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ORIGINAL PAPER

Ginkgo biloba extract enhances glucose tolerance in hyperinsulinism-induced hepatic cells

Lei Zhou · Qingjie Meng · Tao Qian · Zaiqing Yang

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Abstract *Ginkgo biloba*, an herbal medication, is capable of lowering glucose, fat, and lipid peroxide in diabetic patients. In the current study, we tested the hypothesis that *Ginkgo biloba* extract (GBE) prevented hyperinsulinism-induced glucose intolerance in hepatocytes. We investigated the effects of GBE on glucose consumption, glucokinase activity, and mRNA levels of key genes in glucose metabolism and the insulin signaling pathway. To better show its efficacy, we included a control group that was treated with rosiglitazone, a type of thiazolidinedione (TZD). The data indicated that GBE repressed glucose uptake under normal conditions, while it dramatically improved glucose tolerance under insulin-resistant conditions. Furthermore, after analyzing gene expression, we suggest that GBE chiefly exerts its effects by stimulating IRS-2 transcription. It should be noted that, unlike rosiglitazone, GBE did not stimulate excessive glucose uptake as it improved glucose tolerance. It is said that GBE treatment could avoid drug-induced obesity. Our data suggest that GBE has the potential to prevent insulin resistance and is a promising anti-diabetic drug.

Keywords Herb · Thiazolidinedione · Diabetes · Insulin resistance · Hepatocytes

L. Zhou · Q. Meng · T. Qian · Z. Yang (✉)
Key Laboratory of Agricultural Animal Genetics, Breeding and Reproduction of the Ministry of Education, College of Life Science and Technology, Huazhong Agricultural University, Wuhan 430070, Hubei, People's Republic of China
e-mail: yangzq@mail.hzau.edu.cn

L. Zhou
e-mail: zhoulei@mail.hzau.edu.cn

Abbreviations

GBE	*Ginkgo biloba* extract
G6Pase	Glucose-6-phosphatase
IRS	Insulin receptor substrate
GLUT	Glucose transporter
PPAR	Peroxisome proliferator-activated receptor
SREBP	Sterol regulatory element-binding protein
TZD	Thiazolidinedione

Introduction

It is estimated that by the year 2020 there will be approximately 300 million people affected by type 2 diabetes mellitus in the world. Of even greater concern is the difficulty associated with preventing its global spread, and most of the increase is expected occur in developing countries. We urgently need to understand and control this disease as soon as possible.

Ginkgo biloba, an herb, has been used in traditional Chinese medicine for thousands of years. *Ginkgo biloba* extract (GBE), which is the leaf extract of *Ginkgo biloba*, is being widely studied and applied for its beneficial properties in the treatment or prevention of human diseases. GBE has been reported to lower blood glucose, fat, and lipid peroxide and prevent atherosclerosis in animal models and humans. In previous studies, GBE was found to have anti-inflammatory action [1, 2] and to stimulate skin microcirculation [3]. It has also been used as a therapeutic agent for some cardiovascular and neurological disorders. Recently, in vitro and in vivo evidence has increasingly demonstrated that GBE has potential efficacy in lipid metabolism, glucose metabolism, and in treating

diabetes mellitus. Saponara and Bosisio [4] showed that GBE inhibited cAMP phosphodiesterase in rat adipose tissue. Dell'Agli and Bosisio [5] illustrated that the biflavones of *Ginkgo biloba* stimulated lipolysis in fully differentiated 3T3-L1 adipocytes. Boveris et al. [6] obtained the same results as Dell'Agli and Bosisio and further proved that GBE inhibited lipid peroxidation. By investigating a diabetic rodent model, Nian et al. [7] indicated that GBE lowered the postprandial blood-glucose level; decreased the levels of total cholesterol (TC), triglycerides (TG), and low-density lipoprotein (LDL); promoted super oxide dismutase (SOD) activity in serum; and relieved damage to the pancreatic islets. Moreover, Wei et al. [8] found GBE could protect endothelial cells from damage by decreasing serum lipid levels and suppressing inflammatory response. It is well known that oxidation-modified LDL plays an important role in the pathogenesis of artherosclerosis with type 2 diabetes mellitus. Maitra et al. [9] found GBE was also capable of inhibiting the oxidation of LDL. In addition, early detection of retina pathology is pivotal in monitoring visual complications in diabetic patients. Researchers showed GBE prevented diabetic retinopathy, and they thought it was a good adjuvant for patients with long-lasting diabetes mellitus [10]. All data suggested that GBE was valuable in diabetic therapy.

The liver is an important organ for glucose metabolism and energy homeostasis, and hepatic insulin resistance is a key component in the development of type 2 diabetes mellitus. In this process, PPARs, GLUT, G6Pase, IRS, and SREBPs play crucial roles. GLUT2 is the primary glucose-transporter isoform in the liver and is pivotal in glucose homeostasis by mediating bidirectional transport of glucose [11]. PPARs and SREBPs are well characterized transcription factors. PPARγ was deemed the main isoform in adipocytes, but it has also been found to mediate lipid metabolism and energy homeostasis by changing its expression in the liver [12]. SREBP1c is crucial for the regulation of lipogenic gene. Recent studies have also found that it is interrelated with insulin action [13]. G6Pase as the last enzyme in hepatic glucogenesis is an important determinant of hepatic glucose fluxes. IRS-2 is the main isoform in liver. It compensates for the lack of IRS-1 in the IRS-$1^{-/-}$ model [14]. Hepatic insulin signaling is mediated mainly through IRS-2 [15].

To our knowledge, no systematic study has illustrated the molecular mechanism by which GBE improves insulin sensitivity and enhances glucose tolerance in the insulin resistance model. So, in this study we tested the hypothesis that GBE is involved in modulation of insulin action and enhances glucose tolerance. To better interpret its molecular mechanism, we assayed the expression and glucokinase activity of the genes listed above.

Materials and methods

Materials

The powder form of GBE was purchased from Greensky Biological Tech (Hangzhou, China). The active component of GBE is represented by 24% ginkgo flavone glycosides, 6% ginkgolides, and less than 1 ppm ginkgolic acid. TRIzol was obtained from Sangon (Shanghai, China). The Mammalian Cell Protein Extraction kit was purchased from Shenergy Biocolor BioScience & Technology (Shanghai, China). The glucose assay kit was obtained from Shenergy-diasys Diagnostic Technology (Shanghai, China).

Cell culture and treatment

The L-02 cell line was derived from adult human liver [16]. The cells were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) at 5% CO_2 and 37°C. For the relevant experiments, the density of cells was about 5×10^5 cells/well in 24-well culture plates for RNA extraction or 5×10^6 cells/dish in 60-mm Petri dishes for metabolite concentration assay. There were two groups of cells in the experiments, those mimicking normal physiological status (N) and those mimicking insulin-resistant status owing to hyperinsulinemic treatment in vitro (A) [17–19]. The subgroups of normal cells included the following: controls (NC), cells treated with 10 μM rosiglitazone (NRT), and cells treated with 10 mg/l GBE (NGT) group. All normal cells were cultured in DMEM supplemented with 10% FBS and treated with 10 nM insulin. The abnormal group included the following three subgroups: controls (AC), cells treated with 10 μM rosiglitazone (ART), and cells treated with 10 mg/l GBE (AGT). All abnormal cells were cultured in DMEM supplemented with 10% FBS and given 100 nM insulin. All treatments are listed in Table 1.

RNA isolation

Total RNA was isolated from L-02 cells using TRIzol after culturing the cells for 36 h. All of the RNA samples were treated with DNase I to digest the genomic DNA and stored at −80°C.

Semiquantitative RT-PCR

Semiquantitative RT-PCR with 3-phosphate dehydrogenase (GAPDH) as an internal control was performed to determine the levels of PPARγ, IRS-2, GLUT2, SREBP1c, and G6Pase mRNA in L-02 cells. A 4 μl RNA sample was reverse transcribed with oligo(dT)18. cDNA (2 μl) was

Table 1 Cell treatments by group

Reagent	Normal conditions			Abnormal conditions (hyperinsulinism-induced)		
	NC	NRT	NGT	AC	ART	AGT
Insulin (10 nM)	+	+	+			
Insulin (100 nM)				+	+	+
Rosiglitazone		+			+	
Gingko biloba extract			+			+

Table 2 The primers used for semiquantitative RT-PCR

Gene	Size (bp)	Forward and reverse primer (5′–3′)	Accession number
PPARγ	195	F: TCTCCAGTGATATCGACCAGC	BT007281
		R: TTTTATCTTCTCCCATCATTAAGG	
IRS-2	383	F: CACCTCCCCACGACAGTTGC	NM_003749
		R: GGTGGGACAAGAAGTCAATGCTG	
GLUT2	398	F: TTTTCAGACGGCTGGTATCAGC	J03810
		R: CACAGAAGTCCGCAATGTACTGG	
SREBP1c	248	F: CACCGTTTCTTCGTGGATGG	BC057388
		R: CCCGCAGCATCAGAACAGC	
G6Pase	244	F: CGACCTACAGATTTCGGTGCTTG	NM_000151
		R: AGATAAAATCCGATGGCGAAGC	
GAPDH	452	F: ACCACAGTCCATGCCATCAC	BC083511
		R: TCCACCACCCTGTTGCTGTA	

used for PCR amplification with 1 U Taq DNA polymerase. The PCR products were run on a 1% agarose gel containing ethidium bromide and viewed under UV light. All primers are listed in Table 2. Preliminary experiments were carried out with various PCR cycles to determine the linear range of amplification for all genes. The results are always normalized by the expression of GAPDH.

Protein isolation and concentration assay

Proteins in L-02 cells were isolated using the Mammalian Cell Protein Extraction kit. Protein concentrations were determined by Bradford assay [20].

Glucose concentration assay

The glucose concentration in medium of L-02 cells was assayed using the glucose assay kit. Absorbance was assayed at 340 nm using Beckman Coulter DU 800 UV/visible spectrophotometer. All sample concentrations were normalized by each protein amount. The consumption of glucose was calculated as the beginning glucose concentration minus the ending glucose concentration.

Glucokinase assay

Enzymatic activity was assayed as described previously [21], using NAD as coenzyme and glucose-6-phosphate dehydrogenase as coupling enzyme. The assay buffer contained 100 mM triethanolamine hydrochloride (Tris-HCL, pH 7.8), 5 mM $MgCl_2$, 5 mM ATP, 150 mM KCl, 2 mM dithiothreitol, 0.2% bovine serum albumin, 1 mM NAD, and 1 U/ml of G6PDH. Correction for low hexokinase activity was applied by subtracting the activity measured at 0.5 mM glucose from the activity measured at 100 mM glucose. Absorbance was measured at 340 nm using Beckman Coulter DU 800 UV/visible spectrophotometer. Enzymatic activity was normalized by the protein amount.

Statistical analysis

Results are expressed as mean ± SE of three independent experiments in triplicate. Statistical differences between the means were assessed by a two-tailed Student's t test; $p < 0.05$ was considered significant.

Results

In normal status, GBE suppressed glucose consumption but had little effect on glucokinase activity

All cells were cultured in DMEM. NC was treated with 10 nM insulin; NRT was treated with 10 nM insulin and rosiglitazone; NGT was treated with 10 nM insulin and

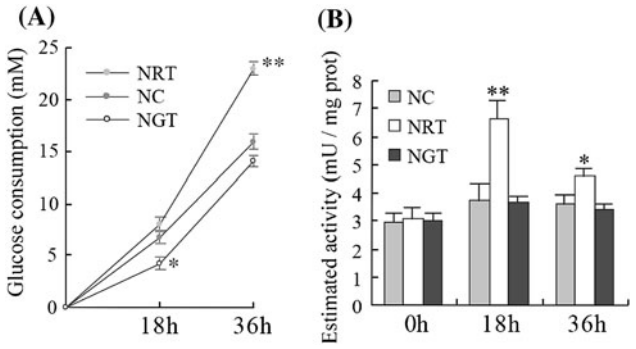

Fig. 1a, b The effects of GBE and rosiglitazone on glucose consumption and glucokinase activity in normal status cells. After plating, L-02 cells were cultured for 36 h as described in "Materials and methods." After 0, 18, and 36 h, glucose concentration in medium (**a**) and glucokinase activity (**b**) were determined. Data were normalized by protein amount and are given as the mean ± SE of three independent experiments in triplicate. $*p < 0.05$, $**p < 0.01$ compared with control group. *NC* Normal status control, *NRT* normal status cells treated with rosiglitazone, *NGT* normal status cells treated with GBE

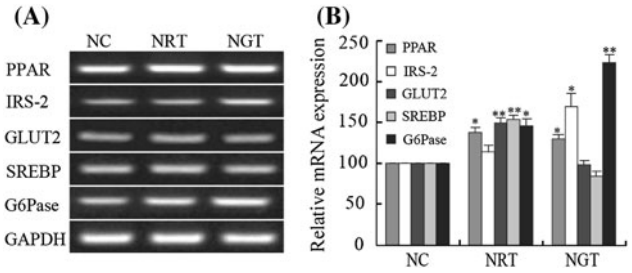

Fig. 2a, b The effects of GBE and rosiglitazone on gene expression in normal status cells. After plating, hepatocytes were cultured for 36 h as described in "Materials and methods." After 36 h, total RNA was extracted and analyzed for the expression of PPARγ, IRS-2, GLUT2, SREBP1c, and G6Pase (**a**). The quantification of blots for these gene expressions, obtained in three independent experiments in triplicate, is shown (**b**). GAPDH was used as an internal control to normalize the expression levels of these genes. $*p < 0.05$, $**p < 0.01$ compared with control group. *NC* Normal status control, *NRT* normal status cells treated with rosiglitazone, *NGT* normal status cells treated with GBE

GBE (as described in "Materials and methods"). The glucose consumption and glucokinase activity were determined at 0, 18, and 36 h. All results were normalized by protein amount. The data indicated that GBE inhibited glucose consumption (36% reduction, 18 h, $p < 0.05$; 9% reduction, 36 h) (Fig. 1a), whereas it had little effect on glucokinase activity (no effect, 18 h; 5% reduction, 36 h) (Fig. 1b) compared with control (NC). The trend was toward a decrease over time. The decline in GBE concentration was a possible explanation for this phenomenon. In contrast to GBE, rosiglitazone stimulated glucose consumption (20% induction, 18 h; 44% induction, 36 h, $p < 0.01$) (Fig. 1a) and glucokinase activity (180% induction, 18 h, $p < 0.01$; 27% induction, 36 h, $p < 0.05$) (Fig. 1b) compared with control (NC). The results suggested that in normal status cells, GBE decreased the risk of obesity by suppressing excessive glucose consumption.

In normal status, GBE increased mRNA levels of PPARγ, IRS-2, and G6Pase

To further understand the mechanism by which GBE regulated glucose consumption, the mRNA levels of PPARγ, G6Pase, GLUT2, SREBP1c, and IRS-2 genes were measured. All cells were treated as described in "Materials and methods." After 36 h, cell RNA was isolated. The results from semiquantitative RT-PCR (Fig. 2a) demonstrated that GBE stimulated expressions of PPARγ (30% induction; $p < 0.05$), IRS-2 (69% induction; $p < 0.05$), and G6Pase (120% induction; $p < 0.01$) observably (Fig. 2b). PPARγ is involved in the insulin signaling pathway and could regulate insulin sensitivity. The increase in its expression would make cells sensitive to insulin. IRS-2 is a crucial molecule in intracellular insulin signal transduction. It is able to elevate intracellular insulin sensitivity by enhancing its expression. The increases in PPARγ and IRS-2 indicated that GBE was able to enhance insulin sensitivity. As a result, cells would increase glucose uptake. G6Pase as a key enzyme catalyzed the last step in the hepatic glucose synthesis reaction. Its increase might lead to augmented glucose output in the liver. In contrast to GBE, rosiglitazone increased all gene expressions (Fig. 2b).

In insulin resistance, GBE improved glucose consumption

All cells were cultured in DMEM. AC cells were treated with 100 nM insulin, ART with 100 nM insulin and rosiglitazone, and AGT with 100 nM insulin and GBE (as described in "Materials and methods"). The glucose consumption and glucokinase activity were determined at 0, 18, and 36 h. All results were normalized by protein amount. The data showed that 100 nM insulin inhibited glucose consumption absolutely (Fig. 3a), whereas GBE stimulated glucose consumption dramatically (Fig. 3a), suggesting that GBE had potential efficacy in preventing insulin resistance. In addition, GBE enhanced glucokinase activity, but the difference was not significant, suggesting glucokinase does not play a key role in GBE treatment. Rosiglitazone had a similar efficacy to GBE. It markedly stimulated glucose consumption (Fig. 3a) and glucokinase activity (Fig. 3b).

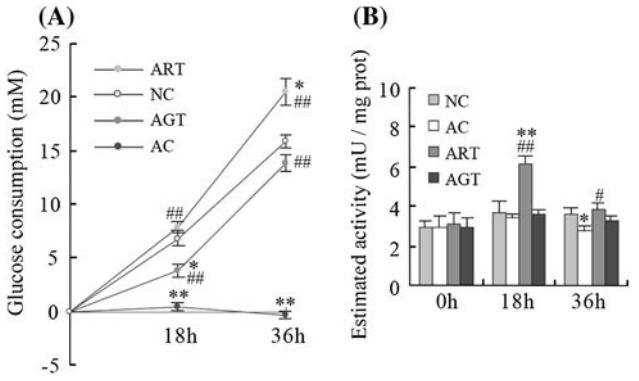

Fig. 3a, b The effects of GBE and rosiglitazone on glucose consumption and glucokinase activity in cells induced with hyperinsulinism. After 0, 18, and 36 h, glucose concentration in medium (**a**) and glucokinase activity (**b**) were determined. Data were normalized by protein amount and are given as mean ± SE of three independent experiments in triplicate. $^{*}p < 0.05$, $^{**}p < 0.01$ compared with the normal control group (NC); $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ compared with the hyperinsulinism-induced control group (AC). *ART* Cells induced with hyperinsulinism and treated with rosiglitazone, *AGT* cells induced with hyperinsulinism and treated with GBE

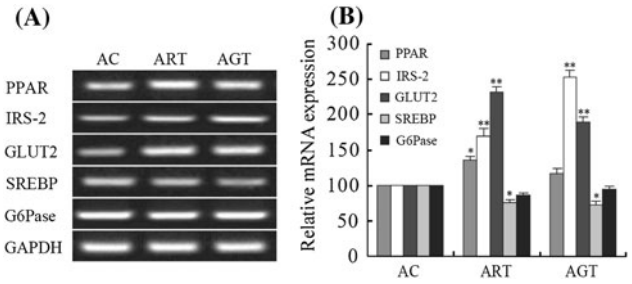

Fig. 4a, b The effects of GBE and rosiglitazone on gene expression in cells induced with hyperinsulinism. After plating, hepatocytes were cultured for 36 h as described in "Materials and methods." After 36 h, total RNA was extracted and analyzed for the expression of PPARγ, IRS-2, GLUT2, SREBP1c, and G6Pase (**a**). The quantification of blots for these gene expressions, obtained in three independent experiments in triplicate is shown (**b**). GAPDH was used as an internal control to normalize the expression levels of these genes. $^{*}p < 0.05$, $^{**}p < 0.01$ compared with the hyperinsulinism-induced control group (AC). *ART* Cells induced with hyperinsulinism and treated with rosiglitazone, *AGT* cells induced with hyperinsulinism and treated with GBE

In insulin resistance, GBE increased expressions of IRS-2 and GLUT2 and decreased SREBP1c expressions

To further understand the mechanism by which GBE improved glucose tolerance, the mRNA levels of PPARγ, G6Pase, GLUT2, SREBP1c, and IRS-2 genes were measured. All cells were treated as described in "Materials and methods." After 36 h, cell RNA was isolated. The results from semi-quantitative RT-PCR (Fig. 4a) demonstrated that GBE enhanced expressions of IRS-2 (1.5-fold induction; $p < 0.01$) and GLUT2 (0.9-fold induction; $p < 0.01$) and inhibited SREBP1c expression (27% reduction; $p < 0.05$) compared with control (AC) (Fig. 4b). As in normal status cells, GBE stimulated IRS-2 expression. It was very important to improve insulin sensitivity. Because GLUT2 is a main glucose transporter in the liver, its increased expression was helpful for cells to absorb glucose. SREBP1c is a crucial transcription factor in lipid metabolism; thus, on the one hand, the decrease in its expression would reduce lipid production, but on the other hand, considering that SREBP directly suppresses IRS-2 expression at the transcriptional level, its decrease might be a reason for the increase in IRS-2 expression. Rosiglitazone had a similar effect to GBE on gene expression. However, PPARγ had a higher expression level and IRS-2 had a lower expression level under rosiglitazone treatment.

Discussion

This study proved that GBE is potentially efficacious in preventing insulin resistance; the possible molecular mechanism driving this process was discussed.

TZDs are common drugs used in type 2 diabetic therapy. They are thought to be PPARγ agonists [22] and to improve downstream insulin sensitivity [23]. However, TZDs increase the weight of patients, too [22, 24]. It is unclear whether gaining weight in this way could do further harm to diabetic patients, but it is well known that obesity is a major risk factor for insulin resistance and type 2 diabetes mellitus. By contrast, GBE suppressed excessive glucose consumption while ameliorating glucose tolerance. Moreover, although GBE decreased glucose consumption in normal status cells, it did not induce insulin resistance. Kudolo showed that long-term ingestion of GBE by healthy individuals caused a significant increase in pancreatic beta-cell insulin response [25]. The effect of GBE on glucose (improved glucose tolerance and inhibited excessive glucose consumption) lead us to believe that GBE has a potential role in diabetes therapy.

The liver, as a key metabolic organ, has two functions in glucose metabolism, namely utilization and production of glucose, and it plays a crucial role in regulating energy balance. Hepatic insulin resistance has been deemed chiefly responsible for type 2 diabetes [26]. In this study, GBE ameliorated glucose tolerance in hyperinsulinism-induced hepatocytes, suggesting that GBE can prevent hepatic insulin resistance. To further explore the signaling pathway, we determined mRNA levels of related genes and glucokinase activity. Our data showed that in normal status cells, GBE enhanced expression of PPARγ, IRS-2, and G6Pase; under conditions of insulin resistance, GBE stimulated expressions of IRS-2 and GLUT2 and repressed SREBP1c expression.

Glucokinase is the main hexokinase in the liver and plays a key role in regulating glucose phosphatization. In

our study, its activity did not change after GBE treatment. The data suggested that GBE did not exert its effects on glucokinase. It should be noted that IRS-2 expression was improved after GBE treatment both in normal status and insulin resistance. Because IRS-2 is a crucial element in insulin signaling and researchers have found that a deficiency of IRS-2 causes insulin resistance [27], its expression is understood to be closely related with insulin sensitivity. Thus we suggest that GBE exerts its effects mainly via IRS-2 expression. Moreover, our data indicate that the increase in IRS-2 is followed by a decrease in SRBEP1c, demonstrating a link between them. The results of Ide et al. [28] support our supposition. They found SREBP1c directly suppressed IRS-2 transcription in hepatocytes. So we proposed that GBE improved insulin sensitivity mainly by enhancing IRS-2 transcription. A recent study reported that nobiletin, a polymethoxylated flavone, improves insulin sensitivity in diabetic mice [29]. The similarity in the main components of nobiletin and GBE suggests that plants with rich flavones have a potential role in diabetic therapy.

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References

1. Della Loggia R, Sosa S, Tubaro A, Morazzoni P, Bombardelli E, Griffini A (1996) Anti-inflammatory activity of some *Ginkgo biloba* constituents and of their phospholipid-complexes. Fitoterapia 67:257–264
2. Omar ME, Baiuomy AR, El-Batran S, Arbid MS (2004) Evaluation of the anti-inflammatory, anti-nociceptive and gastric effects of *Ginkgo biloba* in the rat. Pharmacol Res 49:133–142
3. Bombardelli E, Cristoni A, Curri SB (1996) Activity of phospholipid-complex of *Ginkgo biloba* dimeric flavonoids on the skin microcirculation. Fitoterapia 67:265–273
4. Saponara R, Bosisio E (1998) Inhibition of cAMP-phosphodiesterase by biflavones of *Ginkgo biloba* in rat adipose tissue. J Nat Prod 61:1386–1387
5. Dell'Agli M, Bosisio E (2002) Biflavones of *Ginkgo biloba* stimulate lipolysis in 3T3-L1 adipocytes. Planta Med 68:76–79
6. Boveris AD, Galatro A, Puntarulo S (2000) Effect of nitric oxide and plant antioxidants on microsomal content of lipid radicals. Biol Res 33:159–165
7. Nian H, Song B, Wang W (2004) Antioxidative stress effect of *Ginkgo biloba* extract to experimental diabetes rats. J Mudanjiang Med Coll 25:3–6
8. Wei Z, Peng Q, Lau BH, Shah V (1999) *Ginkgo biloba* inhibits hydrogen peroxide-induced activation of nuclear factor kappa B in vascular endothelial cells. Gen Pharmacol 33:369–375
9. Maitra I, Marcocci L, Droy-Lefaix MT, Packer L (1995) Peroxyl radical scavenging activity of *Ginkgo biloba* extract EGb 761. Biochem Pharmacol 49:1649–1655
10. Bernardczyk-Meller J, Siwiec-Proscinska J, Stankiewicz W, Fichna P, Pecold K, Korman E (2004) Influence of Eqb 761 on the function of the retina in children and adolescent with long lasting diabetes mellitus—preliminary report. Klin Oczna 106:569–571
11. Bell GI, Kayano T, Buse JB, Burant CF, Takeda J, Lin D, Fukumoto H, Seino S (1990) Molecular biology of mammalian glucose transporters. Diabetes Care 13:198–208
12. Memon RA, Tecott LH, Nonogaki K, Beigneux A, Moser AH, Grunfeld C, Feingold KR (2000) Up-regulation of peroxisome proliferator-activated receptors (PPAR-alpha) and PPAR-gamma messenger ribonucleic acid expression in the liver in murine obesity: troglitazone induces expression of PPAR-gamma-responsive adipose tissue-specific genes in the liver of obese diabetic mice. Endocrinology 141:4021–4031
13. Horton JD, Goldstein JL, Brown MS (2002) SREBPs: activators of the complete program of cholesterol and fatty acid synthesis in the liver. J Clin Invest 109:1125–1131
14. Patti ME, Sun XJ, Bruening JC, Araki E, Lipes MA, White MF, Kahn CR (1995) 4PS/insulin receptor substrate (IRS)-2 is the alternative substrate of the insulin receptor in IRS-1-deficient mice. J Biol Chem 270:24670–24673
15. Rother KI, Imai Y, Caruso M, Beguinot F, Formisano P, Accili D (1998) Evidence that IRS-2 phosphorylation is required for insulin action in hepatocytes. J Biol Chem 273:17491–17497
16. Yeh H, Chu T, Shen T (1980) Ultrastructure of continuously cultured adult human liver cell. Acta Biol Exp Sin 13:361–364
17. Li C, Ning G, Chen J (1999) Establishing and identifying insulin-resistant HepG2 cell line. Chin J Diabetes 7:198–200
18. Garvey WT, Olefsky JM, Marshall S (1985) Insulin receptor down-regulation is linked to an insulin-induced postreceptor defect in the glucose transport system in rat adipocytes. J Clin Invest 76:22–30
19. Pryor PR, Liu SC, Clark AE, Yang J, Holman GD, Tosh D (2000) Chronic insulin effects on insulin signalling and GLUT4 endocytosis are reversed by metformin. Biochem J 348 Pt 1:83–91
20. Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248–254
21. Fernandez-Mejia C, Vega-Allende J, Rojas-Ochoa A, Rodriguez-Dorantes M, Romero-Navarro G, Matschinsky FM, Wang J, German MS (2001) Cyclic adenosine 3′,5′-monophosphate increases pancreatic glucokinase activity and gene expression. Endocrinology 142:1448–1452
22. Yki-Jarvinen H (2004) Thiazolidinediones. N Engl J Med 351: 1106–1118
23. Miyazaki Y, He H, Mandarino LJ, DeFronzo RA (2003) Rosiglitazone improves downstream insulin receptor signaling in type 2 diabetic patients. Diabetes 52:1943–1950
24. Mori Y, Murakawa Y, Okada K, Horikoshi H, Yokoyama J, Tajima N, Ikeda Y (1999) Effect of troglitazone on body fat distribution in type 2 diabetic patients. Diabetes Care 22:908–912
25. Kudolo GB (2000) The effect of 3-month ingestion of *Ginkgo biloba* extract on pancreatic beta-cell function in response to glucose loading in normal glucose tolerant individuals. J Clin Pharmacol 40:647–654
26. Taniguchi CM, Ueki K, Kahn R (2005) Complementary roles of IRS-1 and IRS-2 in the hepatic regulation of metabolism. J Clin Invest 115:718–727
27. Kubota N, Tobe K, Terauchi Y, Eto K, Yamauchi T, Suzuki R, Tsubamoto Y, Komeda K, Nakano R, Miki H, Satoh S, Sekihara H, Sciacchitano S, Lesniak M, Aizawa S, Nagai R, Kimura S, Akanuma Y, Taylor SI, Kadowaki T (2000) Disruption of insulin receptor substrate 2 causes type 2 diabetes because of liver insulin resistance and lack of compensatory beta-cell hyperplasia. Diabetes 49:1880–1889

28. Ide T, Shimano H, Yahagi N, Matsuzaka T, Nakakuki M, Yamamoto T, Nakagawa Y, Takahashi A, Suzuki H, Sone H, Toyoshima H, Fukamizu A, Yamada N (2004) SREBPs suppress IRS-2-mediated insulin signalling in the liver. Nat Cell Biol 6:351–357

29. Lee YS, Cha BY, Saito K, Yamakawa H, Choi SS, Yamaguchi K, Yonezawa T, Teruya T, Nagai K, Woo JT (2010) Nobiletin improves hyperglycemia and insulin resistance in obese diabetic ob/ob mice. Biochem Pharmacol 79:1674–1683