Eur J Nutr (2011) 50:107–118
DOI 10.1007/s00394-010-0120-0

ORIGINAL CONTRIBUTION

Gastrodia elata Blume water extracts improve insulin resistance by decreasing body fat in diet-induced obese rats: vanillin and 4-hydroxybenzaldehyde are the bioactive candidates

Sunmin Park · Da Sol Kim · Suna Kang

Received: 21 March 2010 / Accepted: 14 June 2010 / Published online: 26 June 2010

Abstract
Background Insulin resistance is a common symptom of metabolic diseases such as obesity, type 2 diabetes and hyperlipidemia.
Aim of the study We investigated whether *Gastrodia elata* Blume water extract(GEB), containing phenolic compounds, had a beneficial action on insulin resistance in male Sprague–Dawley rats fed a high fat diet(HFD) and determined how this effect was produced. In addition, the bioactive candidates involved were identified.
Methods Rats fed HFD were daily administered with 0.3 g GEB(GEB-L), 1 g GEB(GEB-H), or 1 g cellulose(control) per kg body weight for 8 weeks, while rats in the fourth group were fed a low fat diet(LFD). In vitro study, 4 major components of GEB were tested for their impact on fat accumulation.
Results Rats in the control group exhibited a higher weight gain of epididymal and retroperitoneal fat pads than those fed LFD, while GEB prevented such an increment in a dose-dependent manner. GEB-H significantly decreased energy intake partly through potentiating STAT3 phosphorylation and attenuating AMPK phosphorylation in the hypothalamus. GEB-H also increased energy expenditure with the increase in fat oxidation. GEB-H increased whole body glucose disposal rates and decreased hepatic glucose output compared to the control. Among the major components of GEB, 4-hydroxybenzaldehyde and vanillin decreased triglyceride accumulation by modulating the expression of genes involved in fat metabolism in 3T3-L1 adipocytes. They increased insulin-stimulated glucose uptake to reduce insulin resistance.
Conclusions GEB-H, mainly as a result of the action of 4-hydroxybenzaldehyde and vanillin, reduces insulin resistance by decreasing fat accumulation in adipocytes by activating fat oxidation and potentiating leptin signaling in diet-induced obese rats.

Keywords *Gastrodia elata* Blume · Energy expenditure · AMP kinase · Glucose tolerance · Insulin resistance · Euglycemic hyperinsulinemic clamp · Hypothalamus

S. Park (✉) · D. S. Kim · S. Kang
Department of Food and Nutrition, Hoseo University,
165 Sechul-Ri, BaeBang-Yup, Asan-Si,
ChungNam-Do 336-795, South Korea
e-mail: smpark@hoseo.edu

S. Park
Department of Maechatronics Engineering,
Hoseo University, Asan, South Korea

S. Park
Obesity/Diabetes Research Institutes,
Hoseo University, Asan, South Korea

Introduction

Obesity has reached global epidemic proportions among children, adults and the elderly, and it is associated with numerous metabolic diseases including hypertension, type 2 diabetes, dyslipidemia, obstructive sleep apnea, certain cancers and major cardiovascular diseases [1]. A common symptom of these metabolic diseases is insulin resistance, and the reduction in insulin resistance ameliorates these metabolic disturbances. Thus, the reduction in insulin resistance is an important goal to maintain a healthy life.

The hypothalamus is a key integrator of nutrient-induced signals of hunger and satiety, crucial for processing information regarding energy stores and food availability [2, 3]. Food intake is regulated by anorexic and orexigenic

hormones and neurotransmitters in the hypothalamus [2]. Enhancement of leptin signaling in the hypothalamus reduces food intake and consequently decreases body fat. In addition, recent studies have cast new light on the role of the central nervous system in regulating peripheral glucose via a hypothalamic energy sensing device such as AMP kinase (AMPK), which detects nutrient availability and relays a negative feedback signal on food intake and insulin sensitivity [3, 4]. Thus, certain herbs and their ingredients working in the hypothalamus may act to help maintain the regulation of energy, glucose and lipid homeostasis in humans and experimental animals.

Gastrodia elata Blume (GEB), the tuber of the orchid, has been used since ancient times in Korea and China for treating a variety of metabolic disorders. GEB acts effectively as an anticonvulsant, analgesic and sedative against vertigo, general paralysis, epilepsy and tetanus [5, 6]. GEB contains bioactive phenolic compounds such as gastrodin (glucoside of 4-hydroxybenzyl alcohol), 4-hydroxybenzyl alcohol, 4-hydroxybenzaldehyde, vanillin, 1,3-bis(4-hydroxybenzyl)citrate, 1-(4-beta-D-glucopyranosyloxybenzyl)citrate and parishin B [6]. These components are conveyed into the brain through the blood stream, and they have antiepileptic and antidepressant properties [5]. They also have a neuronal protective action due to the presence of aluminium chloride and amyloid β-peptide, and the protection was associated with increased dopamine concentration and decreased dopamine turnover in striatum [7]. In addition, GEB and its primary components protects against ischemic damage by modulating γ-aminobutyric acid (GABA) release and GABAergic activity [8, 9]. Both dopamine and GABA in the brain are involved in regulating body weight by modulating food intake and energy expenditure [10–12]. Thus, GEB and its components work in various parts of the brain by modulating the levels of neurotransmitters such as GABA and dopamine, and it has great potential to assist in decreasing body fat and insulin resistance.

Therefore, we hypothesized that the long-term administration of GEB water extracts would have beneficial effects on insulin resistance by decreasing fat accumulation in male rats with diet-induced obesity. Since body fat accumulation was determined by energy intake and energy expenditure, both pathways were also investigated. As GEB water extracts were believed to improve insulin resistance by decreasing fat storage, its major compounds of GEB, such as 4-hydroxybenzaldehyde, vanillin, 4-hydroxybenzyl alcohol and gastrodin, were tested for triglyceride accumulation, insulin-stimulated glucose uptake and gene expression involved in fatty acid metabolism in 3T3-L1 adipocytes, to determine which elements were prominent in helping to reduce fat accumulation and insulin resistance.

Methods and materials

GEB water extract and total phenols quantification

Dried and ground GEB (2 kg) was extracted three times by refluxing with water (1:5 and then 1:3, wt/vol) at 80 °C for 3 h, and the filtered extracts were lyophilized. The quantity of phenolic compounds in the GEB water extract was measured since they are the major bioactive compounds of GEB. Total phenolics were measured as in Bellido and Beta [13]. Briefly, diluted extracts and standards (catechin) were mixed with 10% Folin–Ciocalteau reagent, and the color changes were measured with absorbance at 740 nm by a spectrophotometer.

Experimental animals and design

Male Sprague–Dawley rats, weighing 225 ± 24 g, were used as experimental animals. They were housed individually in stainless steel cages in a controlled environment (23 °C and with a 12-h light and dark cycle). All surgical and experimental procedures were performed according to the NIH Guidelines and were approved by Hoseo University Animal Care and Use Review Committee. Experimental animals freely consumed water and were assigned a diet during the 8-week experimental period. Low fat diet (LFD) and HFD were prepared by a semi-purified method with a modified AIN-93 formulation for experimental animals [14]. The HFD consisted of 40 energy percent (En%) carbohydrates, 20 En% protein and 40 En% fats while the LFD consisted of 65 En% carbohydrates, 20 En% protein and 15 En% fats. The major carbohydrate, protein and fat sources were starch plus sugar, casein (milk protein) and lard (CJ Co, Seoul).

Eighty male rats were randomly divided into the four groups. Three groups were fed a HFD, and the remaining group consumed a LFD. Since GEB was traditionally used in a 0.1 g water extracts per kg body weight ratio for humans, the dosages for rats were assigned taking into account the different metabolic rates between humans and rats. Rats fed a HFD were orally administered with 0.3 g water extracts of GEB (GEB-L), 1 g water extract of GEB (GEB-H) or 1 g cellulose (control), respectively, per kg of body weight on a daily basis. Rats on a LFD were daily administered 1 g cellulose per kg of body weight as a positive control. Overnight-fasted serum glucose levels, food and water intake and body weight were measured every Tuesday at 10 am.

Energy expenditure by indirect calorimetry

After 7 weeks of the assigned treatment, energy expenditure was assessed at the beginning of the dark phase of the

light–dark cycle after each rat had fasted for 6 h. The rats were placed into metabolic chambers (airflow = 800 ml/min) in a computer-controlled O_2 and CO_2 measurement system (Biopac Systems Inc., Goleta, CA) to determine their calorimetric parameters. The respiratory quotient (RQ) and resting energy expenditure (REE) were measured by the equations provided by Niwa et al. [15]. Average oxygen consumption (VO_2) and average carbon dioxide production (VCO_2) were integrated over periods of 30 min. After the experiment, data were averaged over 1-min intervals, and VO_2 and VCO_2 values were corrected for metabolic body size ($kg^{0.75}$) [16]. Carbohydrate and fat oxidation were calculated from non-protein oxygen consumption, their relative oxidative proportions, and the amount of oxygen consumed per gram of substrate oxidized [17].

Locomotor activity

Locomotor activity was determined using a Linton AM1053 Activity Monitor with AmLogger software (Linton Instruments, Diss, UK). This instrument comprises a three-dimensional array of infrared beams and was placed around clear Perspex cages to monitor animal motion. Breakage of beams was automatically logged as 'mobility' if more than one beam was broken on the *x–y* plane for 1 s (i.e., recorded walking motion), 'rearing' if the motion was in the vertical plane, and 'activity' if any beam was broken in the *x–y* plane. The sum of rearing, mobility and activity measurements was used as a measure of overall locomotion or total locomotor activity. Activity was assessed during the dark phase of the light–dark cycle when the rats were the most active. After allowing 30 min to adapt to a clean Perspex cage, activity was then monitored for an hour. All data are shown as the active time in seconds.

Oral glucose tolerance test (OGTT) and liver glycogen and triglyceride contents

Two days after measuring locomotive activity an OGTT was performed on overnight-fasted animals by orally administering 2 g glucose/kg body weight. Blood samples were taken by tail bleeding at 0, 10, 20, 30, 40, 50, 60, 70, 80, 90 and 120 min after glucose loading, and serum glucose and insulin were measured with a Glucose Analyzer II (Beckman, Palo Alto, CA) and radioimmunoassay kit (Linco Research, Billerica, MA), respectively. The average of the total areas under the curves for the serum glucose and insulin was calculated by the trapezoidal rule.

After the OGTT, 10 rats were freely provided with foods and water for 2 days and next day and then they were deprived of food for 16 h. Blood from the fasted animals was collected for further analysis. The rats were anesthetized with the mixture of ketamine and xylazine, and human regular insulin (5 U/kg body weight) was injected through the inferior vena cava of the rats. Ten minutes later, they were killed by decapitation, and tissues were rapidly collected, frozen in liquid nitrogen, and stored at −70 °C for further experiments. In order to determine the glycogen content in the liver, the supernatants of the lysates were deproteinized with 1.5 N perchloric acid, and the glycogen content was calculated from glucose concentrations derived from glycogen hydrolyzed by α-amyloglucosidase in an acid buffer [18]. Triglyceride was extracted with a chloroform–methanol (2:1, vol/vol) from the liver and resuspended in pure chloroform [19]. Triglyceride concentration was determined using a Trinder kit (Young Dong Pharm.). After separating serum from blood collected from animals that had fasted, serum triglyceride concentrations were also measured by the same kit, and serum leptin and adiponectin levels were also determined by radioimmunoassay kit (Linco Research).

Euglycemic hyperinsulinemic clamp

After the catheterization of the right carotid artery and left jugular vein in the 7th week, a euglycemic hyperinsulinemic clamp was performed on fasted conscious rats ($n = 10$) to determine insulin resistance as previously described [20]. [3-^{3}H] glucose (NEN Life Science, Boston, MA) was continuously infused during a 4-h period at the rate of 0.05 μCi/min. Basal hepatic glucose output was measured in blood collected at 100 and 120 min after initiation of the [3-^{3}H] glucose infusion. A primed continuous infusion of human regular insulin (Humulin; Eli Lilly, Indianapolis, IN) was then initiated at a rate of 20 pmol × kg^{-1} × min^{-1} to raise plasma insulin concentration to approximately 1100 pM after 210–240 min. Glucose solution (25%) was infused as needed to clamp glucose levels at approximately 6 mM. Rates of whole body glucose uptake and basal glucose turnover were determined according to the ratio of the [3-^{3}H] glucose infusion rate to the specific activity of plasma glucose (dpm/μmol) during the final 30 min of the respective experiments. Hepatic glucose production at the hyperinsulinemic clamped state was determined by subtracting the glucose infusion rate from the whole body glucose uptake.

Immunoblot analysis

Six rats from each group that had not been subjected to a euglycemic hyperinsulinemic clamp were used for an immunoblotting assay as previously described [21]. Frozen hypothalamus of each rat was lysed with a 20 mM Tris buffer (pH 7.4) containing 2 mM EDTA, 137 mM NaCl, 1% NP40, 10% glycerol and 12 mM α-glycerol phosphate and protease inhibitors. Lysates containing equal amounts

of protein (30–50 μg) were resolved by SDS–PAGE, and immunoblotting was performed with specific antibodies against phosphorylated signal transducer and activator of transcription (STAT3)^{tyr705} and AMPK^{Thr172} and β-actin. The intensity of protein expression was determined using Imagequant TL (Amersham Biosciences, Piscataway, NJ). Three sets of two samples per each group were determined ($n = 6$).

RNA isolation and reverse transcription polymerase chain reaction (RT-PCR)

The hypothalamus from four rats from each group was collected at the end of treatment. Total RNA was isolated from the hypothalamus using a monophasic solution of phenol and guanidine isothiocyanate (Trizol reagent, Gibco-BRL, Rockville, MD), followed by extraction and precipitation with isopropyl alcohol [22]. The cDNA was synthesized from equal amounts of total RNA with superscript III reverse transcriptase, and polymerase chain reaction (PCR) was performed with high fidelity Taq DNA polymerase. Equal amounts of cDNA were mixed with sybergreen mix, and they were analyzed by a real-time PCR machine (BioRad, Richmond, CA). The expression level of the gene of interest was corrected for that of the house keeping gene, glyceraldehydes 3-phosphate dehydrogenase (GAPDH). The primers used to detect rat leptin receptor, neuropeptide Y (NPY), agouti-related peptide (AgRP), long-form leptin receptor (Ob-Rb), and GAPDH genes were the following: NPY forward 5-ATGCT AGGTAACAAACG-3, reverse 5-ATGTAGTGTCGCAG AG-3; AGRP forward 5-ACCTTGCTGCGACCTGTG-3, reverse 5-GGTTCGTGGTGCCAGTAC-3; Ob-Rb forward 5′-GGTTGGATGAGTTTTGGAA-3′, reverse 5′-AGAC AGTGAGCTGGGAATGG-3′; GAPDH forward 5′-CTC CACTCACGGCAAATTCAAC-3′; reverse 5′-ACTCCAC GACATACTCAGCACC-3′. The primers were designed to sandwich at least one intron in order to distinguish between the products derived from mRNA and genomic DNA.

Triglyceride accumulation and insulin-stimulated glucose uptake and in 3T3-L1 adipocytes

3T3-L1 mouse embryonic fibroblasts were grown, and they were differentiated into adipocytes with differentiation inducers. The effects of 4-hydroxybenzylaldehyde, 4-hydroxybenzyl alcohol, vanillin or gastrodin on triglyceride accumulation were determined by adding these compounds (20 μM) or vehicle (DMSO) with differentiation inducers as previously described [23]. After fully differentiated into adipocytes, the triglyceride contents in the cells were measured using a Trinder kit (Young Dong Pharmaceutical Co., Seoul). The results for each treatment were expressed as the percentage of change in triglyceride concentration from the baseline (vehicle treatment). Insulin-stimulated glucose uptake was measured in 3T3-L1 adipocytes as previously described by Park et al. [23]. Briefly, adipocytes were preincubated with DMSO, 4-hydroxybenzylaldehyde, 4-hydroxybenzyl alcohol, vanillin or gastrodin for 16 h. Insulin-stimulated glucose uptake was measured in Krebs–Ringer-Hepes (KRH) buffer containing either 0.2 or 10 nM insulin with vehicles (DMSO) and assigned respective compounds at 37 °C for 30 min. At the end of the incubation, glucose uptake was measured with 0.1 μCi 2-deoxy-D-[^{3}H] glucose and 1 mM glucose as the final concentrations for 10 min. Non-specific glucose uptake was measured in cells treated with vehicles and methanol and water extracts without insulin. The radioactivity retained by the cell lysates was determined using a Wallac Liquid Scintillation Counter (Waltham, MA, USA).

3T3-L1 adipocytes were treated with 4 different compounds for 8 h and cDAN was synthesized from total RNA isolated from the adipocytes as shown in the above method and the previous study [22]. The expression of proteins such as carnitine palmitoyltransferase-1 (CPT-1), peroxisome proliferator-activated receptor-γ (PPAR-γ), CCAAT/enhancer binding protein-α (C/EBPα), glycerol-3-phosphate acyl transferase-1 (GPAT-1) and glyceraldehyde 3-phosphate dehydrogenase (GPDH) was analyzed by a real-time PCR method [22].

MTT assay

Cytotoxicity of 3T3-L1 fibroblasts during proliferation was measured. 3T3-L1 fibroblasts were plated in a 96-well plate at a density of 4×10^3 cells/well and cultured in culture media supplemented with varying concentrations of 4-hydroxybenzylaldehyde, 4-hydroxybenzyl alcohol, vanillin, or gastrodin. After 2 days of incubation, viable cell numbers were assessed by MTT method using a MTT Cell Growth Assay Kit (Millipore, Billerica, MA). Cell viability and half-maximal inhibitory concentration (IC50) values were calculated from the mean values of data from three wells.

Statistical analysis

Statistical analysis was performed using the SAS statistical analysis program, and all results are expressed as a mean ± standard deviation. The metabolic effects of two different dosages of GEB and the control were determined by a one-way analysis of variance (ANOVA). Significant differences in the main effects among the groups were identified by Tukey's test at $p < 0.05$. The differences

between the rats fed LFD and HFD were determined by two-sample *t*-test.

Results

Yield of water extracts and total phenols quantification

Water extracts of GEB yield 16.3% of solids, and they contained 4.5% of phenolic compounds, calculated as catechin equivalents.

Body weight and body fat

LFD rats had a lower body weight and body fat than HFD rats as shown in Table 1. The increase caused by HFD was prevented by GEB-H treatment, while GEB-L protected against the increase in comparison with the control, but it was not significant (Table 1). However, GEB decreased epididymal and retroperitoneal fat pads in a dose-dependent manner. The differences in body fat were associated with serum leptin levels in an overnight-fasted state; higher body fat led to higher serum leptin levels (Table 1). In contrast to serum leptin levels, serum adiponectin levels were reduced by HFD, and the reduction was reversed by GEB in a dose-dependent manner (Table 1). Overnight-fasted serum triglyceride levels were also higher in the HFD group than the LFD, and the increase in the HFD group was reduced by GEB-H.

Changes of body weight and body fat result from the balance between energy intake and energy expenditure. Furthermore, the ratio of epididymal fat pad weight to body weight was slightly but significantly higher in HFD-fed rats than LFD-fed rats, and the ratio was reduced by GEB-H in the HFD groups (Table 1). The ratio of retroperitoneum fat weight to body weight showed the same significant differences between the groups as the ratio of epididymal fat pad weight to body weight. This indicated that the reduction in body fat was more than that of body weight. The total energy intake was lower in LFD rats compared to HFD and GEB-H prevented the increase in total energy in HFD rats compared to the negative control (Table 1). Energy expenditure was not significantly different between the LFD and HFD groups (Table 2). Analyzing the details of energy expenditure, the fasting RQ was higher in LFD-fed rats than HFD-fed rats but it was not significantly different ($p = 0.07$). VO_2 adjusted to metabolic body size (VO_2/$kg^{0.75}$/min) was not significantly different between HFD and LFD rats while VCO_2 adjusted to metabolic body size was higher in the HFD group than the LFD group. Calculating from these results, carbohydrate oxidation increased in LFD rats compared with HFD rats, while fat oxidation had the opposite effect. GEB-H increased energy expenditure in HFD-fed rats in comparison with the control. GEB-H significantly decreased RQ in HFD rats, while the fasting VO_2 adjusted to metabolic body size was higher in GEB in a dose-dependent manner (Table 2). However, there were no significant changes in VCO_2 adjusted to metabolic body size among the groups fed HFD. In HFD rats, GEB-H had the highest fat oxidation and the lowest carbohydrate oxidation (Table 2). GEB-L also revealed higher fat oxidation and lower carbohydrate oxidation than the control.

After 1 h of adapting to the new environment to measure locomotive activity, all groups exhibited a similar pattern of movement such as the total time spent moving horizontally and rearing during the dark cycle (data not shown). This indicated

Table 1 Metabolic parameters at the end of experimental periods

	Control ($n = 20$)	GEB-L ($n = 20$)	GEB-H ($n = 20$)	LFD ($n = 20$)
Body weight (g)	333 ± 40^{a}	315 ± 33^{ab}	$299 \pm 34^{b*}$	$295 \pm 32^{\dagger}$
Body weight gain (g)	108.3 ± 11.2^{a}	93.6 ± 8.1^{b}	$79.2 \pm 7.3^{c*}$	$74.7 \pm 8.2^{\dagger}$
Epididymal fat pads (g)	5.8 ± 0.7^{a}	4.9 ± 0.6^{b}	$4.1 \pm 0.6^{c*}$	$4.2 \pm 0.6^{\dagger}$
Ratio of epididymal fat pads and body weight	0.017 ± 0.002^{a}	0.016 ± 0.002^{ab}	$0.014 \pm 0.002^{b*}$	$0.014 \pm 0.002^{\dagger}$
Retroperitoneum fat (g)	7.8 ± 0.8^{a}	6.6 ± 0.8^{b}	$5.5 \pm 0.7^{c*}$	$5.7 \pm 0.8^{\dagger}$
Ratio of retroperitoneum fat and body weight	0.023 ± 0.003^{a}	0.021 ± 0.003^{b}	$0.018 \pm 0.003^{b*}$	$0.019 \pm 0.003^{\dagger}$
Caloric intakes (Kcal/day)	129.6 ± 13.6^{a}	120.7 ± 13.7^{ab}	$113.7 \pm 12.7^{b*}$	$108 \pm 12.1^{\dagger}$
Overnight-fasted leptin levels (ng/mL)	4.5 ± 0.6^{a}	3.7 ± 0.6^{b}	$3.2 \pm 0.5^{c*}$	$3.4 \pm 0.6^{\dagger}$
Overnight-fasted adiponectin levels (μg/mL)	4.8 ± 0.7^{c}	6.7 ± 0.9^{b}	$8.2 \pm 1.3^{a*}$	$9.6 \pm 1.2^{\dagger\dagger}$
Overnight-fasted glucose levels (mg/dL)	105 ± 13	102 ± 13	98 ± 12	101 ± 13
Overnight-fasted insulin levels (ng/mL)	1.32 ± 0.32^{a}	1.18 ± 0.25^{ab}	$0.98 \pm 0.17^{b*}$	$1.02 \pm 0.28^{\dagger}$
Overnight-fasted triglyceride levels (mg/dL)	115 ± 21^{a}	106 ± 19^{ab}	$94 \pm 12^{b*}$	$73 \pm 13^{\dagger}$

* Significantly different among the groups of rats fed a high fat diet (HFD) at $p < 0.05$

[a,b] Values on the same row with different superscripts were significantly different at $p < 0.05$

† Significant difference between the control and a low fat diet (LFD) at $p < 0.05$. [††] at $p < 0.01$

Table 2 The parameters of indirect calorimetry at the end of experiment

	Control ($n = 20$)	GEB-L ($n = 20$)	GEB-H ($n = 20$)	Low fat ($n = 20$)
Energy expenditure (kcal/kg$^{0.75}$/day)	106 ± 12.4^{b}	117 ± 12.5^{ab}	129 ± 12.7^{a}	108 ± 12.6
Respiratory quotient	0.84 ± 0.10^{a}	0.78 ± 0.10^{ab}	0.73 ± 0.95^{b*}	0.93 ± 0.11
VO_2 (mL/kg$^{0.75}$/min)	15.1 ± 1.9^{a}	16.8 ± 2.1^{ab}	18.5 ± 2.7^{b*}	15.4 ± 2.0
VCO_2 (mL/kg$^{0.75}$/min)	12.7 ± 1.4	13.1 ± 1.6	13.5 ± 1.6	14.3 ± 1.7†
Carbohydrate oxidation (mg/kg$^{0.75}$/min)	5.1 ± 0.7^{a}	3.1 ± 0.4^{b}	1.1 ± 0.2^{c*}	8.7 ± 1.1†
Fat oxidation (mg/kg$^{0.75}$/min)	6.2 ± 0.8^{a}	9.4 ± 1.2^{b}	12.7 ± 1.6^{c*}	2.8 ± 0.4†

Rat were administered with 3 g water extracts of GEB (GEB-L), 9 g water extract of GEB (GEB-H) or 9 g cellulose (control) per kg body weight for eight weeks, while rats the fourth group were fed a low fat diet (low fat)

* Significantly different among the groups of female rats fed a high fat diet by one-way ANOVA at $p < 0.05$

[a,b] Values on the same row with different superscripts were significantly different by Tukey test at $p < 0.05$

† Significant difference between the control and low fat by two-sample t-test at $p < 0.05$

that dietary fat and GEB treatment did not affect locomotive activity. Thus, LFD lowered body fat and weight gain by decreasing food intake in comparison with HFD without changing energy expenditure. However, GEB-H in HFD rats lowered body fat by decreasing energy intake and increasing energy expenditure, especially fat oxidation in comparison with the control. GEB-L showed a similar tendency to GEB-H in energy regulation, but was less effective (Table 2).

The phosphorylation of STAT3 and AMPK and the expression of orexigenic neuropeptides in the hypothalamus

The phosphorylation of STAT3 was attenuated in the rats fed a HFD in comparison with those fed a LFD, but there was greater phosphorylation of AMPK in HFD-fed rats (Fig. 1a). This was related to higher food intake in HFD-fed rats than LFD-fed rats. GEB potentiated the phosphorylation of STAT3 and attenuated the phosphorylation of AMPK in the hypothalamus in a dose-dependent manner in HFD rats (Fig. 1a). This was consistent with decreased food intake in the GEB group in a dose-dependent manner.

The expression of Ob-Rb mRNA, a long-form of leptin receptor, was higher in the control than LFD and GEB-H significantly decreased the expression (Fig. 1b). This indicated that leptin resistance was induced in HFD in comparison with LFD, and this phenomenon was noticed in serum leptin concentrations. NPY and AgRP, orexigenic peptides, increased in HFD more than LFD and the increase was reversed by GEB-H.

Glucose tolerance

Overnight-fasted serum glucose levels did not differ between rats fed HFD and LFD, and they were not significantly different among the groups of HFD (Table 1). However, serum insulin levels showed a significant decrease in the GEB-H group and LFD group in comparison with the control, which indicated that LFD caused less insulin resistance than HFD, and GEB-H lowered insulin resistance in HFD rats (Table 1).

An OGTT revealed that HFD induced glucose intolerance and that GEB-H restored glucose tolerance in HFD-fed rats (Fig. 2a). LFD and GEB-H with HFD lowered serum glucose levels at their peak and quickly reduced the levels after the peak during an OGTT compared with the control (Fig. 2a). They resulted in a significantly lower area under the curve of serum glucose levels by approximately 17% compared with the control group (Fig. 2b). However, GEB-L did not decrease the area significantly. HFD rats did not alter the area under the curve of serum insulin levels in comparison with LFD rats during the OGTT and also GEB treatments did not alter the area in comparison with the control (Fig. 2b). GEB increased glycogen storage in the liver of the Px rats and decreased their triglyceride contents in a dose-dependent manner (Fig. 2c).

Insulin resistance

As expected, HFD decreased whole body glucose infusion rates to the point of maintaining euglycemia when infusing insulin reached about 1100 pM serum insulin levels during a hyperinsulinemic euglycemic clamp, indicating HFD rats induce insulin resistance. As depicted in Fig. 3a, glucose infusion rates were higher in GEB-H by 24% compared to the control, while glucose uptake was not significantly different among the groups. Consistent with overnight-fasted serum glucose levels, hepatic glucose output in the basal state exhibited no significant differences between the rats fed LFD and HFD, while GEB also did not affect it in the HFD rats (Fig. 3b). However, hepatic glucose output in

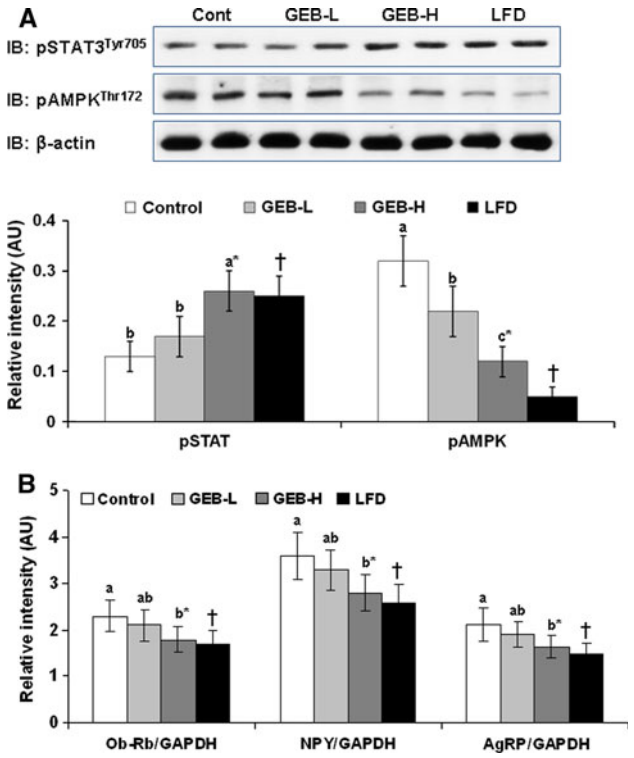

Fig. 1 Relative intensity of phosphorylation of STAT3 and AMPK and mRNA expression of orexigenic neuropeptides in the hypothalamus Control, rats fed a high fat diet (HFD) with cellulose; GEB-L, rats fed HFD with 0.3 g/kg bw of *Gastrodia elata* Blume water extracts (GEB); GEB-H, rats fed with 1 g/kg bw of GEB; LFD, rats fed a low fat diet with 1 g/hg bw of cellulose. **a** Relative intensity of phosphorylation of STAT3 and AMPK proteins corrected by β-actin, a housekeeping protein. **b** mRNA expression of neuropeptide Y (NPY), agouti-related peptide (AgRP) and long-form leptin receptor (Ob-Rb) corrected by glyceraldehydes 3-phosphate dehydrogenase (GAPDH), a housekeeping gene. Three sets of two samples were determined to investigate the differences ($n = 6$) and one set of lot was shown in the figure. *Each bar* represents the mean ± SD. * Significantly different among the groups of rats fed a high fat diet at $p < 0.05$. ^{a,b} Values on the *bar* with different superscripts were significantly different at $p < 0.05$. † Significant difference between the control and the low fat diet (LFD) group at $p < 0.05$

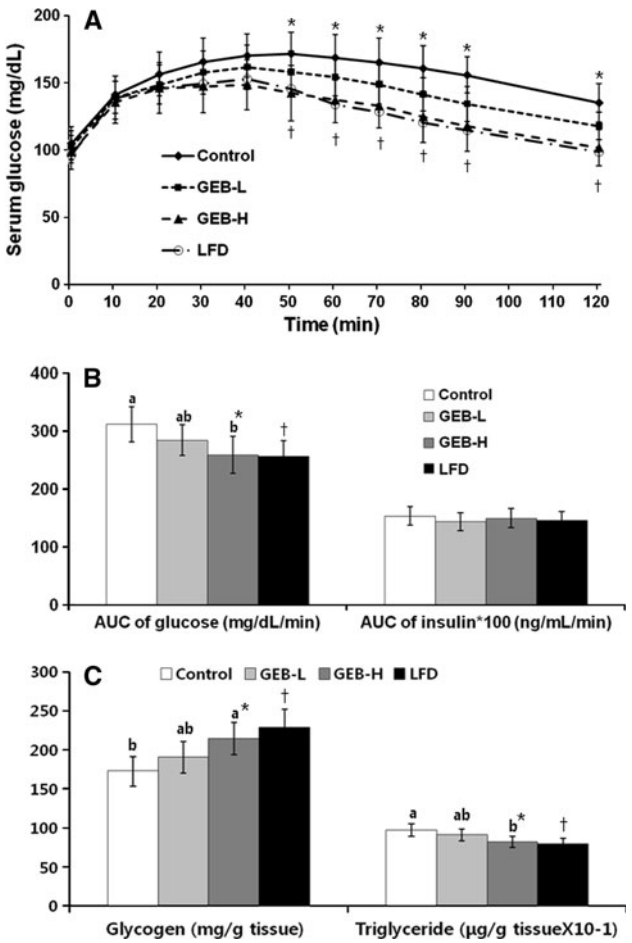

Fig. 2 Changes in serum glucose levels and area under the curve (AUC) of serum glucose and insulin during oral glucose tolerance test. Control, rats fed a high fat diet (HFD) with cellulose; GEB-L, rats fed HFD with 0.3 g/kg bw of *Gastrodia elata* Blume water extracts (GEB); GEB-H, rats fed with 1 g/kg bw of GEB; LFD, rats fed a low fat diet with 1 g/hg bw of cellulose. **a** Serum glucose levels during OGTT. **b** The average of the AUC of glucose and insulin. **c** Hepatic glycogen and triglyceride contents. *Each dot and bar* represents the mean ± SD ($n = 20$). * Significantly different among the groups of rats fed a high fat diet at $p < 0.05$. ^{a,b} Values on the bar with different superscripts were significantly different at $p < 0.05$. † Significant difference between the control and the low fat diet (LFD) group at $p < 0.05$

the hyperinsulinemic clamped state was higher in HFD rats than LFD rats. The increase was prevented by GEB in a dose-dependent manner in the HFD rats (Fig. 3b). Hepatic glucose output was not suppressed significantly in the hyperinsulinemic clamped state and GEB improved the suppression in a dose-dependent manner.

After killing the rats, the glycogen and triglyceride contents were determined since they are associated with insulin resistance and thus glucose homeostasis. Hepatic glycogen storage was higher in LFD than HFD rats, and its storage was increased by GEB-H in HFD-fed rats (Fig. 3c). Conversely, triglyceride accumulation was higher in HFD- than LFD-fed rats while GEB-H lowered the accumulation in HFD-fed rats. Since serum triglyceride concentrations were lower in the GEB-H group than in the control (Table 1), the decrease in triglyceride storage in the liver was not related to increased release and/or decreased uptake into the liver.

Differentiation and triglyceride accumulation in 3T3-L1 adipocytes

A high dose of 4-hydroxybenzaldehyde and vanillin significantly decreased triglyceride accumulation in 3T3-L1 adipocytes in comparison with the control and vanillin especially decreased it (Fig. 4). However, rosiglitazone (2 μM), a commercial PPAR-γ agonist, elevated triglyceride

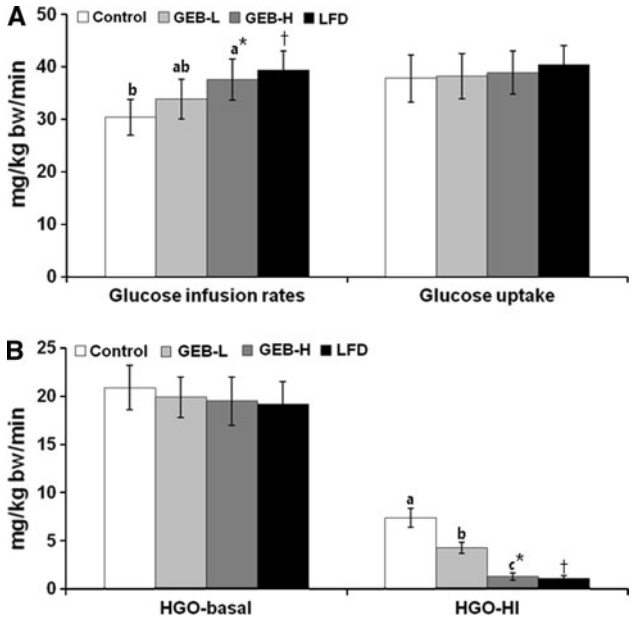

Fig. 3 Metabolic parameters under a euglycemic hyperinsulinemic clamp. Control, rats fed a high fat diet (HFD) with cellulose; GEB-L, rats fed HFD with 0.3 g/kg bw of *Gastrodia elata* Blume water extracts (GEB); GEB-H, rats fed with 1 g/kg bw of GEB; LFD, rats fed a low fat diet with 1 g/hg bw of cellulose. **a** Glucose infusion rates and glucose uptake. **b** Hepatic glucose output (HGO) at basal and hyperinsulinemic (HI) clamp states. *Each bar* represents mean ± SD ($n = 10$). * Significantly different among the groups of rats fed a high fat diet at $p < 0.05$. ^{a,b,c} Values on the *bar* with different superscripts were significantly different at $p < 0.05$. † Significant difference between the control and the low fat diet (LFD) group at $p < 0.05$

storage by 239 ± 31% from the basal level (Fig. 4). Vanillin and 4-hydroxybenzaldehyde increased insulin-stimulated glucose uptake by a mechanism other than PPAR-γ activation. They increased the expression of CPT-1 involved in transferring fatty acid to mitochondria for oxidation and decreased GPAT-1 expression to decrease fatty acid esterification for triglyceride synthesis (Table 3). Vanillin and 4-hydroxybenzaldehyde also decreased the expression of PPAR-γ and C/EBPα, which are the central regulators of adipogenesis and lipid storage in adipocytes but PPAR-γ expression was not significantly different.

Insulin-stimulated glucose uptake in 3T3-L1 adipocytes

Non-specific glucose uptake (no treatment of insulin) occurred at a rate of 6.3 ± 1.4 dpm/μg protein and was not affected by treatment with the major compounds of GEB, 4-hydroxybenzaldehyde, vanillin, 4-hydroxybenzyl alcohol or gastrodin (data not shown). Insulin increased glucose uptake in a dose-dependent manner up to 20 nM while the uptake slowed down after 10 nM and reached a plateau over 20 nM (data not shown). When 0.2 nM insulin plus DMSO (control), 4-hydroxybenzaldehyde, vanillin, 4-hydroxybenzyl alcohol or gastrodin was treated, vanillin and 4-hydroxybenzaldehyde treatment increased insulin-stimulated glucose uptake in a dose-dependent manner in comparison with the control (Fig. 5). This indicated that vanillin and 4-hydroxybenzaldehyde enhanced insulin action to reduce insulin resistance in adipocytes. However, it did not increase glucose uptake as much as the 20 nM insulin treatment and rosiglitazone treatment (Fig. 5).

Cytotoxicity of 3T3-L1 fibroblasts

IC50 represents a concentration of 50% cell cytotoxicity and, in general, over 100 μM of IC50 indicates the compound does not have cytotoxic activities against the cell line. All 4 compounds displayed over 100 μM of IC50 (4-hydroxybenzylaldehyde, 289 ± 42 μM; 4-hydroxybenzyl alcohol, 156 ± 33 μM; vanillin 357 ± 54 μM; gastrodin, 178 ± 31 μM), indicating they had no cytotoxic activities against 3T3-L1 fibroblasts. GEB did not worsen cell cytotoxicity to the extent of 300 μg/mL in 3T3-L1 adipocytes.

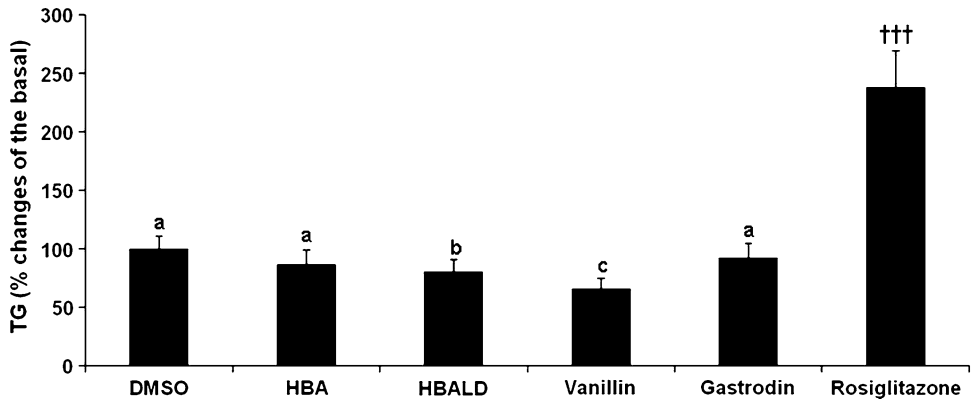

Fig. 4 Triglyceride accumulation in 3T3-L1 adipocytes. HBA, 4-hydroxybenzyl alcohol; HBALD, 4-hydroxybenzylaldehyde. Vehicle (DMSO), HBA, HBALD, vanillin, or gastrodin (20 μM), or rosiglitazone (2 μM) were treated. Each result is expressed as the mean ± SD ($n = 4$). ^{a,b,c} Indicate a significant difference at $p < 0.05$. †††Significantly different from the vehicle at $P < 0.001$

Table 3 The mRNA expression of genes regulating fatty acid metabolism in 3T3-L1 adipocytes

	Vehicle (DMSO)	HBA	HBALD	Vanillin	Gastrodin
Carnitine palmitoyltransferase-1	1.0 ± 0.5^{c}	1.1 ± 0.4^{c}	1.9 ± 0.6^{b}	2.5 ± 0.7^{a}	1.2 ± 0.5^{c}
Glycerol-3-phosphate acyl transferase-1	1.0 ± 0.4^{a}	0.9 ± 0.5^{a}	0.5 ± 0.3^{b}	0.3 ± 0.3^{b}	1.0 ± 0.5^{a}
CCAAT/enhancer binding protein-α	1.0 ± 0.4^{a}	1.1 ± 0.4^{a}	0.8 ± 0.3^{ab}	0.7 ± 0.3^{b}	1.0 ± 0.4^{a}
Peroxisome proliferator-activated receptor-γ	1.0 ± 0.5	1.0 ± 0.4	0.8 ± 0.4	0.7 ± 0.4	1.1 ± 0.4

Unit: fold of the vehicle

[a,b] Values on the same row with different superscripts were significantly different at $p < 0.05$

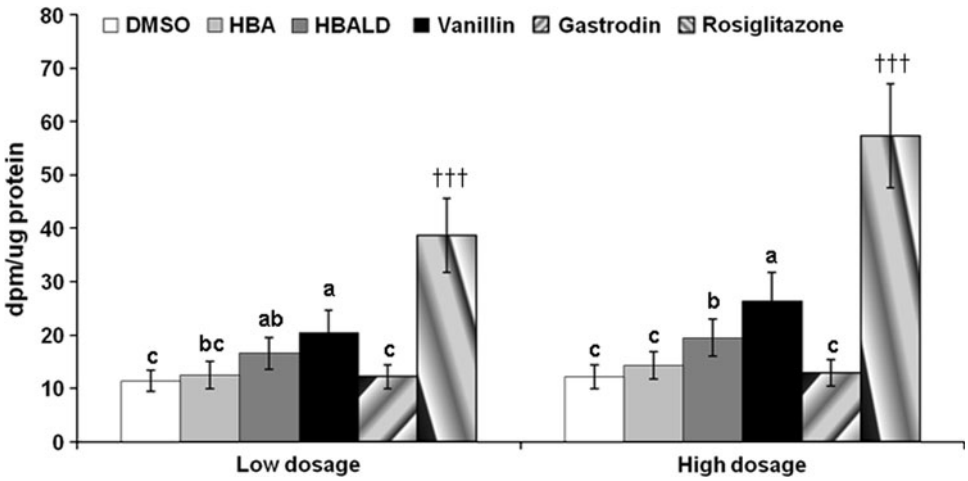

Fig. 5 Insulin-stimulated glucose uptake in 3T3-L1 adipocytes. HBA, 4-hydroxybenzyl alcohol; HBALD, 4-hydroxybenzylaldehyde. Vehicle (DMSO), HBA, HBALD, vanillin, or gastrodin (2 or 20 μM), or rosiglitazone (0.2 or 2 μM) were treated. Each result is expressed as the mean ± SD ($n = 4$). [a,b,c] Indicate a significant difference at $p < 0.05$. [†††] Significantly different from the vehicle at $p < 0.001$

Discussion

Recent studies have shown that the brain, especially the hypothalamus, plays an important role in regulating insulin resistance by modulating food intake and hepatic glucose metabolism [3, 4, 8]. GEB has been used to relieve symptoms associated with neurological ailments such as vertigo, general paralysis and epilepsy due to its anticonvulsant, analgesic and sedative effects. The water extract of GEB used in this study contained 4.5% of phenolic compounds. The major phenolic compounds are 4-hydroxybenzyl alcohol, 4-hydroxybenzylaldehyde, vanillin and gastrodin, and they are known to work in the brain after passing through the blood brain barrier [24]. Here, we can report for the first time that GEB treatment protects against insulin resistance by decreasing body fat by directly working on adipocytes and indirectly through the hypothalamus. In addition, vanillin and 4-hydroxybenzylaldehyde were the likely candidates for being responsible for decreasing fat accumulation in adipocytes. This finding suggests that GEB, especially vanillin and 4-hydroxybenzylaldehyde, can be applied as a remedy to improve insulin action and decrease fat accumulation.

The brain, especially the hypothalamus, is a major site for the integration of central and peripheral signals that regulate energy homeostasis [25]. Leptin is known to relay the status of fat stores and proper leptin signaling in the hypothalamus is critical to normal regulation of food intake and body weight [25]. Hypothalamic leptin receptor signaling is mediated by Janus kinase 2 → STAT3. Hypothalamic leptin resistance, possibly due to defective nutritional regulation of leptin receptor expression and/or reduced STAT3 signaling in the hypothalamus, contributes to the development of obesity associated with high fat feeding [25]. The present study showed that mRNA expression of Ob-Rb in the hypothalamus was higher in HFD than LFD. In spite of increased Ob-Rb expression, the phosphorylation of STAT-3 was attenuated. This indicated that HFD induced leptin resistance in the hypothalamus. GEB-H reversed the leptin resistance. In addition, hypothalamic AMPK activity is known to play an important role in regulating feeding behavior. Decreasing hypothalamic AMPK activity by expressing the dominant negative form of AMPK is associated with hypophagia, whereas increasing AMPK expression by expressing the constitutively active form of AMPK leads to the stimulation of food intake [3, 26]. Activation of AMPK in the hypothalamus is sufficient to increase food intake and body weight by increasing the mRNA expression of orexigenic neuropeptides, NPY and AgRP; suppression of hypothalamic AMPK activity is sufficient to decrease the expression of orexigenic neuropeptides [3, 26]. The present study also found that HFD increased AMPK phosphorylation in the hypothalamus and that GEB reversed the modulation in a

dose-dependent manner. In addition, mRNA expression of NPY and AgRP was elevated in HFD in comparison with LFD and GEB-H decreased the increased expression to the levels of LFD. This supported the fact that GEB regulates energy intake though attenuating leptin resistance and decreases the expression of orexigenic neuropeptides in the hypothalamus.

In addition to energy intake, energy expenditure plays an important role in maintaining body weight. As previous studies have shown, there was no significant difference in energy expenditure between HFD and LFD [27]. The present study showed the same result as Miller et al. [27] and HFD rats exhibited a slight decrease in RQ. As expected from the RQ values, HFD rats increased fat oxidation more than LFD rats in this study, which was associated with the increased availability of fats from the diet. However, both short- and long-term studies have shown that HFD does not stimulate its own fat oxidation when dietary fat is given in amounts above energy requirements [27, 28]. Excess dietary fat is stored in the adipose tissue and energy balance is closely correlated with fat balance, which is indicative of a shift in the oxidized fuels. Furthermore, during carbohydrate overfeeding more of the excess energy is oxidized and less is stored in the body compared with fat overfeeding. However, fat overfeeding has no effect on fat oxidation and the excessive energy stored as fat. As a result, a positive fat balance was much more pronounced after fat overfeeding compared with carbohydrate overfeeding. This study showed that GEB, possibly phenolic compounds, increased energy expenditure to prevent obesity in HFD rats in a dose-dependent manner. GEB also increased fat oxidation more than the control in a dose-dependent manner and decreased carbohydrate oxidation. This indicated that rats fed GEB induced body fat oxidation by increasing β-oxidation. The increase in fat oxidation is known to elevate serum adiponectin levels in animal studies and this is consistent with the results of the present study. Adiponectin is very highly expressed in adipose tissues, and it can increase β-oxidation of fatty acids in tissues and causes weight loss in mice [29]. The plasma adiponectin level in humans decreases with an increase in body weight or in conditions of obesity [30], which seems to be correlated with insulin resistance and hyperinsulinemia [31, 32]. As such, GEB exhibits a beneficial effect on improving the serum leptin and adiponectin levels, thus enhancing β-oxidation.

Insulin resistance refers to a decreased capacity to circulate insulin to regulate nutrient metabolism, especially glucose utilization [33]. Animals, including humans, produce hyperinsulinemia to normalize blood glucose levels in an insulin resistant state, but they do not develop diabetes as long as pancreatic β-cells produce enough insulin to compensate [33]. Animals become diabetic when insulin secretion does not compensate for insulin resistance. Insulin resistance is a failure of insulin action in the liver, skeletal muscles and fats that occurs in people who are obese or who have type 2 diabetes and causes the abnormal regulation of glucose metabolism. The mechanism of insulin resistance remains unclear but recent studies have shown that the hypothalamus has an integral role in regulating energy and glucose metabolism. GEB is known to work in the brain and regulate the levels of neurotransmitters such as GABA and dopamine, which are involved in regulating energy and glucose metabolism through the hypothalamus [5–7]. The major components of GEB, 4-hydroxybenzylaldehyde, has been revealed to work in the brain to maintain GABAergic activity by inhibiting GABA transaminase expression after global ischemic [34]. As a result, GEB help protect against obesity by directly working in adipocytes and indirectly in the hypothalamus. This study found that GEB attenuated the phosphorylation of AMPK in the hypothalamus, which is also known to be involved in energy and glucose metabolism. Thus, there is a strong possibility that GEB, via the hypothalamus, may relieve the adverse effect of HFD on energy and glucose metabolism.

PPAR-γ and C/EBPα, which are mainly found in adipose tissue, are the key transcription factors in adipogenesis and lipogenesis [22]. A PPAR-γ agonist such as thiazolidinedione, which is used to treat type 2 diabetes, induces differentiation of human preadipocytes and increases subcutaneous adiposity [35]. The present study also showed PPAR-γ agonist (rosiglitazone) increased triglyceride accumulation while vanillin and 4-hydroxybenzylaldehyde decreased triglyceride accumulation in 3T3-L1 adipocytes although they increased insulin-stimulated glucose uptake among the major GEB components. The reduction in adiposity by vanillin and 4-hydroxybenzylaldehyde was partly mediated by their suppression of C/EBPα and PPAR-γ, but PPAR-γ expression was not significantly reduced. In addition, the signal compounds attenuate the synthesis of glycerol lipids and augment fatty acid oxidation by downregulating GPAT-1 and upregulating CPT-1 expression, respectively, which results in a diminution of the cytosolic long-chain fatty acyl CoA [22]. This explained why they could reduce body fat by directly acting in adipocytes in vivo. Cell-based experiments showed that GEB decreased body fat possibly by increasing fat oxidation and attenuating fat synthesis. It is difficult to distinguish which mechanism was more important in reducing body fat. Since GEB increased energy expenditure through fat oxidation in rats and vanillin and 4-hydroxybenzylaldehyde, the components of GEB, did not decrease the expression of PPAR-γ expression, GEB might, according to the present study, reduce body fat mainly by fat oxidation.

In conclusion, the protective effect of GEB on body fat was associated with a decreased food intake via potentiating leptin signaling and attenuating the phosphorylation of AMPK in the hypothalamus. The decrease in body fat was also associated with increased energy expenditure, especially, in fat oxidation. In addition to energy homeostasis, insulin resistance disturbed by HFD was attenuated by GEB-H. Vanillin and 4-hydroxybenzaldehyde, the major components of GEB, decreased fat accumulation by increasing fatty acid oxidation and decreasing adipogenesis in 3T3-L1 adipocytes and enhanced insulin-stimulated glucose uptake. These results suggest that GEB supplementation, if used to treat obese men with diet-induced obesity, might relieve insulin resistance directly by decreasing fat accumulation in adipocytes mainly through activating fat oxidation and indirectly by decreasing food intake due to the activation of leptin signaling in the hypothalamus. It needs to be investigated in further studies whether vanillin and 4-hydroxybenzylaldehyde decrease fat accumulation and insulin resistance and which animal tissue is responsible for reducing them. The mechanism by which this action takes place also needs to be determined.

Acknowledgments This study was supported by a grant of the Korea Health 21 R&D Project, Ministry of Health & Welfare, Republic of Korea (HMP-08-A-0-80958). All authors have no conflicts of interest.

References

1. Lavie CJ, Milani RV, Ventura HO (2009) Obesity and cardiovascular disease: risk factor, paradox, and impact of weight loss. J Am Coll Cardiol 53:1925–1932
2. Figlewicz DP, Benoit SC (2009) Insulin, leptin, and food reward: update 2008. Am J Physiol Regul Integr Comp Physiol 296:R9–R19
3. Minokoshi Y, Alquier T, Furukawa N, Kim YB, Lee A, Xue B, Mu J, Foufelle F, Ferré P, Birnbaum MJ, Stuck BJ, Kahn BB (2004) AMP-kinase regulates food intake by responding to hormonal and nutrient signals in the hypothalamus. Nature 428:569–574
4. Ropelle ER, Pauli JR, Fernandes MF, Rocco SA, Marin RM, Morari J, Souza KK, Dias MM, Gomes-Marcondes MC, Gontijo JA, Franchini KG, Velloso LA, Saad MJ, Carvalheira JB (2008) A central role for neuronal AMP-activated protein kinase (AMPK) and mammalian target of rapamycin (mTOR) in high-protein diet-induced weight loss. Diabetes 57:594–605
5. Ojemanna LM, Nelsonb WL, Shinc DS, Rowed AO, Buchanane RA (2006) Tian ma, an ancient Chinese herb, offers new options for the treatment of epilepsy and other conditions. Epilepsy Behav 8:376–383
6. Yang XD, Zhu J, Yang R, Liu JP, Li L, Zhang HB (2007) Phenolic constituents from the rhizomes of Gastrodia elata. Nat Prod Res 21:180–186
7. Shuchang H, Qiao N, Piye N, Mingwei H, Xiaoshu S, Feng S, Sheng W, Opler M (2008) Protective effects of Gastrodia elata on aluminium-chloride-induced learning impairments and alterations of amino acid neurotransmitter release in adult rats. Restor Neurol Neurosci 26:467–473
8. Huang NK, Chern Y, Fang JM, Reed GW, Peters JC, Hill JO (2007) Neuroprotective principles from Gastrodia elata. J Nat Prod 70:571–574
9. Shuaib A, Murabit MA, Kanthan R, Howlett W, Wishart T (1996) The neuroprotective effects of gamma-vinyl GABA in transient global ischemia: a morphological study with early and delayed evaluations. Neurosci Lett 204:1–4
10. Gainetdinov RR (2007) Mesolimbic dopamine in obesity and diabetes. Am J Physiol Regul Integr Comp Physiol 293:R601–R602
11. Goudie AJ, Cooper GD, Halford JC (2005) Antipsychotic-induced weight gain. Diabetes Obes Metab 7:478–487
12. Tong Q, Ye CP, Jones JE, Elmquist JK, Lowell BB (2008) Synaptic release of GABA by AgRP neurons is required for normal regulation of energy balance. Nat Neurosci 11:998–1000
13. Bellido GG, Beta T (2009) Anthocyanin composition and oxygen radical scavenging capacity (ORAC) of milled and pearled purple, black, and common barley. J Agric Food Chem 57:1022–1028
14. American Institute of Nutrition (1993) AIN-93 purified diets for laboratory rodents: final report of the American Institute of Nutrition ad hoc writing committee on the reformulation of the AIN-76A rodent diet. J Nutr 123:1939–1951
15. Niwa H, Ogawa Y, Kido Y, Abe Y, Kobayashi M, Mori T, Tanaka T (1989) The rate of lipid oxidation in septic rat models. Jpn J Surg 19:439–445
16. Lusk G (1924) Analysis of the oxidation of mixtures of carbohydrate and fat. J Biol Chem 59:41
17. Labayen I, Forga L, Martinez JA (1999) Nutrient oxidation and metabolic rate as affected by meals containing different proportions of carbohydrate and fat, in healthy young women. Eur J Nutr 38:158–166
18. Frontoni S, Choi SB, Banduch D, Rossetti L (1991) In vivo insulin resistance induced by amylin primarily through inhibition of insulin-stimulated glycogen synthesis in skeletal muscle. Diabetes 40:568–573
19. Sebokova E, Klimes I, Moss R, Stolba P, Wiersma MM, Mitkova A (1993) Muscle GLUT 4 protein levels and impaired triglyceride metabolism in streptozotocin diabetic rats. Effect of a high sucrose diet and fish oil supplementation. Ann NY Acad Sci 683:218–227
20. Kim JK, Kim YJ, Fillmore JJ, Chen Y, Moore I, Lee J, Yuan M, Li ZW, Karin M, Perret P, Shoelson SE, Shulman GI (2001) Prevention of fat-induced insulin resistance by salicylate. J Clin Invest 108:437–446
21. Park S, Hong SM, Ahn IS (2009) Long-term ICV infusion of insulin, but not glucose, modulates body weight and hepatic insulin sensitivity through modifying hypothalamic insulin signaling pathway in type 2 diabetic rats. Neuroendocrinology 89:387–399
22. Ejaz A, Wu D, Kwan P, Meydani M (2009) Curcumin inhibits adipogenesis in 3T3–L1 adipocytes and angiogenesis and obesity in C57/BL mice. J Nutr 139:919–925
23. Park S, Ahn IS, Kwon DY, Ko BS, Jun WK (2008) Ginsenoside Rb1 and Rg1 suppress triacylglycerol accumulation in 3T3–L1 adipocytes and enhance β-cell insulin secretion and viability in Min6 cells via PKA dependent pathways. Biosci Biotechnol Biochem 72:2815–2823
24. Lin LC, Chen YF, Lee WC, Wu YT, Tsai TH (2008) Pharmacokinetics of gastrodin and its metabolite p-hydroxybenzyl alcohol in rat blood, brain and bile by microdialysis coupled to LC-MS/MS. J Pharm Biomed Anal 48:909–917
25. Sahu A (2004) Minireview: a hypothalamic role in energy balance with special emphasis on leptin. Endocrinology 145:2613–2620
26. Xue B, Kahn BB (2006) AMPK integrates nutrient and hormonal signals to regulate food intake and energy balance through effects in the hypothalamus and peripheral tissues. J Physiol 574:73–83

27. Miller WC, Lindeman AK, Wallace J, Niederpruem M (1990) Diet composition, energy intake, and exercise in relation to body fat in men and women. Am J Clin Nutri 52:426–430
28. Torre-Villavazo I, Tovar AR, Ramos-Barragán VE, Cerbón-Cervantes MA, Torres N (2008) Soy protein ameliorates metabolic abnormalities in liver and adipose tissue of rats fed a high-fat diet. J Nutr 138:462–468
29. Fruebis J, Tsao TS, Javorschi S, Ebbets-Reed D, Lodish HF (2001) Proteolytic cleavage product of 30-kDa adipocyte complement-related protein increases fatty acid oxidation in muscle and causes weight loss in mice. Proc Natl Acad Sci USA 98:2005–2010
30. Arita Y, Kihara S, Ouchi N, Takahashi M, Maeda K, Miyagawa J, Hotta K, Shimomura I, Nakamura T, Miyaoka K, Kuriyama H, Nishida M, Yamashita S, Okubo K, Matsubara K, Muraguchi M, Ohmoto Y, Funahashi T, Matsuzawa Y (1999) Paradoxical decrease of an adipose-specific protein, adiponectin, in obesity. Biochem Biophys Res Commun 257:79–83
31. Hotta K, Funahashi T, Arita Y, Takahashi M, Matsuda M, Okamoto Y, Iwahashi H, Kuriyama H, Ouchi N, Maeda K, Nishida M, Kihara S, Sakai N, Nakajima T, Hasegawa K, Muraguchi M, Ohmoto Y, Nakamura T, Yamashita S, Hanafusa T, Matsuzawa Y (2000) Plasma concentrations of a novel, adipose-specific protein, adiponectin, in type 2 diabetic patients. Arterioscler Thromb Vasc Biol 20:1595–1599
32. Hotta K, Funahashi T, Bodkin NL, Ortmeyer HK, Arita Y, Hansen BC, Matsuzawa Y (2001) Circulating concentrations of the adipocyte protein adiponectin are decreased in parallel with reduced insulin sensitivity during the progression to type 2 diabetes in rhesus monkeys. Diabetes 50:1126–1133
33. DeFronzo RA (2004) Pathogenesis of type 2 diabetes mellitus. Med Clin North Am 88:787–835
34. Ha JH, Shin SM, Lee SK, Kim JS, Shin US, Huh K, Kim JA, Yong CS, Lee NJ, Lee DU (2001) In vitro effects of hydroxybenzaldehydes from Gastrodia elata and their analogues on GABAergic neurotransmission, and a structure-activity correlation. Planta Med 67:877–880
35. Gurnell M (2005) Peroxisome proliferator-activated receptor gamma and the regulation of adipocyte function: lessons from human genetic studies. Best Pract Res Clin Endocrinol Metab 19:501–523