

Increased glucagon-like peptide-1 secretion may be involved in antidiabetic effects of ginsenosides

Can Liu, Mian Zhang, Meng-yue Hu, Hai-fang Guo, Jia Li, Yun-li Yu, Shi Jin, Xin-ting Wang, Li Liu and Xiao-dong Liu

Key Laboratory of Drug Metabolism and Pharmacokinetics, China Pharmaceutical University, Nanjing 210009, China

Correspondence should be addressed to L Liu; X-d Liu

Emails

liulee@yeah.net;
xdliu@cpu.edu.cn

Abstract

Panax ginseng is one of the most popular herbal remedies. Ginsenosides, major bioactive constituents in *P. ginseng*, have shown good antidiabetic action, but the precise mechanism was not fully understood. Glucagon-like peptide-1 (GLP1) is considered to be an important incretin that can regulate glucose homeostasis in the gastrointestinal tract after meals. The aim of this study was to investigate whether ginseng total saponins (GTS) exerts its antidiabetic effects via modulating GLP1 release. Ginsenoside Rb1 (Rb1), the most abundant constituent in GTS, was selected to further explore the underlying mechanisms in cultured NCI-H716 cells. Diabetic rats were developed by a combination of high-fat diet and low-dose streptozotocin injection. The diabetic rats orally received GTS (150 or 300 mg/kg) daily for 4 weeks. It was found that GTS treatment significantly ameliorated hyperglycemia and dyslipidemia, accompanied by a significant increase in glucose-induced GLP1 secretion and upregulation of proglucagon gene expression. Data from NCI-H716 cells showed that both GTS and Rb1 promoted GLP1 secretion. It was observed that Rb1 increased the ratio of intracellular ATP to ADP concentration and intracellular Ca^{2+} concentration. The metabolic inhibitor azide (3 mM), the K_{ATP} channel opener diazoxide (340 μ M), and the Ca^{2+} channel blocker nifedipine (20 μ M) significantly reversed Rb1-mediated GLP1 secretion. All these results drew a conclusion that ginsenosides stimulated GLP1 secretion both *in vivo* and *in vitro*. The antidiabetic effects of ginsenosides may be a result of enhanced GLP1 secretion.

Key Words

- ▶ glucagon-like peptide-1 (GLP1)
- ▶ ginsenosides
- ▶ Rb1
- ▶ type 2 diabetes
- ▶ ginseng total saponins (GTS)

Journal of Endocrinology
(2013) 217, 185–196

Introduction

Historical records on traditional medicinal systems reveal that *Panax ginseng* (*P. ginseng*, C A Meyer) has been used to treat diabetes for a long time (Xie *et al.* 2005c). Ginsenosides, major bioactive constituents in *P. ginseng*, are thought to be the main components responsible for the antidiabetic actions (Attele *et al.* 1999, 2002). Ginsenoside Rb1 (Rb1) was also reported to be the most abundant constituent among more than 30 ginsenosides in ginseng

root despite different sources and species (Washida & Kitanaka 2003, Lim *et al.* 2005). A series of clinical trials and animal experiments have demonstrated that both *P. ginseng* and ginsenosides exerted insulinotropic effects and improved long-term glycemic control in type 2 diabetes (Kimura *et al.* 1981a,b, Yokozawa *et al.* 1985, Sotaniemi *et al.* 1995, Vuksan *et al.* 2000, Kim & Park 2003, Yun *et al.* 2004, Ma *et al.* 2008, Vuksan *et al.* 2008,

Xiong *et al.* 2010). Several studies have been carried out to investigate the mechanisms of this glucose-lowering effect. Experiments using rats showed that Rb1 treatment reduces intracellular triglyceride accumulation in liver (Park *et al.* 2002). An *in vitro* study also demonstrated that Rb1 may suppress triglyceride accumulation in 3T3-L1 adipocytes and enhance insulin release in Min-6 cells via PKA pathways (Park *et al.* 2008b). It was also reported that Rb1 significantly stimulates basal and insulin-mediated glucose uptake in 3T3-L1 adipocytes and C2C12 myotubes via promoting GLUT1 and GLUT4 translocations to the cell surface (Shang *et al.* 2008). However, the real mechanisms of the antihyperglycemic effect have not been thoroughly elucidated due to poor absorption and low concentration of ginsenosides in plasma.

It is generally accepted that poor membrane permeability across the intestinal wall is the main factor limiting systemic exposure of most ginsenosides (Liu *et al.* 2009). Studies showed that Rb1 and Rb2 are poorly absorbed (Odani *et al.* 1983a,b), with bioavailabilities <1%. The peak concentrations of Rb1 and Rb2 following an oral dose (50 mg/kg) were only 5.51 and 0.35 $\mu\text{mol/l}$ respectively. The findings show that poor absorption and low plasma concentration of ginsenosides after oral administration could not fully characterize its glucose-lowering potency. In fact, this pharmacokinetic–pharmacological paradox is not special to ginsenosides but is common in many natural compounds.

However, it is noteworthy that local high concentration of ginsenosides in intestine may interact with intestinal epithelium, where numerous endocrine cells are located. Glucagon-like peptide-1 (GLP1), secreted by enteroendocrine L-cells, is one of the most important incretins in the regulation of glucose homeostasis and insulin secretion (Baggio & Drucker 2007, Ranganath 2008). Our recent studies revealed that, berberine, another poorly absorbed natural product, may reduce blood glucose levels partly via stimulating GLP1 release (Lu *et al.* 2009, Yu *et al.* 2010). This indicated that, similar to berberine, ginsenosides may exert antidiabetic activity via stimulating GLP1 secretion.

The aim of this study was to investigate whether ginseng total saponins (GTS) treatment could enhance GLP1 secretion and ameliorate glucose and lipid homeostasis in type 2 diabetic rats induced by a combination of high-fat diet and low-dose streptozotocin (STZ). The effects and mechanisms of GTS and Rb1 on GLP1 secretion were also investigated in cultured NCI-H716 cells, a widely used human enteroendocrine L-cell line.

Materials and methods

Materials

GTS (ginsenosides content >80%) from one batch and purified ginsenosides Rb1, Rb2, Rc, Rd, Re, Rg1, Rf, and Rg2 (purity >99%) were purchased from Jilin University (Changchun, China). Pentobarbital, digoxin, STZ, ADP, and ATP were obtained from Sigma–Aldrich. Primary anti-insulin antibody was purchased from Abcam Co. (Cambridge, MA, USA). Rabbit anti-GLP1 antibody was bought from Phoenix Pharmaceuticals (Burlingame, CA, USA).

Analysis of ginsenosides by LC–MS

To investigate the chemical composition of GTS, a validated LC–MS method was employed to analyze the contents of eight major ginsenosides namely Rb1, Rb2, Rc, Rd, Re, Rf, Rg1, and Rg2 (Xie *et al.* 2005a). Briefly, stock solutions were prepared at 2 mg/ml in DMSO and diluted with 95% methanol before experiments. Chromatographic separation was performed using a Waters Symmetry C₁₈ column (5.0 μm , 2.1 \times 150 mm). The mobile phase was composed of a mixture of NH₄Cl (0.15 mM in water i) and acetonitrile ii) at a flow rate of 0.2 ml/min. The gradient conditions were as follows: 0–3 min at 25% B, 3–10 min at 25–60% B, 10–18 min at 60–90% B, 18–20 min at 90–25% B, and 20–25 min at 25% B. Analysis in the mass spectrometer with ESI probe was operated in the selected ion monitoring model: m/z [M+Cl]²⁻ 589.25 for Rb1, [M+Cl]⁻ 1113.5 for Rb2 and Rc, [M+Cl]⁻ 981.45 for Rd and Re, [M+Cl]⁻ 835.4 for Rg1 and Rf, [M+Cl]⁻ 819.4 for Rg2, and [M+Cl]⁻ 815.35 for digoxin (internal standard).

The contents (w/w) in GTS of the ginsenosides Rb1, Rb2, Rc, Rd, Re, Rf, Rg1, and Rg2 were measured to be 21, 18, 16, 9, 11, 1, 5, and 3% respectively. The result showed that content of eight ginsenosides was 84% and ginsenoside Rb1 was the most abundant constituent in GTS.

Animals

Male Sprague Dawley rats (weighing 100–120 g), purchased from Sino-British Sipper & BK Lab Animal Ltd. (Shanghai, China), were housed in a controlled environment of temperature (23 \pm 1 °C) and relative humidity (50 \pm 5%) with 12 h light:12 h darkness cycle. Water and food were allowed *ad libitum*. All animal experimental procedures were approved by the Animal Ethics Committee of China Pharmaceutical University.

Induction of experimental diabetes in rats and GTS treatment

Experimental type 2 diabetic rats were induced by combination of high-fat diet and low-dose STZ injection according to a method described previously (Reed *et al.* 2000, Chen *et al.* 2011). Following an acclimation period of 3 days, the rats were assigned randomly to three groups: control (CON) group, high-fat diet-fed (HFD) group, and diabetic (DM) group. The CON rats were fed on normal chow while both HFD rats and DM rats were fed on high-fat diet, which contains 15% w/w lard, 5% w/w sesame oil, 20% w/w sucrose, 2.5% w/w cholesterol, and 57.5% w/w normal chow. After 4 weeks of dietary manipulation, the rats were fasted overnight. DM rats received an i.p. injection of STZ (35 mg/kg, dissolved in citrate buffer at pH 4.5). Both HFD and CON rats received an equivalent volume of citrate buffer. On day 7 post-STZ injection, only rats with fasting blood glucose (FBG) levels higher than 11.1 mM were selected as diabetic rats for further studies.

Diabetic rats were divided into three groups randomly: diabetic control (DM) group, low-dose treatment group (DM-GL), and high-dose treatment group (DM-GH), which received oral daily dose of vehicle, 150 mg/kg GTS, or 300 mg/kg GTS for 4 weeks respectively.

Oral glucose tolerance test

On day 21 of the treatment, an oral glucose tolerance test (OGTT) was performed. Rats were fasted for 6 h followed by an oral dose of glucose (2 g/kg). Plasma glucose and insulin levels were determined at 0 (baseline), 15, 30, 60, and 120 min after glucose administration.

Sample collection and GLP1 assessment

On day 28 of the treatment, rats were fasted for 6 h. At 2 h after last treatment, portal vein catheter implantation was performed under pentobarbital anesthesia (60 mg/kg, i.p.). Serial blood samples (about 300 μ l) were collected in Eppendorf tubes containing EDTA and dipeptidyl peptidase-IV (DPP-IV) inhibitor (10 μ l/ml, Linco Research, St Charles, MO, USA) via the catheter before and at 10, 20, and 30 min following glucose loading (2.5 g/kg via gavage). Blood samples were centrifuged at 1500g for 10 min and stored at -80°C for assessing insulin and GLP1 concentration.

Then the rats were killed immediately. The pancreas, segments of distal ileum, and proximal colon were rapidly harvested and stored at -80°C until processed. Parts of

ileum and colon were used for immunohistochemistry and quantitative RT-PCR analysis, and the rest were used for GLP1 assessment.

GLP1 levels were measured using a GLP1(7–36) amide active ELISA kit (Linco Research). Plasma insulin was measured by iodine [^{125}I] insulin RIA kit (BNIBT Co., Beijing, China). Homeostatic model assessment (HOMA) was used to assess the longitudinal changes in insulin resistance (HOMA-IR).

Measurement of Rb1 concentration in intestine

Normal male Sprague Dawley rats (280–300 g) received an oral dose of GTS (300 mg/kg). Rats were killed and intestinal contents and mucosa in segments of distal ileum and proximal colon were harvested at 2 and 4 h following GTS administration. Contents and mucosa were homogenized in PBS and ginsenosides were extracted by *n*-butanol saturated with water. Ginsenoside Rb1 concentration in plasma, intestinal contents, and mucosa were measured by LC–MS.

Immunohistochemistry

β -Cell mass in pancreas as well as L-cell number in ileum were measured using immunohistochemistry carried out as described previously (Lu *et al.* 2009, Yu *et al.* 2009, 2010). For ileum tissue, results are expressed as the number of L-cells per mucosal area (number/ mm^2). For pancreas, β -cell volume density was estimated by calculating the proportion of cells immunoreactive for insulin per sectional area of total pancreas. The β -cell mass was calculated by multiplying the β -cell volume density by the weight of the pancreas.

Cell culture and GLP1 secretion studies

Human NCI-H716 cells were obtained from the American Type Culture Collection (Manassas, VA, USA). Cells were maintained in suspension in RPMI-1640 at 37°C under 5% CO_2 . Two days before the experiments, cell adhesion and endocrine differentiation were initiated by growing cells (1.5×10^6 /well) in dishes coated with Matrigel (Becton Dickinson Co., Bedford, MA, USA) in high-glucose DMEM. On the day of the experiment, supernatants were replaced by Krebs–Ringer bicarbonate (KRB) buffer (128.8 mM NaCl, 4.8 mM KCl, 1.2 mM KH_2PO_4 , 1.2 mM MgSO_4 , 2.5 mM CaCl_2 , 5 mM NaHCO_3 , and 10 mM HEPES, pH 7.4) containing 0.2% w/v BSA with or without test agents. After incubation at 37°C for 2 h, the supernatant was collected with the addition of 50 $\mu\text{g/ml}$

phenylmethylsulphonyl fluoride for GLP1 analysis. No damage on cells was found for all the agents within tested concentrations.

Measurement of intracellular ATP and ADP

The intracellular ATP and ADP levels were determined by the HPLC method described previously with minor modifications (Volonté *et al.* 2004, Varum *et al.* 2011). In brief, differentiated cells (1.5×10^6 /well) were incubated with KRB buffer containing 0.2% BSA with or without Rb1 for 0.5 or 1 h. Then cells were scraped off and sonicated. ATP and ADP were extracted with 5% w/v 5-sulfosalicylic acid followed by centrifugation. Supernatants were neutralized with 2 M NaOH. All steps were carried out on ice. Separation was achieved using a Waters Symmetry C₁₈ column (5.0 μ m, 4.6 \times 150 mm). The mobile phase (adjusted pH to 6.5) was 8% v/v methanol containing 70 mM NaH₂PO₄, and 3 mM tetrabutyl ammonium bromide at a flow rate of 1 ml/min. The u.v.–VIS 20A detector (Shimadzu, Kyoto, Japan) was set a wavelength at 220 nm. Results are given as the ratio of 2 (ATP:ADP ratio).

Measurement of intracellular Ca²⁺ level

NCI-H716 cells were grown and differentiated on Matrigel-coated glass-bottomed dishes. Before starting the experiment, cells were washed with KRB buffer three times and loaded with the Ca²⁺ indicator Fluo-3 AM (5 μ mol/l, Beyotime Biotechnology, Jiangsu, China) in the dark for 45 min at 37 °C. Then cells were washed three times with KRB buffer. At the beginning of the experiment, the mean baseline fluorescence was acquired, followed by the stimulation of tested agents. Ca²⁺ transient was recorded by a laser scanning confocal microscope (TCS SP5, Leica, Germany) with Leica application suite (Advanced Fluorescence 2.2.0). Fluorescence was monitored at the emission wavelength of 525 nm, with the excitation wavelength at 488 nm (Reimer *et al.* 2001, Li *et al.* 2012a,b, Lupescu *et al.* 2012).

Quantitative RT-PCR

Quantitative RT-PCR was used to measure *Gcg* mRNA (for proglucagon gene) and *Pcsk1* mRNA (for prohormone convertase 3 (*Pc3*) gene) in rat ileum (on day 28) or human NCI-H716 cells (incubated with Rb1 for 24 h). Briefly, total RNA from frozen tissue or cells was isolated using TRIzol reagent (SunShine Biotechnology, Nanjing, China) according to the manufacturer's instruction. The quality and purity of RNA were determined by u.v. spectrophotometry

at 260 and 280 nm. Quantitative RT-PCR was performed with an input of cDNA converted from 2 μ g of total RNA. Primer sequences of rat mRNA: forward 5'-ACACGGAGGA GAACGCCAGA-3', reverse 5'-GCGGGAGTCCAGGTA TTTGC-3' for proglucagon gene; forward 5'-CTTCT TTTCTCTCAGCCCTTCCTAC-3', reverse 5'-CATTTCATGA CAAACTGCCTCTTC-3' for *Pc3* gene; and forward 5'-GGGAAATCGTGC GTGACATT-3', reverse 5'-GCGGCA GTGGCCATCTC-3' for β -actin gene. Primer sequences of human mRNA: forward 5'-TGTCAGCGTAATATCTGTGA GGC-3', reverse 5'-AGCAGGTGAAGAGAGAGCAAGC-3' for proglucagon gene; forward 5'-CAGAAGGCTTTTGAATA TGGTGT-3', reverse 5'-GGAGGCACTGCTGATGGAGAT-3' for *Pc3* gene; and forward 5'-CAGTCGGTTGGAGCGAGCA T-3', reverse 5'-GGACTTCCTGTAAACAACGCATCT-3' for β -actin gene.

After denaturing at 95 °C for 2 min, the amplification was obtained by 40 cycles of 95 °C for 5 s and 60 °C for 30 s. Melting curves were performed to investigate the specificity of the PCR reaction. Relative quantification of each gene expression was calculated according to a comparative Ct method using the formula: $RQ = 2^{-\Delta Ct}$.

Statistical analysis

Results were expressed as mean \pm s.e.m. Statistical differences among groups were evaluated by one-way ANOVA. Differences between groups were estimated using Student–Newman–Keuls multiple comparison *post hoc* test when necessary. A *P* value of <0.05 indicated a significant difference.

Result

Features of DM rats and HFD rats

Physiological and biochemical parameters were measured in CON rats, HFD rats, DM rats, and DM rats treated with GTS (Table 1 and Fig. 1). In response to STZ injection, levels of glucose, triglyceride (TG), total cholesterol (TC), and free fatty acids (FFA) in plasma of DM rats were significantly higher than those in CON rats, accompanied by reductions in body weight gain. DM rats also developed diabetic symptoms such as polyphagia, polyuria, and polydipsia. High levels of insulin as well as HOMA-IR were observed in DM rats (Table 1). As shown in Fig. 1C, the OGTT results also showed that DM rats had significantly higher postprandial glucose concentrations induced by glucose loading (2 g/kg, post ovulation (p.o.)), leading to significant increase in area under concentration–

Table 1 Alterations in body weight, parameters of glucose, and lipid metabolism in diabetic rats after 4 weeks of GTS treatment. DM-GL rats and DM-GH rats were treated with GTS 150 and 300 mg/kg per day for 4 weeks respectively. Data shown are mean \pm S.E.M. ($n=5$)

Parameter	CON	HFD	DM	DM-GL	DM-GH
Body weight (g)	420.2 \pm 10.7	428.3 \pm 13.1	301.5 \pm 7.9 [†]	304.1 \pm 14.6 [†]	299.4 \pm 13.1 [†]
Liver weight (% of body weight)	3.38 \pm 0.07	4.6 \pm 0.06 [†]	4.8 \pm 0.13 [†]	4.75 \pm 0.16 [†]	4.71 \pm 0.15 [†]
TG (mmol/l)	1.73 \pm 0.19	2.21 \pm 0.28	10.81 \pm 2.21 [†]	8.28 \pm 1.92 [*]	4.91 \pm 0.52 ^{†,‡}
TC (mmol/l)	1.74 \pm 0.05	2.42 \pm 0.08 [†]	27.98 \pm 3.42 [†]	21.56 \pm 3.94 [†]	21.88 \pm 2.42 [†]
FFA (g/l)	0.49 \pm 0.09	1.27 \pm 0.41	1.45 \pm 0.25 [*]	1.65 \pm 0.52	0.83 \pm 0.21
FBG (mmol/l)	7.63 \pm 1.16	10.11 \pm 0.26	23.19 \pm 1.24 [†]	19.12 \pm 2.09 [†]	19.31 \pm 0.81 ^{†,‡}
Fasted insulin level (mIU/l)	24.78 \pm 3.34	37.44 \pm 1.75 [*]	30.46 \pm 5.30	26.58 \pm 2.52	28.99 \pm 2.55
HOMA-IR	7.89 \pm 0.30	16.89 \pm 1.07 [†]	30.5 \pm 4.29 [†]	22.74 \pm 3.75 [†]	24.82 \pm 2.34 [†]

* $P < 0.05$, [†] $P < 0.01$ vs CON rats and [‡] $P < 0.05$, vs DM rats.

time curve (AUC) values of glucose. These indexes were similar to the physiopathological state of type 2 diabetes, indicating that the DM rats may be considered as type 2 diabetic rats (Reed *et al.* 2000, Srinivasan *et al.* 2005). HFD rats showed both higher plasma insulin concentrations and HOMA-IR index, inferring the development of IR (Table 1).

Plasma glucose, insulin, and lipid levels

Four-week GTS treatment significantly decreased energy intake (Fig. 1A) and normalized TG, TC, and FFA levels

but failed to influence body weight and liver weight (Table 1). Further study showed that GTS treatment dramatically decreased FBG levels especially in the last 2 weeks (Fig. 1B and Table 1). Although fasting insulin level remained unchanged, the rats treated with GTS showed lower HOMA-IR index compared with DM rats (Table 1). Data from the OGTT conducted on day 21 showed that GTS treatment significantly reduced postprandial blood glucose levels induced by glucose loading (2 g/kg, p.o.; Fig. 1C), resulting in lower AUC of glucose

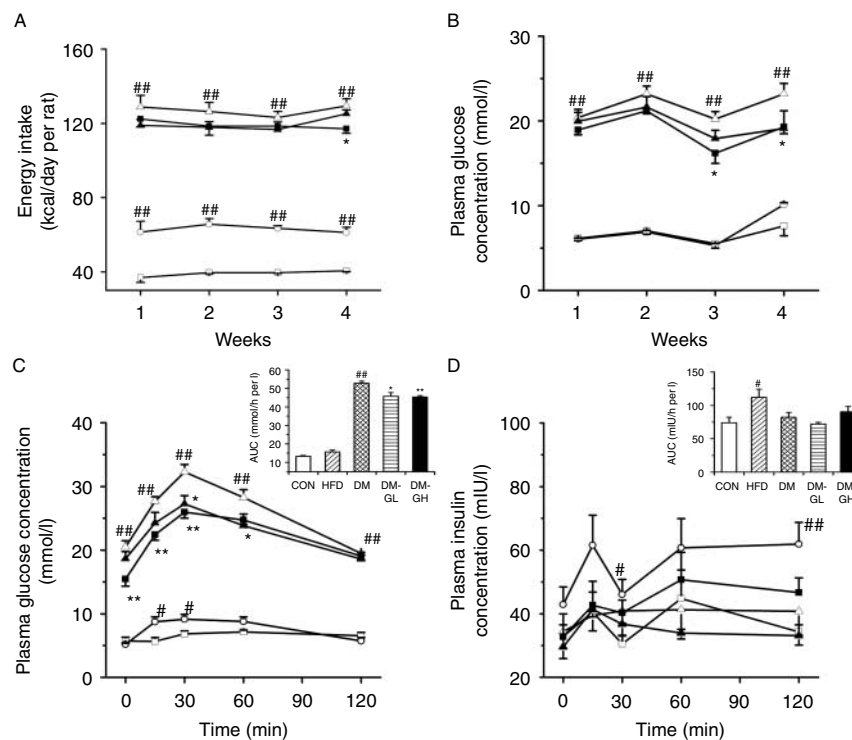


Figure 1

Effects of GTS on energy intake (A) and fasted plasma glucose (B) in DM rats during 4 weeks of treatment; postprandial plasma glucose (C) and insulin levels (D) on OGTT on day 21. DM-GL rats and DM-GH rats were treated with GTS 150 and 300 mg/kg per day for 4 weeks respectively. Symbols represent

as follows: open square, CON rats; open circle, HFD rats; open triangle, DM rats; filled triangle, DM-GL rats; and filled square, DM-GH rats. Values are expressed as mean \pm S.E.M. ($n=5$), * $P < 0.05$, ** $P < 0.01$ vs CON rats and * $P < 0.05$, ** $P < 0.01$ vs DM rats.

(Fig. 1C inset) compared with those in DM rats. Moreover, higher doses of GTS treatment stimulated an increment in insulin levels (Fig. 1D) induced by glucose loading. However, the increment did not reach statistical significance. All these results further verified the antidiabetic effect of GTS.

On day 28, glucose levels and insulin levels in plasma of portal vein were also monitored together with GLP1 following glucose loading (2.5 g/kg, p.o.). It was apparent that compared with DM rats, GTS treatment remarkably decreased plasma glucose concentrations (Fig. 2A), which was in line with the results of the OGTT (Fig. 1C). GTS treatment also displayed a trend to increase insulin level induced by glucose loading, with a significant increase in DM-GH rats (Fig. 2B).

Measurement of pancreas insulin levels was also performed at 30 min after glucose loading on day 28 (Fig. 2C). High doses of GTS treatment increased pancreas insulin levels when compared with control rats, although no significance was found. The β -cell volume density corresponds to the ratio of insulin immunoreactivity area to pancreatic parenchymal area. The β -cell mass was

calculated by multiplying the β -cell volume density by the weight of the pancreas (Yu *et al.* 2010). The data from immunohistochemistry showed that high-dose GTS treatment significantly increased β -cell mass by 2.52-fold compared with DM rats (Fig. 2D).

GLP1 secretion in rats

Assessment of GLP1 levels in portal plasma was conducted after glucose loading (2.5 g/kg, p.o.) on day 28 (Fig. 3A) and AUC from 0 to 30 min (AUC₀₋₃₀) was estimated using the linear trapezoidal rule. The results confirmed that DM rats exhibited greater basal GLP1 levels than CON rats, which was consistent with those previously reported (Cani *et al.* 2005). Obviously, glucose loading induced GLP1 release in all experimental rats. Compared with DM rats, high-dose GTS treatment did not produce appreciable effects on basal GLP1 levels but enhanced glucose-induced GLP1 release, which continued rising to the peak at 20 min (96.4 ± 7.85 pM in DM-GH rats vs 57.1 ± 11.5 pM in DM rats, $P < 0.05$) and stayed high to the end of the experiment. A similar increase was obtained in DM-GL group, although

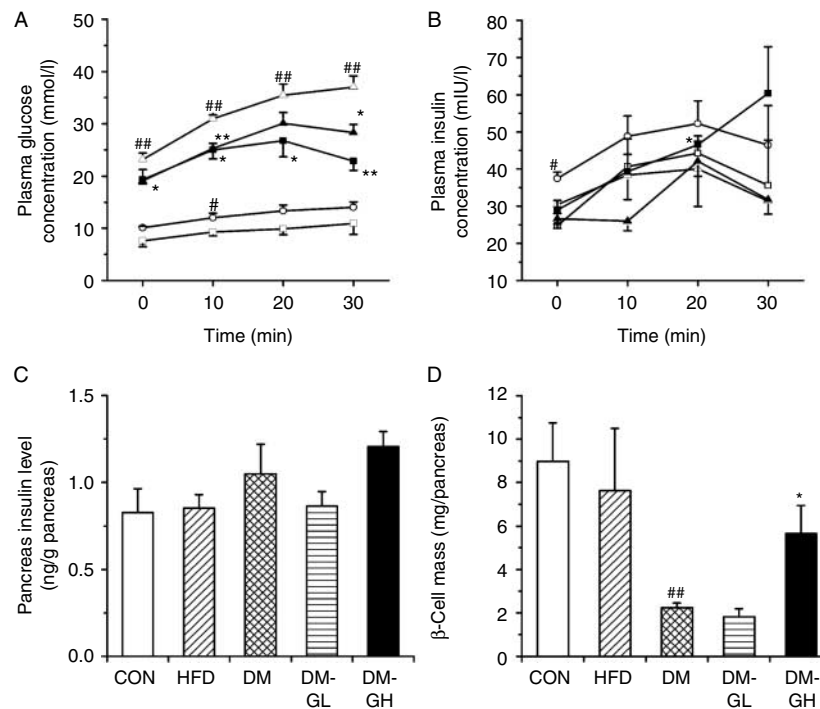
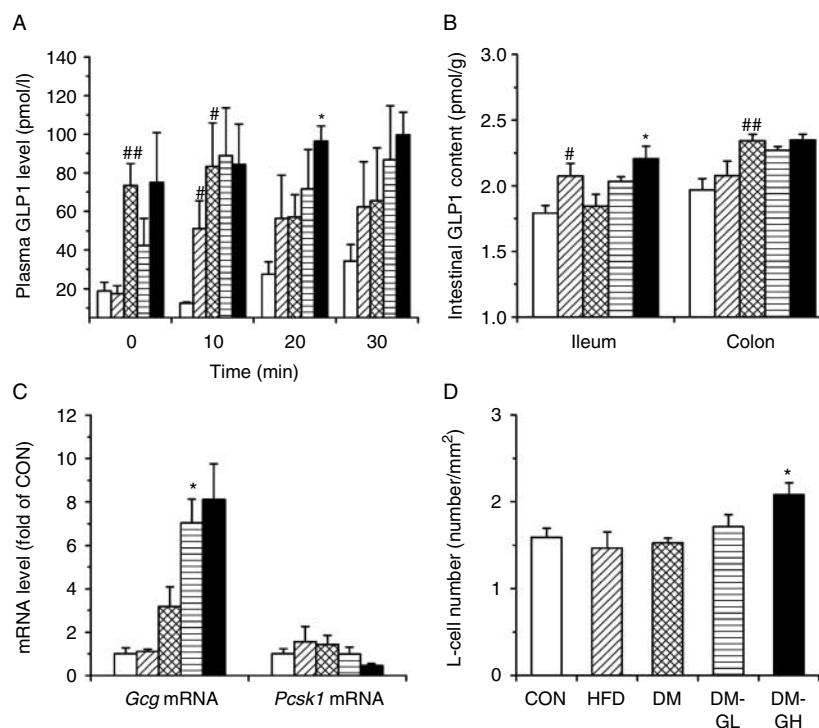


Figure 2

Effects of 4-week GTS treatment on glucose (A) and insulin levels (B) in portal vein, pancreas insulin level (C), and β -cell mass (D) in DM rats. DM-GL rats and DM-GH rats were treated with GTS 150 and 300 mg/kg per day for 4 weeks respectively. Plasma samples were collected before and at 10, 20, and 30 min following glucose loading (2.5 g/kg, p.o.) on day 28. Pancreas samples were obtained at 30 min after glucose loading. β -Cell proliferation

was presented by β -cell mass, detected by immunohistochemistry. Symbols represent as follows: open square, CON rats; open circle, HFD rats; open triangle, DM rats; filled triangle, DM-GL rats; and filled square, DM-GH rats. Values are expressed as means \pm s.e.m. ($n = 5$), $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ vs CON rats and $^*P < 0.05$, $^{**}P < 0.01$ vs DM rats.

**Figure 3**

Effects of 4-week GTS treatment on GLP1 secretion. (A) Portal vein GLP1 level, (B) GLP1 content in intestinal tract, (C) *Gcg* mRNA (for proglucagon gene) and *Pcsk1* mRNA (for *Pc3* gene) expression in ileum, and (D) intestinal L-cell number. DM-GL rats and DM-GH rats were treated with GTS 150 and 300 mg/kg per day for 4 weeks respectively. Plasma samples were collected before and at 10, 20, and 30 min following glucose loading (2.5 g/kg, p.o.)

no significance was found. Meanwhile, GTS treatment also increased the AUC₀₋₃₀ of GLP1 (44.69 ± 6.50 pmol/h per l in DM-GH rats vs 34.97 ± 6.94 pmol/h per l in DM rats). These results indicated that GTS treatment enhanced GLP1 secretion induced by glucose loading.

GLP1 levels in ileum and colon was measured at 30 min after glucose loading (Fig. 3B). High-dose GTS treatment significantly increased GLP1 concentrations in the ileum. However, the enhancement did not occur in the colon.

The proglucagon gene is the precursor gene of GLP1. GLP1(7-36) amide is produced by a tissue-specific post-translational process of proglucagon peptide by PC3 (Rouillé *et al.* 1995). Levels of *Gcg* mRNA (for proglucagon gene) and *Pcsk1* mRNA (for *Pc3* gene) in ileum segments were assessed. The results showed that diabetes upregulated expression of *Gcg* mRNA. GTS treatment further increased *Gcg* mRNA expression (Fig. 3C). By contrast, GTS treatment did not elicit a marked alteration in *Pcsk1* mRNA expression.

The L-cells were identified in the microvilli of the intestinal lumen by immunohistochemistry. Compared with DM rats, high-dose GTS treatment significantly

increased the number of GLP1-positive L-cells in the ileum (Fig. 3D). Besides, no obvious alteration was observed in DM rats compared with CON rats.

Rb1 level in intestine after oral GTS administration

Rb1 level in plasma, intestinal contents, and mucosa were investigated at 2 and 4 h following GTS administration (300 mg/kg, p.o.). The results showed that Rb1 in plasma was only 204.9 ± 47.5 ng/ml at 2 h and 225.1 ± 15.8 ng/ml at 4 h following GTS administration. At 2 h, the Rb1 concentration in ileal mucosa was 873.3 ± 80.5 µg/g mucosa while a higher Rb1 concentration appeared in ileal contents (4.27 ± 0.81 mg/g content). At 4 h, the Rb1 concentration in ileal mucosa and in contents was 298.1 ± 36.5 µg/g mucosa and 728.1 ± 284.4 µg/g content respectively. For colon, the Rb1 concentration in mucosa and contents was only 14.1 ± 9.9 µg/g mucosa and 48.2 ± 13.3 µg/g content at 2 h and then increased to 118.8 ± 19.8 µg/g mucosa and 2.34 ± 0.68 mg/g content at 4 h respectively.

GLP1 secretion in NCI-H716 cells

Rb1, Rb2, Rc, Rd, and Re were found to be major ginsenosides in GTS. The effects of the five major ginsenosides on GLP1 secretion were investigated in the NCI-H716 cell line. The results showed that tested agents (100 μ M) possessed similar efficacy enhancing GLP1 secretion except Re (Fig. 4A). Extent of increase in GLP1 release induced by GTS (100 and 200 μ g/ml) was similar to that by Rb1. This study showed that Rb1 was the most abundant constituent in GTS; therefore, Rb1 was selected to be the representative constituent for further studies.

The following study showed that ginsenoside Rb1 dose dependently stimulated GLP1 secretion (Fig. 4A). GLP1 secretion from the cells was glucose dependent (Fig. 4B). Rb1 (100 μ M) increased GLP1 secretion by 1.56-fold at 5.5 mM glucose concentration compared with 1.41-fold without glucose, whereas Rb1 did not produce further increments at 11.1 mM glucose concentration.

GCG mRNA and PCSK1 mRNA expression in NCI-H716 cells

As mentioned earlier, both *GCG* mRNA and *PCSK1* mRNA were related to GLP1 production. Data from quantitative RT-PCR analysis showed that Rb1 treatment significantly increased *GCG* mRNA as well as *PCSK1* mRNA expression compared with control cells, which indicated that Rb1 may affect GLP1 biosynthesis (Fig. 4C).

Cellular energy metabolism, intracellular Ca^{2+} level, and K_{ATP} channel in NCI-H716 cells

Some reports showed that GLP1 release is involved in cellular energy metabolism and intracellular Ca^{2+} levels. The intracellular levels of ATP and ADP were assessed, and the ratio of ATP and ADP levels (ATP:ADP) was calculated. Consistent with our expectation, ginsenoside Rb1 significantly elevated the ATP:ADP ratio (Fig. 5A). The metabolic inhibitor azide (3 mM) may reverse the increase in GLP1 secretion induced by Rb1 (Fig. 5C).

In addition, Rb1 remarkably increased intracellular Ca^{2+} levels while this augmentation is slow and reversible (Fig. 5B). Furthermore, the presence of 20 μ M nifedipine, an L-type Ca^{2+} channel blocker, abrogated the Rb1-induced increase in GLP1 secretion (Fig. 5C). Substitution of extracellular medium by Ca^{2+} -free KRB buffer also abolished the stimulation of GLP1 secretion induced by Rb1. Similarly, adding 340 μ M diazoxide, a K_{ATP} channel opener, also attenuated the rise of GLP1 secretion induced

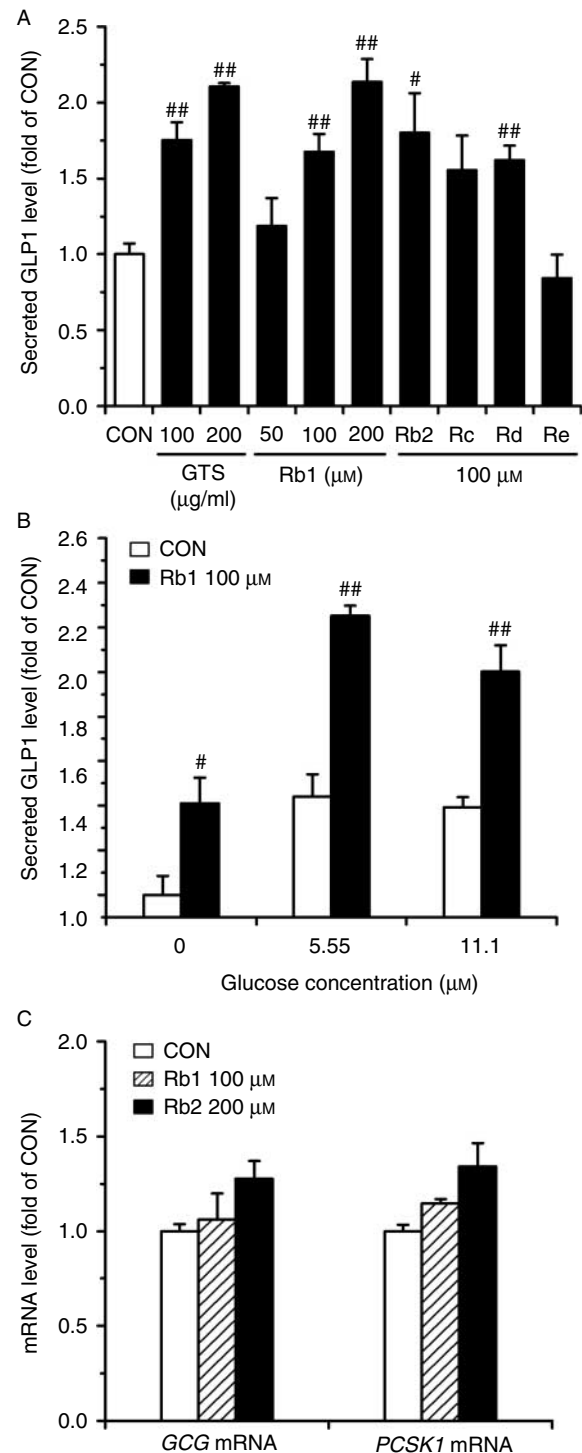
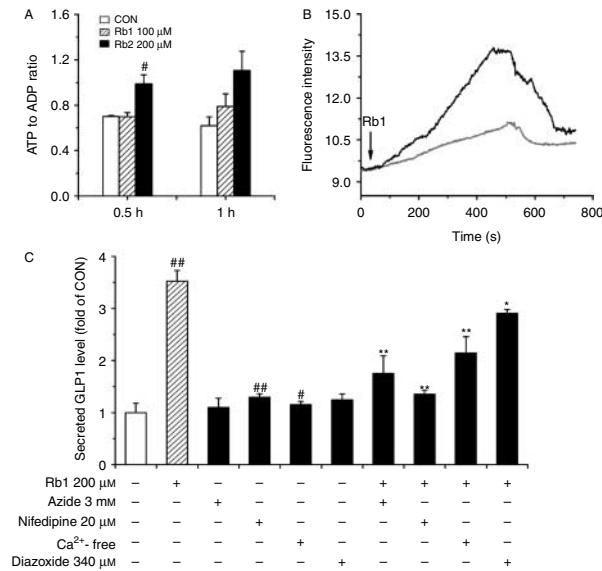


Figure 4

Effects of GTS and ginsenosides (Rb1, Rb2, Rc, Rd, and Re) on GLP1 secretion in NCI-H716 cells (A). Effects of Rb1 incubation with glucose on GLP1 secretion (B). *GCG* mRNA and *PCSK1* mRNA gene expression in NCI-H716 cells (C). Values are expressed as mean \pm s.e.m. ($n=5-8$), [#] $P<0.05$ and ^{##} $P<0.01$ vs CON.

**Figure 5**

Effects of Rb1 on cellular energy metabolism (ATP:ADP ratio; A) and cytosolic Ca²⁺ transient in NCI-H716 cells induced by ginsenoside Rb1 100 μM (gray line) and 200 μM (black line; B). Effects of azide (3 mM), nifedipine (20 μM), diazoxide (340 μM), and Ca²⁺-free KRB buffer on GLP1 secretion induced by Rb1 (200 μM) in NCI-H716 cells (C). Values are expressed as mean ± s.e.m. (n = 5–8), #P < 0.05, ##P < 0.01 vs CON and *P < 0.05, **P < 0.01 vs Rb1.

by Rb1 (Fig. 5C). All these results indicated that GLP1 release stimulated by Rb1 may be involved in cellular energy metabolism, intracellular Ca²⁺ level, and K_{ATP} channel.

Discussion

As they account for over 60% of postprandial insulin release, incretin hormones have become a promising treatment for controlling type 2 diabetes, especially when traditional hypoglycemic agents appear to be ineffective (Salehi *et al.* 2010). Based on incretin hormones, current drug development focuses on GLP1 analogs as well as DPP-IV inhibitors (Reimann 2010). However, these newly approved chemicals have displayed some untoward reactions (Elashoff *et al.* 2011, Knezevich *et al.* 2012). One alternative approach is to seek some natural compounds that may stimulate intestinal L-cells and trigger endogenous GLP1 release. The major finding of this study was that ginsenosides may be a potent stimulator of GLP1 secretion.

According to our results, GTS treatment may lower both FBG and postprandial plasma glucose, accompanied by an increase in insulin levels in plasma and pancreatic

tissue, as well as β-cell mass. GTS treatment may partly improve lipid homeostasis and reduce HOMA-IR index. All these results verified the therapeutic value of GTS with regard to type 2 diabetes.

As we expected, 300 mg/kg GTS treatment significantly increased GLP1 release induced by glucose loading (2.5 g/kg, p.o.). Peripherally released GLP1 may access the brain and participate in the regulation of anorexic response (Lu *et al.* 2009), which indicated that suppression of food intake induced by GTS may partly be due to enhancement of GLP1 release. It was well known that GLP1 exerts important effects on regulating glucose homeostasis via stimulating insulin secretion, stimulating β-cell proliferation, inhibiting food intake, etc. (Holst 2007), which was observed in this study. It was noticed that GTS promoted proglucagon gene expression and L-cell proliferation in the intestine of diabetic rats. These findings implied that enhancement of GLP1 release may be partially attributed to promoted GLP1 biosynthesis and L-cell function.

Our results indicated that a high concentration of Rb1 existed in distal intestine, which may directly interact with intestinal epithelial endocrine L-cells. As mentioned earlier, ginsenosides are poorly absorbed while high concentrations of ginsenosides in the intestine may be responsible for GLP1 secretion. Compared with the ileum, the Rb1 concentration in the colon contents was very low at 2 h, which indicated that GTS did not reach the colon at that time. This fact also explained that GTS treatment did not influence GLP1 levels in the colon (Fig. 3B).

NCI-H716 cells served as an *in vitro* model of the intestinal L-cell to further investigate the effect of GTS on regulating GLP1 release. *In vitro* study demonstrated that GTS may provoke an increase in GLP1 secretion from the NCI-H716 cells, in accordance with *in vivo* findings. We further evaluated the effects of five major ginsenosides in GTS. All the tested ginsenosides enhanced GLP1 secretion except Re, which indicated that ginsenosides were the major compounds stimulating GLP1 secretion. Rb1, the most abundant constituent in GTS, served as the representative constituent for further experiments. Rb1 stimulated GLP1 secretion in a dose-dependent manner and exhibited a synergistic effect with glucose in GLP1 secretion. *GCG* and *PCSK1* mRNA expression were also promoted in NCI-H716 cells treated with Rb1.

GLP1 secretion induced by glucose was under modulation of many factors including cellular energy metabolism, closure of K_{ATP} channels (Reimann & Gribble 2002), electrogenic coupled Na⁺ and glucose entry via sodium-coupled glucose co-transporters (Gribble *et al.* 2003),

sweet taste receptors, and G-protein-coupled receptor pathways (Jang *et al.* 2007, Chu *et al.* 2008). Some studies have demonstrated that ginseng may enhance glucose-stimulated insulin secretion via increasing ATP levels, affecting activity of K_{ATP} channels and Ca^{2+} channels (Luo & Luo 2006, Park *et al.* 2008a). We discovered that Rb1 significantly increased the ATP:ADP ratio and cytosolic Ca^{2+} levels in NCI-H716 cells. Further study proved that the enhancement of GLP1 secretion induced by Rb1 was partly diminished by the presence of azide, diazoxide, and nifedipine. These results implied that an elevated ATP:ADP ratio stimulated by Rb1 could result in the closure of K_{ATP} channels and membrane depolarization and in turn trigger the opening of voltage-gated calcium channels. Then elevation in the cytosolic free Ca^{2+} concentration triggers the exocytotic response of GLP1. Besides, as ginsenosides are amphipathic, the possibility that Rb1 increased cytosolic Ca^{2+} acting as a detergent could not be ruled out.

It is of interest that hyperglycemia was effectively attenuated by GTS treatment while insulin level was only marginally increased (Fig. 1C and D). Similarly, β -cell mass markedly increased in the DM-GH group while pancreatic insulin content just increased moderately compared with DM rats (Fig. 2C and D). These phenomena may be attributed to the ameliorated IR. Similar reports showed that plasma glucose level and plasma insulin level may decrease simultaneously when the diabetic conditions are improved efficiently (Attele *et al.* 2002, Xie *et al.* 2005b, Yoon *et al.* 2007, Xiong *et al.* 2010, Yang *et al.* 2010). In our experiment, increased GLP1 secretion induced by ginsenosides may mediate these effects. It is well known that GLP1 exhibits potent insulinotropic and glucose-lowering actions. In addition, chronic GLP1 enhancement may strongly inhibit glucagon secretion, reduce food intake, slow gastric emptying, exert insulin-like effects, and increase insulin sensitivity and β -cell function (Young *et al.* 1999, Holst 2007). These factors may result in a great amelioration in IR. For instance, GLP1 increases glycogen synthesis and glucose transport in skeletal muscles and liver (Redondo *et al.* 2003, Acitores *et al.* 2004). The existence of GLP1 can also amplify insulin signaling in adipocytes (Gao *et al.* 2007). Moreover, lipid homeostasis improved by GTS treatment (Table 1) and augmented GLP1 release may further ameliorate IR. Meanwhile, we cannot exclude the possible role of absorbed ginsenosides on IR and glucose homeostasis. In our experiment, although the absolute amount of insulin did not increase, poor potency of insulin was attenuated after the treatment. Improved insulin sensitivity may require less insulin to maintain glucose

disposal (Xiong *et al.* 2010). Thus, it is reasonable to consider that GLP1 as well as β -cell mass markedly increased in the DM-GH group while insulin content in plasma and pancreas just increased moderately. In addition, enhanced insulin level may have appeared obviously due to the insulinotropic action of GLP1 as well as ginsenosides (Kimura *et al.* 1981b, Rotshteyn & Zito 2004, Park *et al.* 2008b) during the treatment.

In conclusion, this study suggested that modulating GLP1 secretion as well as relative gene expression may be one of the mechanisms by which ginsenosides exert their antidiabetic effect. The underlying molecular mechanism involved elevation of the ATP:ADP ratio, subsequent K_{ATP} channel closure, and the opening of Ca^{2+} channels. These findings may suggest that it is endogenous GLP1 that acts as a critical mediator of this antidiabetic action, which shed some light on the pharmacokinetic–pharmacological paradox of ginsenosides.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

Funding

This work has received grants from National Youth Science Foundation of China (no. 81102503), Fundamental Research Funds for the Central Universities (no. JKY2011018), and National Science Foundation of China (no. 81273587).

References

- Acitores A, González N, Sancho V, Valverde I & Villanueva-Peñacarrillo ML 2004 Cell signalling of glucagon-like peptide-1 action in rat skeletal muscle. *Journal of Endocrinology* **180** 389–398. (doi:10.1677/joe.0.1800389)
- Attele AS, Wu JA & Yuan CS 1999 Ginseng pharmacology: multiple constituents and multiple actions. *Biochemical Pharmacology* **58** 1685–1693. (doi:10.1016/S0006-2952(99)00212-9)
- Attele AS, Zhou YP, Xie JT, Wu JA, Zhang L, Dey L, Pugh W, Rue PA, Polonsky KS & Yuan CS 2002 Antidiabetic effects of *Panax ginseng* berry extract and the identification of an effective component. *Diabetes* **51** 1851–1858. (doi:10.2337/diabetes.51.6.1851)
- Baggio LL & Drucker DJ 2007 Biology of incretins: GLP-1 and GIP. *Gastroenterology* **132** 2131–2157. (doi:10.1053/j.gastro.2007.03.054)
- Cani PD, Daubioul CA, Reusens B, Remacle C, Catillon G & Delzenne NM 2005 Involvement of endogenous glucagon-like peptide-1(7–36) amide on glycaemia-lowering effect of oligofructose in streptozotocin-treated rats. *Journal of Endocrinology* **185** 457–465. (doi:10.1677/joe.1.06100)
- Chen GM, Hu N, Liu L, Xie SS, Wang P, Li J, Xie L, Wang GJ & Liu XD 2011 Pharmacokinetics of verapamil in diabetic rats induced by combination of high-fat diet and streptozotocin injection. *Xenobiotica* **41** 494–500. (doi:10.3109/00498254.2011.558933)
- Chu ZL, Carroll C, Alfonso J, Gutierrez V, He H, Lucman A, Pedraza M, Mondala H, Gao H, Bagnol D *et al.* 2008 A role for intestinal endocrine cell-expressed G protein-coupled receptor 119 in glycemic control by

- enhancing glucagon-like peptide-1 and glucose-dependent insulinotropic peptide release. *Endocrinology* **149** 2038–2047. (doi:10.1210/en.2007-0966)
- Elashoff M, Matveyenko AV, Gier B, Elashoff R & Butler PC 2011 Pancreatitis, pancreatic, and thyroid cancer with glucagon-like peptide-1-based therapies. *Gastroenterology* **141** 150–156. (doi:10.1053/j.gastro.2011.02.018)
- Gao H, Wang X, Zhang Z, Yang Y, Yang J, Li X & Ning G 2007 GLP-1 amplifies insulin signaling by up-regulation of IR β , IRS-1 and Glut4 in 3T3-L1 adipocytes. *Endocrine* **32** 90–95. (doi:10.1007/s12020-007-9011-4)
- Gribble FM, Williams L, Simpson AK & Reimann F 2003 A novel glucose-sensing mechanism contributing to glucagon-like peptide-1 secretion from the GLUTag cell line. *Diabetes* **52** 1147–1154. (doi:10.2337/diabetes.52.5.1147)
- Holst JJ 2007 The physiology of glucagon-like peptide 1. *Physiological Reviews* **87** 1409–1439. (doi:10.1152/physrev.00034.2006)
- Jang HJ, Kokrashvili Z, Theodorakis MJ, Carlson OD, Kim BJ, Zhou J, Kim HH, Xu X, Chan SL, Juhaszova M *et al.* 2007 Gut-expressed α -gustducin and taste receptors regulate secretion of glucagon-like peptide-1. *PNAS* **104** 15069–15074. (doi:10.1073/pnas.0706890104)
- Kim SH & Park KS 2003 Effects of *Panax ginseng* extract on lipid metabolism in humans. *Pharmacological Research* **48** 511–513. (doi:10.1016/S1043-6618(03)00189-0)
- Kimura M, Waki I, Chujo T, Kikuchi T, Hiyama C, Yamazaki K & Tanaka O 1981a Effects of hypoglycemic components in ginseng radix on blood insulin level in alloxan diabetic mice and on insulin release from perfused rat pancreas. *Journal of Pharmacobio-Dynamics* **4** 410–417. (doi:10.1248/bpb1978.4.410)
- Kimura M, Waki I, Tanaka O, Nagai Y & Shibata S 1981b Pharmacological sequential trials for the fractionation of components with hypoglycemic activity in alloxan diabetic mice from ginseng radix. *Journal of Pharmacobio-Dynamics* **4** 402–409. (doi:10.1248/bpb1978.4.402)
- Knezevich E, Crnic T, Kershaw S & Drincic A 2012 Liraglutide-associated acute pancreatitis. *American Journal of Health-System Pharmacy* **69** 386–389. (doi:10.2146/ajhp110221)
- Li J, Wang P, Yu S, Zheng Z & Xu X 2012a Calcium entry mediates hyperglycemia-induced apoptosis through Ca²⁺/calmodulin-dependent kinase II in retinal capillary endothelial cells. *Molecular Vision* **18** 2371–2379.
- Li X, Chen Z, Jiang Z, Li Y & Zhang Y 2012b Zinc reverses glycine-dependent inactivation of NMDARs in cultured rat hippocampal neurons. *Science China. Life sciences* **55** 1075–1081. (doi:10.1007/s11427-012-4421-9)
- Lim W, Mudge KW & Vermeylen F 2005 Effects of population, age, and cultivation methods on ginsenoside content of wild American ginseng (*Panax quinquefolium*). *Journal of Agricultural and Food Chemistry* **53** 8498–8505. (doi:10.1021/jf051070y)
- Liu H, Yang J, u F, Gao X, Ma X, Huang Y, Xu F, Niu W, Wang F, Mao Y *et al.* 2009 Absorption and disposition of ginsenosides after oral administration of *Panax notoginseng* extract to rats. *Drug Metabolism and Disposition* **37** 2290–2298. (doi:10.1124/dmd.109.029819)
- Lu SS, Yu YL, Zhu HJ, Liu XD, Liu L, Liu YW, Wang P, Xie L & Wang GJ 2009 Berberine promotes glucagon-like peptide-1 (7–36) amide secretion in streptozotocin-induced diabetic rats. *Journal of Endocrinology* **200** 159–165. (doi:10.1677/JOE-08-0419)
- Luo JZ & Luo L 2006 American ginseng stimulates insulin production and prevents apoptosis through regulation of uncoupling protein-2 in cultured β -cells. *Evidence-Based Complementary and Alternative Medicine* **3** 365–372. (doi:10.1093/ecam/nel026)
- Lupescu A, Jilani K, Zbidah M, Lang E & Lang F 2012 Enhanced Ca²⁺ entry, ceramide formation, and apoptotic death of erythrocytes triggered by plumbagin. *Journal of Natural Products* **75** 1956–1961. (doi:10.1021/np300611r)
- Ma SW, Benzie IF, Chu TT, Fok BS, Tomlinson B & Critchley LA 2008 Effect of *Panax ginseng* supplementation on biomarkers of glucose tolerance, antioxidant status and oxidative stress in type 2 diabetic subjects: results of a placebo-controlled human intervention trial. *Diabetes, Obesity & Metabolism* **10** 1125–1127. (doi:10.1111/j.1463-1326.2008.00858.x)
- Odani T, Tanizawa H & Takino Y 1983a Studies on the absorption, distribution, excretion and metabolism of ginseng saponins. II. The absorption, distribution and excretion of ginsenoside Rg1 in the rat. *Chemical & Pharmaceutical Bulletin* **31** 292–298. (doi:10.1248/cpb.31.292)
- Odani T, Tanizawa H & Takino Y 1983b Studies on the absorption, distribution, excretion and metabolism of ginseng saponins. III. The absorption, distribution and excretion of ginsenoside Rb1 in the rat. *Chemical & Pharmaceutical Bulletin* **31** 1059–1066. (doi:10.1248/cpb.31.1059)
- Park KH, Shin HJ, Song YB, Hyun HC, Cho HJ, Ham HS, Yoo YB, Ko YC, Jun WT & Park HJ 2002 Possible role of ginsenoside Rb1 on regulation of rat liver triglycerides. *Biological & Pharmaceutical Bulletin* **25** 457–460. (doi:10.1248/bpb.25.457)
- Park MW, Ha J & Chung SH 2008a 20(S)-ginsenoside Rg3 enhances glucose-stimulated insulin secretion and activates AMPK. *Biological & Pharmaceutical Bulletin* **31** 748–751. (doi:10.1248/bpb.31.748)
- Park S, Ahn IS, Kwon DY, Ko BS & Jun WK 2008b Ginsenosides Rb1 and Rg1 suppress triglyceride accumulation in 3T3-L1 adipocytes and enhance β -cell insulin secretion and viability in Min6 cells via PKA-dependent pathways. *Bioscience, Biotechnology, and Biochemistry* **72** 2815–2823. (doi:10.1271/bbb.80205)
- Ranganath LR 2008 Incretins: pathophysiological and therapeutic implications of glucose-dependent insulinotropic polypeptide and glucagon-like peptide-1. *Journal of Clinical Pathology* **61** 401–409. (doi:10.1136/jcp.2006.043232)
- Redondo A, Trigo MV, Acitores A, Valverde I & Villanueva-Peñacarrillo ML 2003 Cell signalling of the GLP-1 action in rat liver. *Molecular and Cellular Endocrinology* **204** 43–50. (doi:10.1016/S0303-7207(03)00146-1)
- Reed MJ, Meszaros K, Entes LJ, Claypool MD, Pinkett JG, Gadbois TM & Reaven GM 2000 A new rat model of type 2 diabetes: the fat-fed, streptozotocin-treated rat. *Metabolism* **49** 1390–1394. (doi:10.1053/meta.2000.17721)
- Reimann F 2010 Molecular mechanisms underlying nutrient detection by incretin-secreting cells. *International Dairy Journal* **20** 236–242. (doi:10.1016/j.idairyj.2009.11.014)
- Reimann F & Gribble FM 2002 Glucose-sensing in glucagon-like peptide-1-secreting cells. *Diabetes* **51** 2757–2763. (doi:10.2337/diabetes.51.9.2757)
- Reimer RA, Darimont C, Gremlich S, Nicolas-Métral V, Rüegg UT & Macé K 2001 A human cellular model for studying the regulation of glucagon-like peptide-1 secretion. *Endocrinology* **142** 4522–4528. (doi:10.1210/en.142.10.4522)
- Rotshteyn Y & Zito SW 2004 Application of modified *in vitro* screening procedure for identifying herbals possessing sulfonylurea-like activity. *Journal of Ethnopharmacology* **93** 337–344. (doi:10.1016/j.jep.2004.04.007)
- Rouillé Y, Martin S & Steiner DF 1995 Differential processing of proglucagon by the subtilisin-like prohormone convertase PC2 and PC3 to generate either glucagon or glucagon-like peptide. *Journal of Biological Chemistry* **270** 26488–26496. (doi:10.1074/jbc.270.44.26488)
- Salehi M, Aulinger B, Prigeon RL & D'Alessio DA 2010 Effect of endogenous GLP-1 on insulin secretion in type 2 diabetes. *Diabetes* **59** 1330–1337. (doi:10.2337/db09-1253)
- Shang W, Yang Y, Zhou L, Jiang B, Jin H & Chen M 2008 Ginsenoside Rb1 stimulates glucose uptake through insulin-like signaling pathway in 3T3-L1 adipocytes. *Journal of Endocrinology* **198** 561–569. (doi:10.1677/JOE-08-0104)
- Sotaniemi EA, Haapakoski E & Rautio A 1995 Ginseng therapy in non-insulin-dependent diabetic patients. *Diabetes Care* **18** 1373–1375. (doi:10.2337/diacare.18.10.1373)

- Srinivasan K, Viswanad B, Asrat L, Kaul CL & Ramarao P 2005 Combination of high-fat diet-fed and low-dose streptozotocin-treated rat: a model for type 2 diabetes and pharmacological screening. *Pharmacological Research* **52** 313–320. (doi:10.1016/j.phrs.2005.05.004)
- Varum S, Rodrigues AS, Moura MB, Momcilovic O, Easley CA, Ramalho-Santos J, Van Houten B & Schatten G 2011 Energy metabolism in human pluripotent stem cells and their differentiated counterparts. *PLoS ONE* **6** e20914. (doi:10.1371/journal.pone.0020914)
- Volonté MG, Yuln G, Quiroga P & Consolini AE 2004 Development of an HPLC method for determination of metabolic compounds in myocardial tissue. *Journal of Pharmaceutical and Biomedical Analysis* **35** 647–653. (doi:10.1016/j.jpba.2004.02.002)
- Vuksan V, Sievenpiper JL, Koo VY, Francis T, Beljan-Zdravkovic U, Xu Z & Vidgen E 2000 American ginseng (*Panax quinquefolius* L) reduces postprandial glycemia in nondiabetic subjects and subjects with type 2 diabetes mellitus. *Archives of Internal Medicine* **160** 1009–1013. (doi:10.1001/archinte.160.7.1009)
- Vuksan V, Sung MK, Sievenpiper JL, Stavro PM, Jenkins AL, Di Buono M, Lee KS, Leiter LA, Nam KY, Arnason JT *et al.* 2008 Korean red ginseng (*Panax ginseng*) improves glucose and insulin regulation in well-controlled, type 2 diabetes: results of a randomized, double-blind, placebo-controlled study of efficacy and safety. *Nutrition, Metabolism, and Cardiovascular Diseases* **18** 46–56. (doi:10.1016/j.numecd.2006.04.003)
- Washida D & Kitanaka S 2003 Determination of polyacetylenes and ginsenosides in *Panax* species using high performance liquid chromatography. *Chemical & Pharmaceutical Bulletin* **51** 1314–1317. (doi:10.1248/cpb.51.1314)
- Xie HT, Wang GJ, Sun JG, Tucker I, Zhao XC, Xie YY, Li H, Jiang XL, Wang R, Xu MJ *et al.* 2005a High performance liquid chromatographic–mass spectrometric determination of ginsenoside Rg3 and its metabolites in rat plasma using solid-phase extraction for pharmacokinetic studies. *Journal of Chromatography. B, Analytical Technologies in the Biomedical and Life Sciences* **818** 167–173. (doi:10.1016/j.jchromb.2004.12.028)
- Xie JT, Mehendale SR, Li X, Quigg R, Wang X, Wang CZ, Wu JA, Aung HH, A Rue P, Bell GI *et al.* 2005b Anti-diabetic effect of ginsenoside Re in *ob/ob* mice. *Biochimica et Biophysica Acta* **1740** 319–325. (doi:10.1016/j.bbdis.2004.10.010)
- Xie JT, Mehendale S & Yuan CS 2005c Ginseng and diabetes. *American Journal of Chinese Medicine* **33** 397–404. (doi:10.1142/S0192415X05003004)
- Xiong Y, Shen L, Liu KJ, Tso P, Wang G, Woods SC & Liu M 2010 Antiobesity and antihyperglycemic effects of ginsenoside Rb1 in rats. *Diabetes* **59** 2505–2512. (doi:10.2337/db10-0315)
- Yang CY, Wang J, Zhao Y, Shen L, Jiang X, Xie ZG, Liang N, Zhang L & Chen ZH 2010 Anti-diabetic effects of *Panax notoginseng saponins* and its major anti-hyperglycemic components. *Journal of Ethnopharmacology* **130** 231–236. (doi:10.1016/j.jep.2010.04.039)
- Yokozawa T, Kobayashi T, Oura H & Kawashima Y 1985 Studies on the mechanism of the hypoglycemic activity of ginsenoside-Rb2 in streptozotocin-diabetic rats. *Chemical & Pharmaceutical Bulletin* **33** 869–872. (doi:10.1248/cpb.33.869)
- Yoon SH, Han EJ, Sung JH & Chung SH 2007 Anti-diabetic effects of compound K versus metformin versus compound K-metformin combination therapy in diabetic *db/db* mice. *Biological & Pharmaceutical Bulletin* **30** 2196–2200. (doi:10.1248/bpb.30.2196)
- Young AA, Gedulin BR, Bhavsar S, Bodkin N, Jodka C, Hansen B & Denaro M 1999 Glucose-lowering and insulin-sensitizing actions of exendin-4: studies in obese diabetic (*ob/ob*, *db/db*) mice, diabetic fatty Zucker rats, and diabetic rhesus monkeys (*Macaca mulatta*). *Diabetes* **48** 1026–1034. (doi:10.2337/diabetes.48.5.1026)
- Yu YL, Lu SS, Yu S, Liu YC, Wang P, Xie L, Wang GJ & Liu XD 2009 Huang-lian-jie-du-decoction modulates glucagon-like peptide-1 secretion in diabetic rats. *Journal of Ethnopharmacology* **124** 444–449. (doi:10.1016/j.jep.2009.05.027)
- Yu YL, Liu L, Wang XT, Liu X, Liu X, Xie L & Wang G 2010 Modulation of glucagon-like peptide-1 release by berberine: *in vivo* and *in vitro* studies. *Biochemical Pharmacology* **79** 1000–1006. (doi:10.1016/j.bcp.2009.11.017)
- Yun SN, Moon SJ, Ko SK, Im BO & Chung SH 2004 Wild ginseng prevents the onset of high-fat diet induced hyperglycemia and obesity in ICR mice. *Archives of Pharmacological Research* **27** 790–796. (doi:10.1007/BF02980150)

Received in final form 2 February 2013

Accepted 26 February 2013

Accepted Preprint published online 26 February 2013