2 expression of Hep G2 cells (Figure 7A). In addition, Nrf2 siRNA knockdown attenuated the inhibition of PTP1B expression by resveratrol in MG-treated Hep G2 cells (Figure 7B). Conversely, glyoxalase and HO-1 expression in MG-treated Hep G2 cells were both reduced by a Nrf2 knockdown with siRNA treatment, and Nrf2 expression was not affected by resveratrol treatment (Figure 7B).

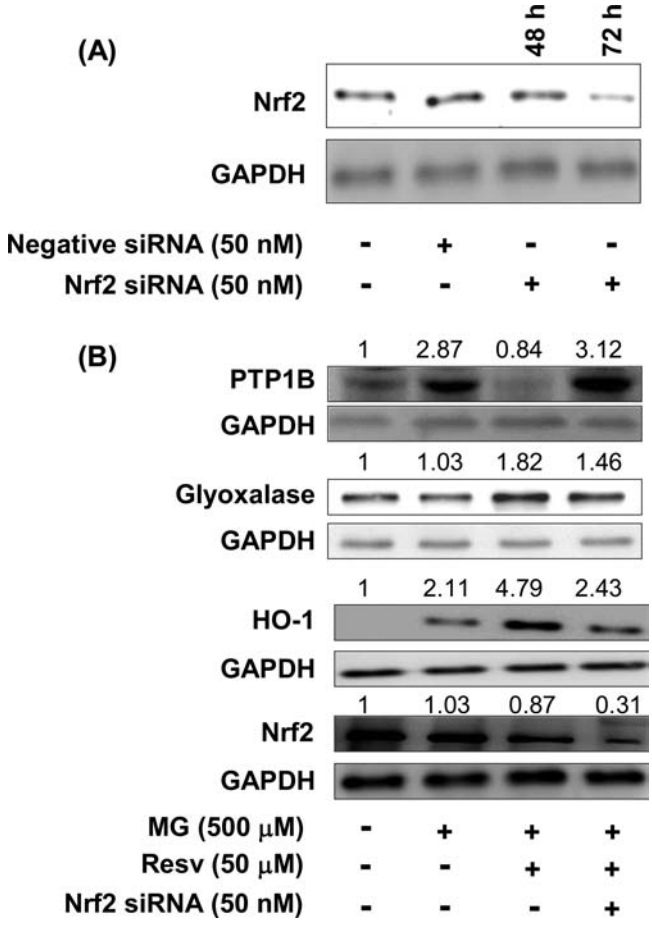


**Figure 7.** (A) Inhibition of Nrf2 expression in Hep G2 cells treated with Nrf2 siRNA. (B) Effects of resveratrol on PTP1B, glyoxalase, HO-1, and Nrf2 expressions in Hep G2 cells treated with MG for 24 h. Resv = resveratrol.

**Effects of Nrf2 Knockdown on Glucose Uptake.** We found that Nrf2 knockdown significantly attenuated the membrane GLUT2 level of Hep G2 cells in the resveratrol group treated with MG for 24 h compared to the resveratrol group, although cytosolic GLUT2 levels of Hep G2 cells were not affected by MG, resveratrol, or Nrf2 siRNA (Figure 8A), suggesting that Nrf2 knockdown promoted downregulation of GLUT2 translocation caused by MG induction. In addition, the glucose uptake activity of MG-treated Hep G2 cells was investigated. Results indicated that the reversion of glucose uptake by resveratrol in MG-treated Hep G2 cells was also attenuated by Nrf2 knockdown (Figure 8B).


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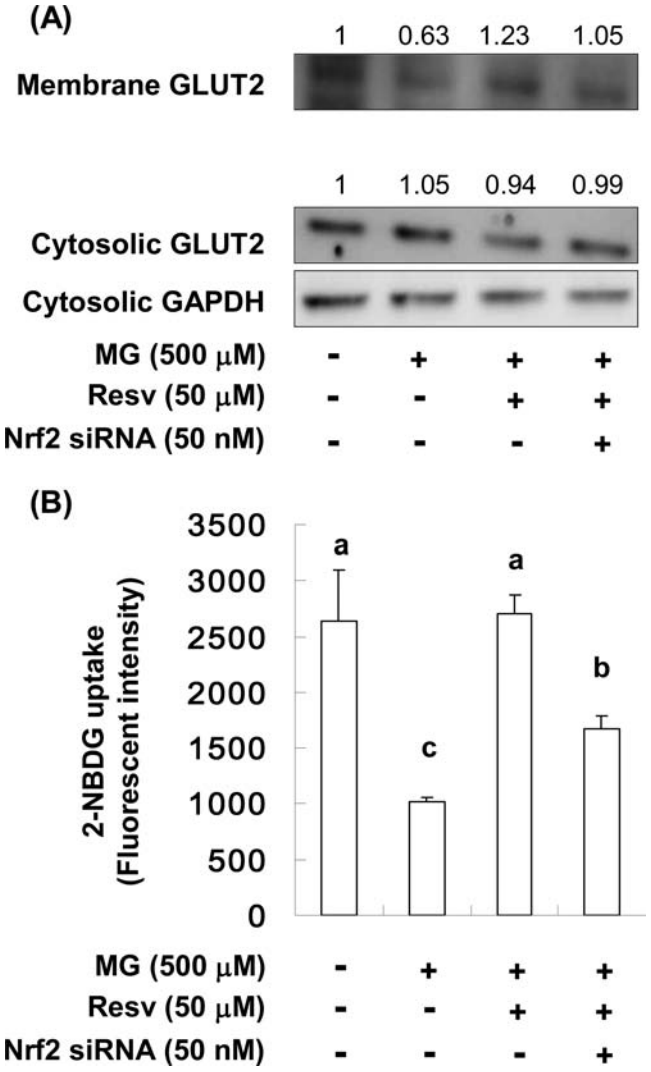


**Figure 8.** Effects of reveratrol on (A) GLUT2 translocation and (B) glucose uptake in MG-treated Hep G2 cells. Significant differences were indicated with different letters (a–c; $p < 0.05$). Resv = resveratrol.

## DISCUSSION

Type-2 diabetes (T2-D) is a chronic disease associated with carbohydrate metabolism and is caused by a deficiency in insulin secretion or ineffective insulin action. Medicinal plants constitute a common alternative treatment for T2-D in many parts of the world. Hyperglycemia, the primary distinguishing feature of T2-D, is a deficiency in the hepatic control of glucose homeostasis. Glucokinase (GK) is a glucose phosphorylating enzyme containing pancreatic $\beta$ cells and hepatocytes, which are critical for glucose homeostasis.[35] In the liver, the enzyme is a vital regulator of glucose storage and disposal.[36] It is known that $\beta$ cells are responsible for insulin secretion, while the role of hepatocytes is thought to be in hepatic glucose uptake.[37] Glucose is taken up into the hepatocytes through GLUT2, and liver-type glucokinase (L-GK) traps glucose within the cytoplasm via phosphorylation.[38] Therefore, GLUT2 and L-GK play important roles in the liver as the glucose-sensing apparatus.[39] In addition, several studies have demonstrated that, by activating insulin signaling and inhibiting the inflammatory response, there will be enhancements to glucose uptake and improvements to insulin resistance in hepatocytes.[40−42] Subsequently, with an impaired insulin signaling pathway, there will also be an inhibition of glucose uptake and glycogen synthesis, which eventually results in insulin resistance in hepatocytes.[43]

The impairment of the ability of insulin to trigger downstream metabolic actions in the liver is defined as hepatic insulin resistance.[44] Recently, it has been discovered that Akt proteins, a family of docking molecules connecting IR activation to essential downstream kinase cascades, are subject to molecular lesions that cause hepatic insulin resistance.[45] Ser/Thr phosphorylation of IRS attenuates the insulin-stimulating activities of phosphatidylinositol 3 kinase (PI3K) and Akt by affecting IRS phosphorylation at tyrosine residues.[46] In theory, Tyr phosphorylation of IRS may activate PKB/Akt signaling to promote GLUT translocation to the membrane, thereby improving insulin sensitivity; however, in practice, PTP1B attenuates IRS Tyr phosphorylation, resulting in insulin resistance.[32] In addition, ER stress results in ROS generation, which, in turn, leads to insulin resistance; MG can also induce oxidative stress.[31] Insulin resistance has already been identified as a leading cause to pre-diabetes and T2-D.[47] Phytoalexin resveratrol is a polyphenol produced by several plants and holds many biological applications, which include those of significant anti-diabetic activity.[48,49] Studies of *in vivo* and *in vitro* models with attenuating effects on insulin resistance have shown that resveratrol may take multiple pathways toward improving insulin sensitivity. Through these pathways, resveratrol can cause an increase in AMP kinase activity,[50,51] activate SIRT1,[52−55] enhance PPAR$\gamma$ activity,[56] elevate caveolin-3 (CAV-3) expression,[47] and activate Nrf2.[57] In addition, a recent study with human subjects has found that the insulin resistance attenuating effect of resveratrol also occurs via the activation of the Akt signaling pathway.[58] In the present study, we also found that resveratrol effectively attenuated oxidative stress and MG-induced insulin resistance in Hep G2 cells.

Abnormal cellular accumulation of MG invariably occurs in diabetes.[20] Furthermore, MG reduces glucose tolerance in rodents,[59] suggesting that postprandial MG production in normoglycemic individuals could result in glucose intolerance and, consequently, greater MG accumulation. As a redox-dependent transcription factor, Nrf2 controls the expression and coordinates the induction of a number of genes, including those that express stress response proteins and detoxifying enzymes. Therefore, the nuclear abundance of Nrf2 is tightly regulated through the control of the mechanisms of nuclear export and degradation of Nrf2.

Recently, antioxidants, such as quercetin and phenolic acid, have been investigated for their ability to attenuate oxidative damage by activating Nrf2.[60,61] Furthermore, Nrf2 activation has been found to be essential for HO-1 and glyoxalase expression.[13,14,16] In concurrence with previous studies, our study concludes that resveratrol can activate, at least in part, the ERK signaling pathway, which, in turn, enhances Nrf2 nuclear translocation and activation.[62] These results provide further evidence indicating that resveratrol promotes HO-1 and glyoxalase expression by mediating Nrf2 activation via ERK phosphorylation to inhibit oxidative stress and scavenge for MG, thereby attenuating insulin resistance. The potential mechanism of resveratrol-mediated protection against MG-induced insulin resistance is shown in Figure 9.

In conclusion, to study the significance of glycoxidative stress on the pathology of diabetes, the effects of antioxidant supplements that inhibit protein modifications have been

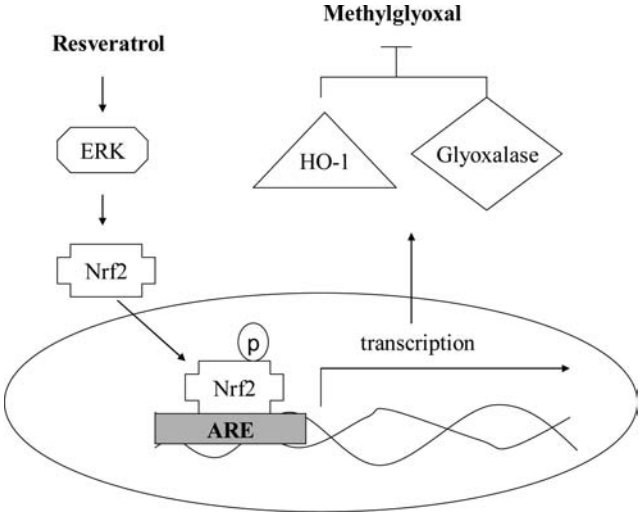


**Figure 9.** Mechanism of insulin resistance inhibition by resveratrol in MG-treated Hep G2 cells. Resveratrol promotes the expression of HO-1 and glyoxalase by mediating Nrf2 activation via ERK phosphorylation. This inhibits oxidative stress and scavenge for MG, effectively attenuating insulin resistance.

examined under diabetic conditions. Antioxidant supplements have been proposed to be effective in treating complications secondary to diabetes. This hypothesis is supported by clinical results, indicating that the development of diabetes and accumulation of MG may be reduced by the intake of natural antioxidants through the diet.

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### Notes

The authors declare no competing financial interest.

## REFERENCES

(1) Day, J. F.; Thorpe, S. R.; Baynes, J. W. Nonenzymatically glucosylated albumin: *In vitro* preparation and isolation from normal human serum. *J. Biol. Chem.* **1979**, *254*, 595−597.

(2) Bonnefont-Rousselot, D. Glucose and reactive oxygen species. *Curr. Opin. Clin. Nutr. Metab. Care* **2002**, *5*, 561−568.

(3) Wu, C. H.; Huang, S. M.; Lin, J. A.; Yen, G. C. Inhibition of advanced glycation endproduct formation by foodstuffs. *Food Funct.* **2011**, *2*, 224−234.

(4) Huang, S. M.; Wu, C. H.; Yen, G. C. Effects of flavonoids on the expression of the pro-inflammatory response in human monocytes induced by ligation of the receptor for AGEs. *Mol. Nutr. Food Res.* **2006**, *50*, 1129−1139.

(5) Lin, J. A.; Fang, S. C.; Wu, C. H.; Huang, S. M.; Yen, G. C. Anti-inflammatory effect of the 5,7,40-trihydroxy-6-geranyl flavanone isolated from the fruit of *Artocarpus communis* in S100B-induced human monocytes. *J. Agric. Food Chem.* **2011**, *59*, 105−111.

(6) Chan, W. H.; Wu, H. J.; Hsuuw, Y. D. Curcumin inhibits ROS formation and apoptosis in methylglyoxal-treated human hepatoma G2 cells. *Ann. N. Y. Acad. Sci.* **2005**, *1042*, 372−378.

(7) Ponugoti, B.; Dong, G.; Graves, D. T. Role of forkhead transcription factors in diabetes-induced oxidative stress. *Exp. Diabetes Res.* **2012**, DOI: 10.1155/2012/939751.

(8) Fiory, F.; Lombardi, A.; Miele, C.; Giudicelli, J.; Beguinot, F.; Van Obberghen, E. Methylglyoxal impairs insulin signalling and insulin action on glucose-induced insulin secretion in the pancreatic beta cell line INS-1E. *Diabetologia* **2011**, *54*, 2941−2952.

(9) Gugliucci, A. "Blinding" of AMP-dependent kinase by methylglyoxal: A mechanism that allows perpetuation of hepatic insulin resistance? *Med. Hypotheses* **2009**, *73*, 921−924.

(10) Guha, M.; Bai, W.; Nadler, J. L.; Natarajan, R. Molecular mechanisms of tumor necrosis factor alpha gene expression in monocytic cells via hyperglycemia-induced oxidant stress-dependent and -independent pathways. *J. Biol. Chem.* **2000**, *275*, 17728−17739.

(11) Shanmugam, N.; Kim, Y. S.; Lanting, L.; Natarajan, R. Regulation of cyclooxygenase-2 expression in monocytes by ligation of the receptor for advanced glycation end products. *J. Biol. Chem.* **2003**, *278*, 34834−34844.

(12) Shanmugam, N.; Reddy, M. A.; Guha, M.; Natarajan, R. High glucose-induced expression of proinflammatory cytokine and chemokine genes in monocytic cells. *Diabetes* **2003**, *52*, 1256−1264.

(13) Keum, Y. S.; Owuor, E. D.; Kim, B. R.; Hu, R.; Kong, A. N. Involvement of Nrf2 and JNK1 in the activation of antioxidant responsive element (ARE) by chemopreventive agent phenethyl isothiocyanate (PEITC). *Pharm. Res.* **2003**, *20*, 1351−1356.

(14) Kobayashi, M.; Yamamoto, M. Molecular mechanisms activating the Nrf2−Keap1 pathway of antioxidant gene regulation. *Antioxid. Redox Signaling* **2005**, *7*, 385−394.

(15) Xue, M.; Rabbani, N.; Momiji, H.; Imbasi, P.; Anwar, M. M.; Kitteringham, N.; Park, B. K.; Souma, T.; Moriguchi, T.; Yamamoto, M.; Thornalley, P. J. Transcriptional control of glyoxalase 1 by Nrf2 provides a stress-responsive defence against dicarbonyl glycation. *Biochem. J.* **2012**, *443*, 213−222.

(16) Vander-Jagt, D.; Hunsaker, L. Methylglyoxal metabolism and diabetic complications: Roles of aldose reductase, glyxalase-I, betaine aldehyde dehydrogenase and 2 oxoaldehyde dehydrogenase. *Chem.-Biol. Interact.* **2003**, *143/144*, 341−351.

(17) Desai, K.; Wu, L. Methylglyoxal and advanced glycation endproducts: New therapeutic horizons? *Recent Pat. Cardiovasc. Drug Discovery* **2007**, *2*, 89−99.

(18) Weng, C. J.; Chen, M. J.; Yeh, C. T.; Yen, G. C. Hepatoprotection of quercetin against oxidative stress by induction of metallothionein expression through activating MAPK and PI3K pathways and enhancing Nrf2 DNA-binding activity. *New Biotechnol.* **2011**, *28*, 767−777.

(19) Yeh, C. T; Yen, G. C. Induction of hepatic antioxidant enzymes by phenolic acids in rats is accompanied by increased levels of multidrug resistance-associated protein 3 mRNA expression. *J. Nutr.* **2006**, *136*, 11−15.

(20) Wu, C. H.; Huang, S. M.; Yen, G. C. Silymarin: A novel antioxidant with antiglycation and antiinflammatory properties in vitro and in vivo. *Antioxid. Redox Signaling* **2011**, *14*, 353−366.

(21) He, X.; Wang, L.; Szklarz, G.; Bi, Y.; Ma, Q. Resveratrol inhibits paraquat-induced oxidative stress and fibrogenic response by activating the nuclear factor erythroid 2-related factor 2 pathway. *J. Pharmacol. Exp. Ther.* **2012**, *342*, 81−90.

(22) Palsamy, P.; Subramanian, S. Resveratrol protects diabetic kidney by attenuating hyperglycemia-mediated oxidative stress and renal inflammatory cytokines via Nrf2−Keap1 signaling. *Biochim. Biophys. Acta* **2011**, *1812*, 719−731.

(23) Turan, B.; Tuncay, E.; Vassort, G. Resveratrol and diabetic cardiac function: Focus on recent in vitro and in vivo studies. *J. Bioenerg. Biomembr.* **2012**, *44*, 281−296.

(24) Dhar, A.; Dhar, I.; Jiang, B.; Desai, K. M.; Wu, L. Chronic methylglyoxal infusion by minipump causes pancreatic $\beta$-cell dysfunction and induces type 2 diabetes in Sprague-Dawley rats. *Diabetes* **2011**, *60*, 899−908.

(25) Untereiner, A. A.; Dhar, A.; Liu, J.; Wu, L. Increased renal methylglyoxal formation with down-regulation of PGC-1$\alpha$-FBPase pathway in cystathionine $\gamma$-lyase knockout mice. *PLoS One* **2011**, *6*, No. e29592.

(26) Pashikanti, S.; de Alba, D. R.; Boissonneault, G. A.; Cervantes-Laurean, D. Rutin metabolites: Novel inhibitors of nonoxidative advanced glycation end products. *Free Radical Biol. Med.* **2010**, *48*, 656−663.

(27) Dhar, A.; Dhar, I.; Desai, K. M.; Wu, L. Methylglyoxal scavengers attenuate endothelial dysfunction induced by methylglyoxal and high concentrations of glucose. *Br. J. Pharmacol.* **2010**, *161*, 1843−1856.
(28) Huang, F. J.; Chin, T. Y.; Chan, W. H. Resveratrol protects against methylglyoxal-induced apoptosis and disruption of embryonic development in mouse blastocysts. *Environ. Toxicol.* **2011**, DOI: 10.1002/tox.20734.
(29) Lee, B. H.; Hsu, W. H.; Liao, T. H.; Pan, T. M. The *Monascus* metabolite monascin against TNF-$\alpha$-induced insulin resistance via suppressing PPAR-$\gamma$ phosphorylation in C2C12 myotubes. *Food Chem. Toxicol.* **2011**, *49*, 2609−2617.
(30) Huang, T. C.; Chung, Y. L.; Wu, M. L.; Chuang, S. M. Cinnamaldenhyde enhances Nrf2 nuclear translocation to upregulate phase II detoxifying enzyme expression in Hep G2 cells. *J. Agric. Food Chem.* **2011**, *59*, 5164−5171.
(31) Dhar, A.; Desai, K. M.; Wu, L. Alagebrium attenuates acute methylglyoxal-induced glucose intolerance in Sprague-Dawley rats. *Br. J. Pharmacol.* **2010**, *159*, 166−175.
(32) Wang, N.; Zhang, D. L.; Mao, X. Q.; Zou, F.; Jin, H.; Ouyang, J. P. Astragalus polysaccharides decreased the expression of PTP1B through relieving ER stress induced activation of ATF6 in a rat model of type 2 diabetes. *Mol. Cell. Endocrinol.* **2009**, *307*, 89−98.
(33) Ogborne, R. M.; Rushworth, S. A.; O'Connell, M. A. Epigallocatechin activates haem oxygenase-1 expression via protein kinase C$\delta$ and Nrf2. *Biochem. Biophys. Res. Commun.* **2008**, *373*, 584−588.
(34) Wu, C. C.; Hsu, M. C.; Hsieh, C. W.; Lin, J. B.; Lai, P. H.; Wung, B. S. Upregulation of heme oxygenase-1 by epigallocatechin-3-gallate via the phosphatidylinositol 3-kinase/Akt and ERK pathways. *Life Sci.* **2006**, *78*, 2889−2897.
(35) Matschinsky, F. M.; Zelent, B.; Doliba, N.; Li, C.; Vanderkooi, J. M.; Nali, A.; Sarabu, R.; Grimsby, J. Glucokinase activators for diabetes therapy. *Diabetes Care* **2011**, *34*, S236−S243.
(36) O'Doherty, R. M.; Lehman, D. L.; Télémaque-Potts, S.; Newgard, C. B. Metabolic impact of glucokinase overexpression in liver: Lowering of blood glucose in fed rats is accompanied by hyperlipidemia. *Diabetes* **1999**, *48*, 2022−2027.
(37) Hariharan, N.; Farrelly, D.; Hanan, D.; Hillyer, D.; Arbeeny, C.; Sabrah, T.; Treloar, A.; Brown, K.; Kalinowski, S.; Mookhtiar, K. Expression of human hepatic glucokinase in transgenic mice liver results in decreased glucose levels and reduced body weight. *Diabetes* **1997**, *46*, 11−16.
(38) Higuchi, N.; Kato, M.; Miyazaki, M.; Tanaka, M.; Kohjima, M.; Ito, T.; Nakamuta, M.; Enjoji, M.; Kotoh, K.; Takayanagi, R. Potential role of branched-chain amino acids in glucose metabolism through the accelerated induction of the glucose-sensing apparatus in the liver. *J. Cell. Biochem.* **2011**, *112*, 30−38.
(39) Kim, H. I.; Ahn, Y. H. Role of peroxisome proliferator-activated receptor gamma in the glucose-sensing apparatus of liver and beta-cells. *Diabetes* **2004**, *53*, S60−S65.
(40) Huang, D. W.; Shen, S. C.; Wu, J. S. Effects of caffeic acid and cinnamic acid on glucose uptake in insulin-resistant mouse hepatocytes. *J. Agric. Food Chem.* **2009**, *57*, 7687−7692.
(41) Zhang, W. Y.; Lee, J. J.; Kim, Y.; Kim, I. S.; Han, J. H.; Lee, S. G.; Ahn, M. J.; Jung, S. H.; Myung, C. S. Effect of eriodictyol on glucose uptake and insulin resistance in vitro. *J. Agric. Food Chem.* **2012**, *60*, 7652−7658.
(42) Shen, S. C.; Chang, W. C.; Chang, C. L. Fraction from wax apple [*Syzygium samarangense* (Blume) Merrill and Perry] fruit extract ameliorates insulin resistance via modulating insulin signaling and inflammation pathway in tumor necrosis factor $\alpha$-treated FL83B mouse hepatocytes. *Int. J. Mol. Sci.* **2012**, *13*, 8562−8577.
(43) Hsieh, M. J.; Lan, K. P.; Liu, H. Y.; Zhang, X. Z.; Lin, Y. F.; Chen, T. Y.; Chiou, H. L. Hepatitis C virus E2 protein involve in insulin resistance through an impairment of Akt/PKB and GSK3ß signaling in hepatocytes. *BMC Gastroenterol.* **2012**, DOI: 10.1186/1471-230X-12-74.
(44) Kim, S. P.; Ellmerer, M.; Can Citters, G. W.; Bergman, R. N. Primacy of hepatic insulin resistance in the development of the metabolic syndrome induced by an isocaloric moderate-fat diet in the dog. *Diabetes* **2003**, *52*, 2453−2460.
(45) Thirone, A. C.; Huang, C.; Klip, A. Tissue-specific roles of IRS proteins in insulin signaling and glucose transport. *Trends Endocrinol. Metab.* **2006**, *17*, 72−78.
(46) Hartman, M. E.; Villela-Bach, M.; Chen, J.; Freund, G. G. Frap dependent serine phosphorylation of IRS-1 inhibits IRS-1 tyrosine phosphorylation. *Biochem. Biophys. Res. Commun.* **2001**, *280*, 776−781.
(47) Tan, Z.; Zhou, L. J.; Mu, P. W.; Liu, S. P.; Chen, S. J.; Fu, X. D.; Wang, T. H. Caveolin-3 is involved in the protection of resveratrol against high-fat-diet-induced insulin resistance by promoting GLUT4 translocation to the plasma membrane in skeletal muscle of ovariectomized rats. *J. Nutr. Biochem.* **2012**.
(48) Xu, Y.; Nie, L.; Yin, Y. G.; Tang, J. L.; Zhou, J. Y.; Li, D. D.; Zhou, S. W. Resveratrol protects against hyperglycemia-induced oxidative damage to mitochondria by activating SIRT1 in rat mesangial cells. *Toxicol. Appl. Pharmacol.* **2012**, *259*, 395−401.
(49) Yu, W.; Fu, Y. C.; Wang, W. Cellular and molecular effects of resveratrol in health and disease. *J. Cell. Biochem.* **2012**, *113*, 752−759.
(50) Baur, J. A.; Pearson, K. J.; Price, N. L.; Jamieson, H. A.; Lerin, C.; Kalra, A.; Prabhu, V. V.; Allard, J. S.; Lopez-Lluch, G.; Lewis, K.; Pistell, P. J.; Poosala, S.; Becker, K. G.; Boss, O.; Gwinn, D.; Wang, M.; Ramaswamy, S.; Fishbein, K. W.; Spencer, R. G.; Lakatta, E. G.; Le Couteur, D.; Shaw, R. J.; Navas, P.; Puigserver, P.; Ingram, D. K.; de Cabo, R.; Sinclair, D. A. Resveratrol improves health and survival of mice on a high-calorie diet. *Nature* **2006**, *444*, 337−7342.
(51) Shang, J.; Chen, L. L.; Xiao, F. X.; Sun, H.; Ding, H. C.; Xiao, H. Resveratrol improves non-alcoholic fatty liver disease by activating AMP-activated protein kinase. *Acta Pharmacol. Sin.* **2008**, *29*, 698−706.
(52) Lagouge, M.; Argmann, C.; Gerhart-Hines, Z.; Meziane, H.; Lerin, C.; Daussin, F.; Messadeq, N.; Milne, J.; Lambert, P.; Elliott, P.; Geny, B.; Laakso, M.; Puigserver, P.; Auwerx, J. Resveratrol improves mitochondrial function and protects against metabolic disease by activating SIRT1 and PGC-1$\alpha$. *Cell* **2006**, *721*, 1109−1122.
(53) Sun, C.; Zhang, F.; Ge, X.; Yan, T.; Chen, X.; Shi, X.; Zhai, Q. SIRT1 improves insulin sensitivity under insulin-resistant conditions by repressing PTP1B. *Cell Metab.* **2007**, *6* (3), 07−319.
(54) Chen, L. L.; Zhang, H. H.; Zhang, J.; Hu, X.; Kong, W.; Hu, D.; Wang, S. X.; Zhang, P. Resveratrol attenuates high-fat diet-induced insulin resistance by influencing skeletal muscle lipid transport and subsarcolemmal mitochondrial $\beta$-oxidation. *Metabolism* **2011**, *60*, 1598−1609.
(55) Lee, J. H.; Song, M. Y.; Song, E. K.; Kim, E.; Moon, W. S.; Han, M. K.; Park, J. W.; Kwon, K. B.; Park, B. H. Overexpression of SIRT1 protects pancreatic beta-cells against cytokine toxicity by suppressing the nuclear factor-kappaB signaling pathway. *Diabetes* **2009**, *58*, 344−351.
(56) Kennedy, A.; Overman, A.; Lapoint, K.; Hopkins, R.; West, T.; Chuang, C. C.; Martinez, K.; Bell, D.; McIntosh, M. Conjugated linoleic acid-mediated inflammation and insulin resistance in human adipocytes are attenuated by resveratrol. *J. Lipid Res.* **2009**, *50*, 225−232.
(57) Bagul, P. K.; Middela, H.; Matapally, S.; Padiya, R.; Bastia, T.; Madhusudana, K.; Reddy, B. R.; Chakravarty, S.; Banerjee, S. K. Attenuation of insulin resistance, metabolic syndrome and hepatic oxidative stress by resveratrol in fructose-fed rats. *Pharmacol. Res.* **2012**, *66*, 260−268.
(58) Brasnyó, P.; Molnár, G. A.; Mohás, M.; Markó, L.; Laczy, B.; Cseh, J.; Mikolás, E.; Szijártó, I. A.; Mérei, A.; Halmai, R.; Mészáros, L. G.; Sümegi, B.; Wittmann, I. Resveratrol improves insulin sensitivity, reduces oxidative stress and activates the Akt pathway in type 2 diabetic patients. *Br. J. Nutr.* **2011**, *106*, 383−389.
(59) Ankrah, N. A.; Appiah-Opong, R. Toxicity of low levels of methylglyoxal: Depletion of blood glutathione and adverse effect on glucose tolerance in mice. *Toxicol. Lett.* **1999**, *109*, 61−67.

(60) He, M.; Siow, R. C.; Sugden, D.; Gao, L.; Cheng, X.; Mann, G. E. Induction of HO-1 and redox signaling in endothelial cells by advanced glycation end products: A role for Nrf2 in vascular protection in diabetes. *Nutr. Metab. Cardiovasc. Dis.* **2011**, *21*, 277−285.
(61) Woodside, J. V.; Yarnell, J. W. G.; McMaster, D.; Young, I. S.; Harmon, D. L; McCrum, E. E.; Patterson, C. C.; Gey, K. F.; Whitehead, A. S.; Evans, A. Effect of B-group vitamins and antioxidant vitamins on hyperhomocysteinemiaa double-blind, randomized, factorial-design, controlled trial. *Am. J. Clin. Nutr.* **1998**, *67*, 858−866.
(62) Chen, C. Y.; Jang, J. H.; Li, M. H.; Surh, Y. J. Resveratrol upregulates heme oxygenase-1 expression via activation of NF-E2-related factor 2 in PC12 cells. *Biochem. Biophys. Res. Commun.* **2005**, *331*, 993−1000.