Anti-Obesity Effects of *Boussingaulti gracilis* Miers var. *pseudobaselloides* Bailey via Activation of AMP-Activated Protein Kinase in 3T3-L1 Cells

Hana Kim and Se-Young Choung

Department of Hygienic Chemistry, College of Pharmacy, Kyung Hee University, Seoul, Korea.

ABSTRACT In a previous study, we demonstrated the anti-obesity and hypolipidemic effects of *Boussingaulti gracilis* Miers var. *pseudobaselloides* Bailey in high-fat diet-induced obese rats. The present study investigated the molecular mechanisms by which *B. gracilis* Miers var. *pseudobaselloides* Bailey ethanol extract (BGE) conferred antidifferentiation and anti-adipogenic effects in the 3T3-L1 preadipocyte differentiation model. BGE treatment significantly and dose-dependently suppressed lipid accumulation and down-regulated the expression of major transcription factors involved in adipogenesis, such as peroxisome proliferator-activated receptor- γ , CCAAT/enhancer binding protein α , sterol regulatory element-binding proteins, and their target genes. It is important that treatment with BGE increased phosphorylation of AMP-activated protein kinase (AMPK), which is one of the rate-limiting enzymes in the fatty acid synthesis pathway, and its direct downstream protein, acetyl-coenzyme A carboxylase. These results suggest that BGE may exert anti-adipogenic effects through regulation of AMPK activity and expression of genes involved in lipogenesis.

KEY WORDS: • AMP-activated protein kinase • antilipogenesis • anti-obesity • *Boussingaulti gracilis* Miers var. *pseudobaselloides* Bailey • β -oxidation

INTRODUCTION

OBESITY, WHICH IS CHARACTERIZED by an altered balance between energy intake and energy expenditure, is often associated with many additional health problems, including increased risk of insulin resistance, nonalcoholic fatty liver, atherosclerosis, degenerative disorders (e.g., dementia), immune-mediated disorders (e.g., asthma), and certain cancers.^{1–3} According to the World Health Organization, overweight and obesity are the fifth leading causes of global mortality, and at least 2.8 million adults die each year as a result of being overweight or obese.¹

Current medications for the treatment of obesity include mixed noradrenergic-serotonergic agents (sibutramine) and absorption-reducing agents (orlistat).^{4–6} However, their use is limited by their side effects. Sibutramine is known to increase blood pressure and may cause cardiac arrhythmias, constipation, and headache with minimum body weight loss.⁴ Furthermore, rimonabant, which is the first selective cannabinoid receptor CB1 blocker, has been officially withdrawn from the market because of its serious psychiatric side effects.⁵ It has also been reported that orlistat, the most commonly used medication to treat obesity, causes steatorrhea and deficiencies in lipid-soluble vitamins and

essential fatty acids.⁶ Thus, interest in natural products for the treatment of obesity is growing rapidly. Natural products derived from medicinal plants have been used as dietary supplements for body weight management in many countries.⁴ For example, resveratrol, a root extract from *Polygonum cuspidatum*, quercetin, rutin, and catechin substantially reduce body weight gain and fat deposition *in vivo* and *in vitro*.^{7–11}

Boussingaultia gracilis Miers var. *pseudobaselloides* Bailey is a South American species of ornamental succulent vine. The fresh leaves of Madeira vine are frequently used as vegetables in Taiwan and as folk medicine as a hypoglycemic agent, an anti-inflammatory analgesic, and a stomachic.¹² Recent studies have demonstrated that *B. gracilis* Miers var. *pseudobaselloides* Bailey ethanol extracts (BGEs) have inhibitory activities against spasmogen-induced contractions of the isolated gastric fundus¹³ and ethanol-induced gastric lesions¹⁴ in rats. In addition, hot water extracts of *B. gracilis* Miers var. *pseudobaselloides* Bailey have antiviral activities against herpes simplex virus and adenovirus type 3.¹⁵ It has also been reported that the butanolic fraction from *B. gracilis* Miers var. *pseudobaselloides* Bailey exhibited antidiabetic effects in alloxan-induced mice.¹⁶

Previously, we have shown that BGE has anti-obesity and hypolipidemic effects in an animal model.¹⁷ BGE treatment effectively rescues biochemical parameters, such as serum lipid and adiponectin levels, and expression of transcription factors related to lipid and glucose metabolism from obese rats. However, the molecular mechanism of obesity

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Address correspondence to: Se-Young Choung, Ph.D., Department of Pharmaceutical Biochemistry, College of Pharmacy, Kyung Hee University, Hoegi-dong, Dongdaemun-gu, Seoul 130-701, Republic of Korea, E-mail: sychoung@khu.ac.kr

inhibition by BGE remains unclear. Intrigued by our previous results, we further investigated the effect of BGE on the differentiation of 3T3-L1 cells and sought to understand the underlying cellular mechanism of BGE. In the current study, we conducted biochemical assays to determine the effect of BGE on lipid accumulation in 3T3-L1 preadipocytes. Reverse transcription (RT)–polymerase chain reaction (PCR) was used to explore whether BGE treatment affects the expression of genes related to lipid metabolism in the process of 3T3-L1 differentiation. Furthermore, we also demonstrate the critical effect of BGE on AMP-activated protein kinase (AMPK) activity, which is a key enzyme governing lipid metabolism.

MATERIALS AND METHODS

Preparation of crude extract

BGE was obtained from the Pharmaceutical Analysis Laboratory of the School of Pharmacy, Shenyang Pharmaceutical University, Shenyang, China. In brief, chopped dried leaves of *B. gracilis* Miers var. *pseudobaselloides* Bailey were extracted three times with a 10-fold volume of 70% fermentation ethanol at 85°C for 4 h. The resulting extract was filtered and then lyophilized to yield 22.5% (of the initial amount) powder, and the extracts were stored in a refrigerator (–30°C) for functional studies. The extract powder contained 29.3% crude protein, 2.5% crude fat, and 13.0% fiber, as well as 53.5 mg/g total flavonoid. The extract was analyzed by the Pharmaceutical Analysis Laboratory of the School of Pharmacy, Shenyang Pharmaceutical University.¹⁷

Materials

Dulbecco's modified Eagle's medium (DMEM) (catalog number SH30243.01), bovine calf serum (catalog number SH30626.03), fetal bovine serum (catalog number SH30396.03), and penicillin–streptomycin solution (catalog number SV30010) were purchased from HyClone Laboratories, Inc. (Logan, UT, USA). Protein assay reagent (protein assay dye reagent concentrate; catalog number 500-0006) was purchased from Bio-Rad (Hercules, CA, USA). Antibodies against phospho-AMPK (Thr172), acetyl-coenzyme A (CoA) carboxylase (ACC), and phospho-ACC (Ser79) were purchased from Cell Signaling Technology (Beverly, MA, USA). Antibodies against β -actin and AMPK were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Immobilon®-P transfer membranes were purchased from Millipore Corp. (Billerica, MA, USA). The easy-spin total RNA extraction kit was purchased from iNtRON (Seongnam, Korea). RT-PCR primers were synthesized by Bioneer (Daejeon, Korea). 6-[4-(2-Piperidin-1-yl-ethoxy)-phenyl]-3-pyridin-4-ylpyrazolo[1,5-*a*]-pyrimidine (compound C) was obtained from Calbiochem (Billerica, MA, USA).

Cell culture and sample treatment

3T3-L1 preadipocyte cells from the American Type Culture Collection (Manassas, VA, USA) were cultured at 37°C in a humidified atmosphere of 5% CO₂ in DMEM

containing 10% bovine calf serum and 1% penicillin–streptomycin solution. To induce differentiation, post-confluent 3T3-L1 preadipocytes (Day 0) were stimulated for 48 h with differentiation medium (DMEM containing 10% fetal bovine serum, 0.5 mM 3-isobutyl-1-methylxanthine, 1 μ M dexamethasone, and 5 μ g/mL insulin). After stimulation, cells were maintained in DMEM supplemented with 10% fetal bovine serum and 5 μ g/mL insulin. Cells were treated with various concentrations of BGE (10, 50, and 100 μ g/mL) and compound C (2 μ M) from Day 0.

Detection of lipid accumulation

3T3-L1 adipocytes (3×10^4 cells per well) were seeded into six-well culture plates. Two days after reaching confluence, cells were treated with various concentrations of BGE for 6 days. Free fatty acid (FFA) and triglyceride (TG) levels were determined enzymatically using commercial kits (Asan Diagnostics, Seoul, Korea). To analyze lipid content, the lipids were extracted by the method of Folch *et al.*¹⁸ FFAs were quantified by the acyl-CoA synthase–acyl-CoA oxidase method¹⁹ using a NEFA-HR kit (Wako Pure Chemical Industries, Osaka, Japan).

RT-PCR

The total RNA was isolated from 3T3-L1 adipocytes using an easy-spin total RNA extraction kit. RNA concentrations were determined using an Optizen 2120UV spectrophotometer (Mecasys, Daejeon, Korea). RT-PCR assays for target mRNA were performed using a Maxime RT-PCR PreMix kit (iNtRON) according to the manufacturer's protocol. Sequences of PCR primers are summarized in Table 1. PCR was carried out as follows: 5 min at 94°C, 30 cycles of (94°C for 1 min, 56°C for 1 min, and 72°C for 5 min), and

TABLE 1. PRIMER SEQUENCES

Gene description	Primer	Sequence (5'–3')
C/EBP α	F	CGCAAGAGCCGAGATAAAGC
	R	CACGGCRCAGCTGTTCCA
PPAR γ	F	CGCTGATGCACTGCCTATGA
	R	AGAGGTCCACAGAGCTGATTCC
SREBP-1	F	AAGGGCCAGGAGTGGGTAAAC
	R	CGCGCCGCGCCCCATTAGG
aP2	F	CTGGAAGACAGCTCCTCCTCG
	R	GCCTCTTCCTTTGGCTCATGC
FAS	F	CAGTTCAGCCATGAAGAGA
	R	TTTGCTGGCAAAGAGAACAC
ACC	F	TCTATTCCGGGTGACTTTC
	R	CTATCAGTCTGTCCAGCCC
GAPDH	F	ATAGAATTGAAGACCCAGAA
	R	CCACTTCTCCTCTATAAGTTTAA

ACC, acetyl-coenzyme A carboxylase; aP2, adipocyte protein 2; C/EBP α , CCAAT/enhancer binding protein α ; F, forward; FAS, fatty acid synthase; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; PPAR γ , peroxisome proliferator-activated receptor γ ; R, reverse; SREBP-1, sterol regulatory element binding protein-1.

a 5-min incubation at 72°C. The *glyceraldehyde 3-phosphate dehydrogenase* gene was used as an internal control.

Western blot analysis

3T3-L1 preadipocytes (3×10^4 cells per well) were seeded into six-well plates, cultured in differentiation medium for 7 days, then treated with or without sample, and incubated at 37°C for differentiation. After the medium was discarded, the adipocytes were rinsed twice with ice-cold phosphate-buffered saline and lysed in cell lysis buffer (80 mM Tris-HCl [pH 7.5], 600 mM NaCl, 100 mM EDTA, 100 mM NaF, and 100 mM Na_3VO_4) containing a protease inhibitor cocktail tablet. Total cell lysates were ultrasonically dispersed for 30 min and centrifuged at 10,000 g at 4°C for 5 min.

For the western immunoblotting, equal amounts of proteins were boiled for 5 min and chilled on ice. Cell lysates (30 μg) were separated by 8% sodium dodecyl sulfate–polyacrylamide gel electrophoresis and electrophoretically transferred to an Immobilon-P transfer membrane. Total protein concentration was determined by the Bradford assay (Bio-Rad). The membranes were blocked by 5% skim milk in Tris-buffered saline with Tween-20 (TBST) (10 mM Tris [pH 7.5], 100 mM NaCl, and 0.1% Tween-20), and then blots were incubated with 1:1000-diluted primary antibody overnight. After being washed with TBST, the membranes were probed with 1:5000-diluted secondary antibody for 1 h and washed with TBST several times. The bound antibody was visualized using an enhanced chemiluminescence detection system (Fuji Film, Tokyo, Japan). The band densities were measured with Science lab version 2.0 software (Fuji Film).

Statistical analysis

Results are expressed as mean $\pm$ SD values of triplicate experiments. Differences between groups were analyzed by Student's *t* test, and *P* values of $<.05$ were considered statistically significant.

RESULTS

BGE suppresses lipid accumulation in 3T3-L1 cells

We recently reported that BGE reduced the increase of body weight, fat pad weights, and serum and hepatic lipid levels induced by feeding high-fat diets to rats.¹⁷ To understand the mode of action of BGE on 3T3-L1 preadipocyte differentiation, we first performed a cell viability assay using 3T3-L1 cells treated with BGE to determine the optimal concentration of BGE for assay. BGE treatment up to 300 $\mu\text{g}/\text{mL}$ showed no significant effect on cell viability (data not shown). To assess the effect of BGE on lipid accumulation during differentiation, we measured TG and FFA levels in lipid droplets of 3T3-L1 preadipocytes treated with different concentrations of BGE (10, 50, and 100 $\mu\text{g}/\text{mL}$) during differentiation. The TG content of intracellular fat droplets was attenuated by BGE treatment in a dose-dependent manner (Fig. 1A). We also found that the

FIG. 1. Effects of *B. gracilis* Miers var. *pseudobaselloides* Bailey ethanol extract (BGE) on lipid accumulation in 3T3-L1 preadipocytes during differentiation: (A) triglycerides and (B) free fatty acids. 3T3-L1 preadipocytes were differentiated into adipocytes in the absence or presence of various concentrations of BGE for 6 days. At the end of differentiation, cellular triglyceride and total free fatty acid levels were measured. Data are mean $\pm$ SD values ($n=3$). ** $P < .01$, *** $P < .001$ versus control (con) cells.

total FFA level was remarkably lowered by BGE treatment in a dose-dependent manner (Fig. 1B). BGE treatment with 100 $\mu\text{g}/\text{mL}$ resulted in a 31% decrease in FFA in the cells, suggesting that BGE treatment can reduce lipid accumulation in 3T3-L1 cells undergoing differentiation to adipocytes.

Effects of BGE on expression of transcription factors related to 3T3-L1 differentiation

Given that BGE treatment lowers lipid accumulation in 3T3-L1 preadipocytes during differentiation, we next determined whether BGE affects the expression of genes involved in lipid accumulation and differentiation. We assessed differentiation of 3T3-L1 cells in the presence or absence of BGE and measured the expression levels of adipogenic genes such as those for peroxisome proliferator-activated receptor- γ (PPAR γ) and CCAAT/enhancer binding protein α (C/EBP α).²⁰ As expected, BGE treatment reduced both PPAR γ and C/EBP α expressions to 37% and 47%, respectively. Moreover, BGE treatment efficiently decreased mRNA levels

FIG. 2. BGE affects the expression of genes related to differentiation. 3T3-L1 cells were treated with BGE during differentiation. Total RNA was extracted at the end of differentiation (Day 6). (A) Reverse transcription–polymerase chain reaction analysis of PPAR γ and C/EBP α expression was performed in 3T3-L1 cells treated with BGE. (B) Relative fold intensities were analyzed using band densities obtained from reverse transcription–polymerase chain reaction. All RNA levels were normalized to that of *GAPDH* mRNA. Data are mean $\pm$ SD values from at least three independent experiments. Experiments were repeated three times with similar results, and a representative result is shown. * $P < .05$, *** $P < .001$ versus control cells.

of C/EBP α even at the lowest concentration of BGE (10 μ g/mL) (Fig. 2).

BGE inhibits the expression of sterol regulatory element-binding protein-1c and its target genes

Based on our finding that BGE treatment inhibits the levels of cellular TG and FFA, we further investigated whether the hypolipidemic effect of BGE involves regulation of genes related to lipid biosynthesis. Sterol regulatory element-binding proteins (SREBPs) are key transcription factors that bind to the sterol regulatory element DNA sequence and regulate the expression of target genes that are involved in biosynthesis of cholesterol and fatty acids.²⁰ Fatty acid synthase (FAS) and ACC are transcriptionally regulated by SREBP-1c.²¹ The gene for adipocyte protein 2 (aP2) (also known as FABP4), a PPAR γ -responsive gene, is also a key regulator of fatty acid synthesis and lipid metabolism.²² The *SREBP-1c* gene and its target gene, *FAS*, were down-regulated in 3T3-L1 cells treated with BGE (Fig. 3). Because SREBP-1c is an essential enzyme for fatty acid synthesis, it is likely that the ability of differentiated 3T3-L1 adipocytes to synthesize fatty acid was impaired by BGE treatment, which is consistent with our finding from the analysis of fatty acid (Fig. 1). Similarly, ACC and aP2 expressions were also affected by BGE treatment. These data suggest that BGE regulates both the SREBP signaling

FIG. 3. Effect of BGE on expression of genes related to lipid biosynthesis in 3T3-L cells. 3T3-L1 preadipocytes were treated with BGE during differentiation. Total RNA was extracted at the end of differentiation (Day 6). (A) Levels of *SREBP1c*, *FAS*, *ACC*, and *aP2* mRNA were determined by reverse transcription–polymerase chain reaction analysis. *GAPDH* mRNA was used as an internal control. (B) Relative fold intensities were analyzed using band densities obtained from reverse transcription–polymerase chain reaction. All RNA levels were normalized to that of *GAPDH* mRNA. Data are mean $\pm$ SD values from at least three independent experiments. * $P < .05$, ** $P < .01$ versus control cells.

pathway and the fatty acid oxidation pathway at the transcription level.

Activation of the AMPK pathway by BGE treatment

Recently, it has been shown that BGE treatment increases AMPK expression in adipose tissue of high-fat diet-induced obese rats.¹⁷ Here, we sought to determine the effect of BGE on the activity of AMPK, which plays a crucial role in lipid metabolism.²³ As shown in Figure 4A, 3T3-L1 preadipocytes treated with BGE showed increased levels of phosphorylated AMPK and ACC compared with control preparations, suggesting that BGE promotes fatty acid oxidation by activating AMPK.

To confirm the effect of BGE on phosphorylation of AMPK, we measured phosphorylated AMPK or ACC levels in the presence of compound C, which is an AMPK inhibitor (Fig. 4B). Consistent with the previous report,¹⁷ compound C treatment resulted in decreased AMPK phosphorylation (Fig. 4B, lane 2). It is interesting that BGE treatment resulted in a 159% increase in AMPK phosphorylation (Fig. 4B, lanes 1 and 3), and the effect of BGE on phosphorylation of AMPK was compromised by compound C treatment.

Finally, to test whether BGE inhibition of TG accumulation involves AMPK phosphorylation, lipid contents in fat droplet were measured in BGE-treated cells in the presence

FIG. 4. Effect of BGE on phosphorylation of AMP-activated protein kinase (AMPK) and ACC. BGE treatment enhanced phosphorylation in a dose-dependent manner. **(A)** During differentiation, cells were treated with various concentration of BGE or vehicle (con). Relative fold intensities were analyzed using band densities obtained from western immunoblotting. All levels were normalized to that of β -actin. P-ACC, phosphorylated ACC; P-AMPK, phosphorylated AMPK. **(B)** Cells were treated with 100 μ g/mL BGE in the absence or presence of compound C (2 μ M) at the end of differentiation. Total proteins were extracted by cell lysis buffer and resolved by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. **(C)** 3T3-L1 cells were treated with BGE in the presence (CC) or absence (con) of compound C (2 μ M). Lipid contents were measured on Day 6. Data are mean $\pm$ SD values from at least three independent experiments. * P < .05, ** P < .01, *** P < .001 versus control cells.

or absence of compound C. As shown in Figure 4C, BGE treatment reduced the TG level by 34%, and the effect of BGE on the TG level was attenuated by compound C treatment, suggesting that activation of AMPK might be the molecular mechanism mediating the anti-obesity effects of BGE.

DISCUSSION

BGE has been used for the treatment of diabetes, inflammation, liver diseases, gastric pain, and cough in many countries.²⁴ Chemical characterization of BGE has been conducted and led to isolation of several sapogenins, including larrea-genin A, 3 β -hydroxy-30-noroleana-12,19-dien-28-oic acid and its ethyl ester, ursolic acid, 28-ethylhydrogen-3 β -hydroxylean-12-ene-28,29-dioate, and 3 β -hydroxy-30-noroleana-12,18-dien-29-oic acid.²⁵ Our recent studies have demonstrated that obese rats treated with BGE for 6 weeks showed decreased body weight gain and serum and hepatic lipid levels compared with rats in the control group.¹⁷ The expression of adipogenesis-related transcription factors, such as PPAR γ and C/EBP α , was significantly decreased by BGE administration in obese rats. In addition, BGE treatment decreased blood glucose and lipid levels, indicating that BGE affects lipid metabolism in obese rats.

An initial finding from the current study is that BGE inhibits the lipid accumulation of 3T3-L1 preadipocytes. During adipocyte differentiation, TG and FFA accumulations in BGE-treated 3T3-L1 cells were lower than those in the nontreated group (Fig. 1). Adipocyte differentiation is regulated by transcriptional factors such as C/EBP α and PPAR γ , which are able to coordinate the expression of genes related to adipocyte differentiation.²⁶ These proteins participate in a transcriptional cascade that controls the expression of several genes that are essential for lipid accumulation in adipocytes during the process of differentiation.^{26,27} We observed that expression of both PPAR γ and C/EBP α was greatly reduced by BGE treatment (Fig. 2), suggesting that the inhibitory effect of BGE on differentiation of preadipocytes might be due to the inhibition of PPAR γ and C/EBP α expression.

Fatty acid metabolism is stringently controlled to balance synthesis (e.g., lipogenesis) and degradation (e.g., β -oxidation) in response to various physiological signals. SREBPs, transcription factors that have been associated with lipogenesis, regulate the expression of ACC, FAS, and acyl-CoA synthase.²⁷ *SREBP-1c* mRNA expression was decreased by BGE treatment. Consequently, the expression of its downstream target genes such as *FAS* and *ACC* was markedly suppressed by BGE (Fig. 3), suggesting that BGE can effectively reduce lipid accumulation by inhibiting lipid synthesis through the SREBP-1c pathway.

AMPK is a "cellular fuel gauge" and acts to simultaneously shut down ATP-consuming biosynthetic processes and facilitate ATP-producing catabolic processes during periods of metabolic stress,²⁸ leading to rapid changes in the control of fatty acid metabolism. AMPK stimulation of fatty acid metabolism occurs as a result of AMPK phosphoryla-

tion and inhibition of ACC.^{22,23,26–28} Our results demonstrate that BGE activates the AMPK pathway to accelerate β -oxidation of FFA. 3T3-L1 cells treated with BGE showed an enhanced phospho-AMPK level compared with the nontreated group (Fig. 4A). Because activity of ACC, which is responsible for producing malonyl-CoA in fatty acid synthesis, is negatively regulated by AMPK, we next examined whether BGE treatment can affect ACC activity. As expected, BGE treatment also increased phosphorylation of ACC (Fig. 4A). Moreover, the increased levels of phosphorylated AMPK and ACC were significantly attenuated by compound C, which is an AMPK inhibitor (Fig. 4B). Thus, it appears that BGE stimulates β -oxidation of FFA by activation of the AMPK pathway.

Previous studies have shown that activation of AMPK in adipocytes leads to decreased fatty acid uptake, decreased TG accumulation, and increased fatty acid oxidation. To confirm the effect of BGE on lipid accumulation via the AMPK pathway in 3T3-L1 preadipocyte differentiation, cells were treated with compound C, and cellular TG levels were monitored. Consistent with western immunoblotting results, the effect of BGE on TG levels was compromised by compound C treatment (Fig. 4C), indicating that activation of AMPK is one of the major molecular mechanisms of BGE in its anti-adipogenic effect.

Recent results showed that the AMPK α subunit interacts with and directly phosphorylates SREBP-1c and SREBP-2, and phosphorylation of SREBP-1c by AMPK represses *de novo* lipogenesis in hepatocytes. In this previous study, *SREBP-1c* mRNA was not sensitive to either hyperinsulinemia or S17834, a synthetic polyphenol, and AMPK activation suppressed the accumulation of nuclear SREBP-1c and -2, subsequently repressing expression of their target genes.²⁹ Thus, it is noteworthy that BGE treatment resulted in a decrease of *SREBP-1c* mRNA level as well as activation of AMPK (Figs. 2B and 3A), suggesting that BGE can efficiently modulate two major regulatory mechanisms of lipid metabolism (the AMPK pathway and the SREBP-1c pathway) not only at the protein level, but also at the transcriptional level.

In conclusion, BGE has anti-adipogenic effects in 3T3-L1 cells by inhibition of lipid synthesis and stimulation of β -oxidation of fatty acid, suggesting that BGE exerts beneficial effects on diabetes.

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AUTHOR DISCLOSURE STATEMENT

No competing financial interests exist.

REFERENCES

1. WHO Expert Consultation: Appropriate body-mass index for Asian populations and its implications for policy and intervention strategies. *Lancet* 2004;363:157–163.

2. Unger RH: Lipid overload and overflow: metabolic trauma and the metabolic syndrome. *Trends Endocrinol Metab* 2003;14:398–403.
3. Wellen KE, Hotamisligil GS: Inflammation, stress, and diabetes. *J Clin Invest* 2005;115:1111–1119.
4. Luque CA, Rey JA: Sibutramine: a serotonin-norepinephrine reuptake-inhibitor for the treatment of obesity. *Ann Pharmacother* 1999; 33:968–978.
5. Leite CE, Mocelin CA, Petersen GO, Leal MB, Thiesen FV: Rimobant: an antagonist drug of the endocannabinoid system for the treatment of obesity. *Pharmacol Rep* 2009;61:217–224.
6. Heck AM, Yanovski JA, Calis KA: Orlistat, a new lipase inhibitor for the management of obesity. *Pharmacotherapy* 2000; 20:270–279.
7. Lagouge M, Argmann C, Grhart-Hines Z, Meziane H, Lerin C, Daussin F, Messadeq N, Milne J, Lambert P, Elliott P, Geny B, Laakso M, Puigserver P, Auwerx J: Resveratrol improves mitochondrial function and protects against metabolic disease by activating SIRT1 and PGC-1 α . *Cell* 2006;127:1–14.
8. Shang J, Chen LL, Xiao FX: [Resveratrol improves high-fat induced nonalcoholic fatty liver in rats]. *Zhonghua Gan Zang Bing Za Zhi* 2008;16:616–619.
9. Shang J, Chen LL, Xiao FX, Sun H, Ding HC, Xiao H: Resveratrol improves non-alcoholic fatty liver disease by activating AMP-activated protein kinase. *Acta Pharmacol Sin* 2008;29:698–706.
10. Ha DT, Trung TN, Phuong TT, Yim N, Chen QC, Bae K: The selected flavonol glycoside derived from Sophorae Flos improves glucose uptake and inhibits adipocyte differentiation via activation AMPK in 3T3-L1 cells. *Bioorg Med Chem Lett* 2010;20: 6076–6081.
11. Rayalam S, Della-Fera MA, Baile CA: Phytochemicals and regulation of the adipocyte life cycle. *J Nutr Biochem* 2008; 19:717–726.
12. Kan WS: *Pharmaceutical Botany*. National Research Institute of Chinese Medicine, Taipei, 1986.
13. Lin WC, Wu SC, Kuo SC: Inhibitory effects of ethanolic extracts of *Boussingaultia gracilis* on the spasmonogen-induced contractions of the rat isolated gastric fundus. *J Ethnopharmacol* 1997;56:89–93.
14. Lin WC, Wu SC, Kuo SC: Prevention of ethanol-induced gastric lesions in rats by ethanolic extracts of *Boussingaultia gracilis*. *Chin Pharm J* 1996;48:259–270.
15. Chiang LC, Cheng HY, Liu MC, Chiang W, Lin CC: In vitro anti-herpes simplex viruses and anti-adenoviruses activity of twelve traditional used medicinal plants in Taiwan. *Biol Pharm Bull* 2003;26:1600–1604.
16. Lin HY: Studies on the chemical constituents and antihyperglycemic effect of *Boussingaultia gracilis* [Master's thesis]. China Medical College, Taichung, Taiwan, 1986.
17. Wang L, Bang CY, Choung SY: Anti-obesity and hypolipidemic effects of *Boussingaultia gracilis* Miers var. *pseudobaselloides* Bailey in obese rats. *J Med Food* 2011;14:17–25.
18. Folch J, Lees M, Sloane Stanley GH: A simple method for the isolation and purification of total lipides from animal tissues. *J Biol Chem* 1957;226:497–509.
19. Shimizu S, Yasui K, Tani Y, Yamada H: Acyl-CoA oxidase from *Candida tropicalis*. *Biochem Biophys Res Commun* 1979;91: 108–113.
20. Eberlé D, Hegarty B, Bossard P, Ferré P, Foufelle F: SREBP transcription factors: master regulators of lipid homeostasis. *Biochimie* 2004;86:839–848.
21. Wilentz RE, Witters LA, Pizer ES: Lipogenic enzymes fatty acid synthase and acetyl-coenzyme A carboxylase are co expressed with sterol regulatory element binding protein and Ki-67 in fetal tissues. *Pediatr Dev Pathol* 2000;3:525–531.
22. Picard F, Kurtev M, Chung N, Topark-Ngarm A, Senawong T, Machado de Oliveira R, Leid M, McBurney MW, Guarente L: Sirt1 promotes fat mobilization in white adipocytes by repressing PPAR- γ . *Nature* 2004;429:771–776.
23. Ruderman N, Prentki M: AMP kinase and malonyl-CoA: targets for therapy of the metabolic syndrome. *Nat Rev Drug Discov* 2004;3:340–351.
24. Chiu NY, Chang KH: *The Illustrated Medicinal Plants of Taiwan*, Vol. 3 (Mandarin Chinese edition). SMG Publishing Inc., Taipei, 1995.
25. Lin HY, Kuo SC, Chao PD, Lin TD: A new sapogenin from *Boussingaultia gracilis*. *J Nat Prod* 1988;51:797–798.
26. Rosen ED, Spiegelman BM: Molecular regulation of adipogenesis. *Annu Rev Cell Dev Biol* 2000;16:145–171.
27. Kolehmainen M, Vidal H, Alhava E, Uusitupa MI: Sterol regulatory element binding protein 1c (SREBP-1c) expression in human obesity. *Obes Res* 2001;9:706–712.
28. Hardie DG, Carling D: The AMP-activated protein kinase—fuel gauge of the mammalian cell? *Eur J Biochem* 1997;246: 259–273.
29. Li Y, Xu S, Mihaylova MM, Zheng B, Hou X, Jiang B, Park O, Luo Z, Lefai E, Shyy JY, Gao B, Wierzbicki M, Verbeuren TJ, Shaw RJ, Cohen RA, Zang M: AMPK phosphorylates and inhibits SREBP activity to attenuate hepatic steatosis and atherosclerosis in diet-induced insulin-resistant mice. *Cell Metab* 2011;13:376–388.