JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

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Black Soybean Seed Coat Extract Ameliorates Hyperglycemia and Insulin Sensitivity via the Activation of AMP-Activated Protein Kinase in Diabetic Mice

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Supporting Information

ABSTRACT: Black soybean seed coat has abundant levels of polyphenols such as anthocyanins (cyanidin 3-glucoside; C3G) and procyanidins (PCs). This study found that dietary black soybean seed coat extract (BE) ameliorates hyperglycemia and insulin sensitivity via the activation of AMP-activated protein kinase (AMPK) in type 2 diabetic mice. Dietary BE significantly reduced blood glucose levels and enhanced insulin sensitivity. AMPK was activated in the skeletal muscle and liver of diabetic mice fed BE. This activation was accompanied by the up-regulation of glucose transporter 4 in skeletal muscle and the down-regulation of gluconeogenesis in the liver. These changes resulted in improved hyperglycemia and insulin sensitivity in type 2 diabetic mice. In vitro studies using L6 myotubes showed that C3G and PCs significantly induced AMPK activation and enhanced glucose uptake into the cells.

KEYWORDS: *black soybean, diabetes, anthocyanin, procyanidins*

INTRODUCTION

Type 2 diabetes is a serious public health problem worldwide. It is strongly associated with coronary heart disease, atherosclerosis, and certain cancers.[1] Recently, considerable attention has been focused on dietary constituents that may be beneficial for the prevention and treatment of diabetes. Therapeutic regimens that target the regulation of adipocytes or the functions of other cells are being used due to their ability to improve insulin sensitivity and glucose homeostasis. Thiazolidinediones, for example, are a class of synthetic peroxisome proliferator-activated receptor (PPAR) ligands that are used as antidiabetic drugs due to their effects on adipocyte differentiation and adipocyte gene activation.[2,3] Another drug, metformin, ameliorates hyperglycemia without stimulating insulin secretion.[4,5] The administration of metformin leads to the activation of AMP-activated protein kinase (AMPK), which has been suggested as a potential target for the treatment of type 2 diabetes.[4,5] Although some drugs have been used as therapeutic regimens for obesity-related metabolic diseases, there is little evidence that food factors themselves can modulate insulin sensitivity directly.

Black soybean (*Glycine max* L.), a type of soybean with a black seed coat, has been used widely as a nutritionally rich food in Asia. Soybean contains many types of phytochemical compounds. For example, soy isoflavones significantly inhibit adipogenesis in 3T3-L1 adipocytes,[6,7] inhibit the down-regulation of adiponectin expression,[8] decrease adipose deposition,[9] prevent nonalcoholic fatty liver disease,[10] and ameliorate insulin sensitivity by modulating hepatic lipid and glucose metabolic enzymes via AMPK activation.[11] Black soybean also contains isoflavones; however, its seed coat, which is different from the coat of yellow soybean, contains abundant levels of polyphenols such as anthocyanins (cyanidin 3-glucoside; C3G) and procyanidins (PCs), although it contains low levels of isoflavones.[12,13] Some studies have suggested that anthocyanins or PCs can function as antiobesity and antidiabetic compounds. For example, C3G significantly suppresses the development of high-fat diet-induced obesity, modulates the expression of adipocytokines in human adipocytes,[14,15] and ameliorates hyperglycemia and improves insulin sensitivity in type 2 diabetic mice.[14,16,17] Grape-seed derived PCs suppress hyperglycemia in streptozotocin-induced type 1 diabetic rats[18] and modulate insulin signaling pathways in vitro.[19] Recently, we demonstrated that dietary black soybean seed coat extract (BE), which is rich in anthocyanins and PCs, significantly decreases the accumulation of body fat in high-fat diet-induced obese mice.[20]

The findings of these studies raise the question of whether dietary BE can ameliorate hyperglycemia and insulin sensitivity in a model of type 2 diabetes. Moreover, the molecular action of BE responsible for the amelioration of hyperglycemia and enhancement of insulin sensitivity is still not fully understood in vivo. Therefore, the present study was designed to examine whether the administration of BE reduces blood glucose levels and improves insulin sensitivity in type 2 diabetic mice. Also,

Received: March 19, 2013
Revised: May 12, 2013
Accepted: May 19, 2013
Published: May 20, 2013

we identified the antidiabetic compounds of BE using in vitro studies.

MATERIALS AND METHODS

Chemicals. The BE (Chrono-Care, Fujicco Co., Ltd., Kobe, Japan) was prepared as follows: black soybeans (local variety) were harvested in autumn 2006 in the Inner Mongolia region of China.[20] The extracts of black soybean hulls obtained with acidic water and ethanol were purified using absorbent resin and powdered by spray-drying. The polyphenol content of BE is shown in Table 1. The composition of PCs in BE was analyzed using HPLC.[20] The chemical structures of C3G and PCs are shown in Figure 1. Anti-glucose transporter (GLUT) 4, anti-GLUT1 and uncoupling protein (UCP) 1 antibodies were obtained from Abcam (Tokyo, Japan). Antiphospho-AMPKα (Thr172), anti-AMPKα, anti-phospho-Akt (Ser473), and Akt antibodies were obtained from Cell Signaling Technology (Beverly, MA, USA). Anti-glyceraldehyde-3-phosphate dehydrogenase (GAPDH) antibody was obtained from Life Technologies, Japan (Tokyo, Japan).

Table 1. Composition of Polyphenols in BE

ingredient	BE (%)
C3G	9.0
total procyanidins	35.5
monomer (PC1; epicatechin)	5.2
dimer (PC2)	4.7
trimer (PC3)	3.2
tetramer (PC4−1, PC4−2)	1.5
total polyphenols	68.7
total isoflavones	0.5

Animals and Diets. Male KK-A^y mice (SPF), age 4 weeks (n = 16) (CLEA Japan, Inc., Tokyo, Japan), were used[21] and maintained at 23 ± 3 °C under an automatic lighting schedule (8:00 a.m.−8:00 p.m. light). The mice were allowed free access to water and a laboratory diet (CE-2; CLEA Japan, Inc.) containing protein, 247 g/kg; fat, 47 g/kg; non-nitrogenous substances, 517 g/kg; crude fiber, 34 g/kg; crude ash, 68 g/kg; energy, 15.1 MJ/kg; and sufficient minerals and vitamins to maintain the health of the mice for 1 week. After 1 week under these conditions, the 5-week-old mice were then divided into two groups (n = 8) and assigned to the control (CE-2) or BE diet (CE-2 supplemented with BE; 22.0 g of BE/kg diet) containing protein, 244 g/kg; fat, 45 g/kg; non-nitrogenous substances, 526 g/kg; crude fiber, 35 g/kg; crude ash, 66 g/kg; and energy, 15.2 MJ/kg. The BE diet was replaced every day to avoid depletion of the polyphenols. The dose of BE was based on a preliminary experiment to show that the supplementation level did not affect food intake. This experimental design was approved by the Animal Experiment Committee, Chubu University, and the care and treatment of mice were in accordance with their guidelines (Permissions 2010034, 2310017).

Measurement of Serum Glucose Concentration. The experimental diets were withdrawn from all mice at 8:00 a.m., and blood was obtained from the tail vein 1 h later. The collected blood was kept at room temperature for 5 min for coagulation. Serum was isolated from the coagulated blood by centrifugation at 1600g for 15 min at 4 °C. The serum glucose concentration was assayed immediately using a commercial assay kit according to the

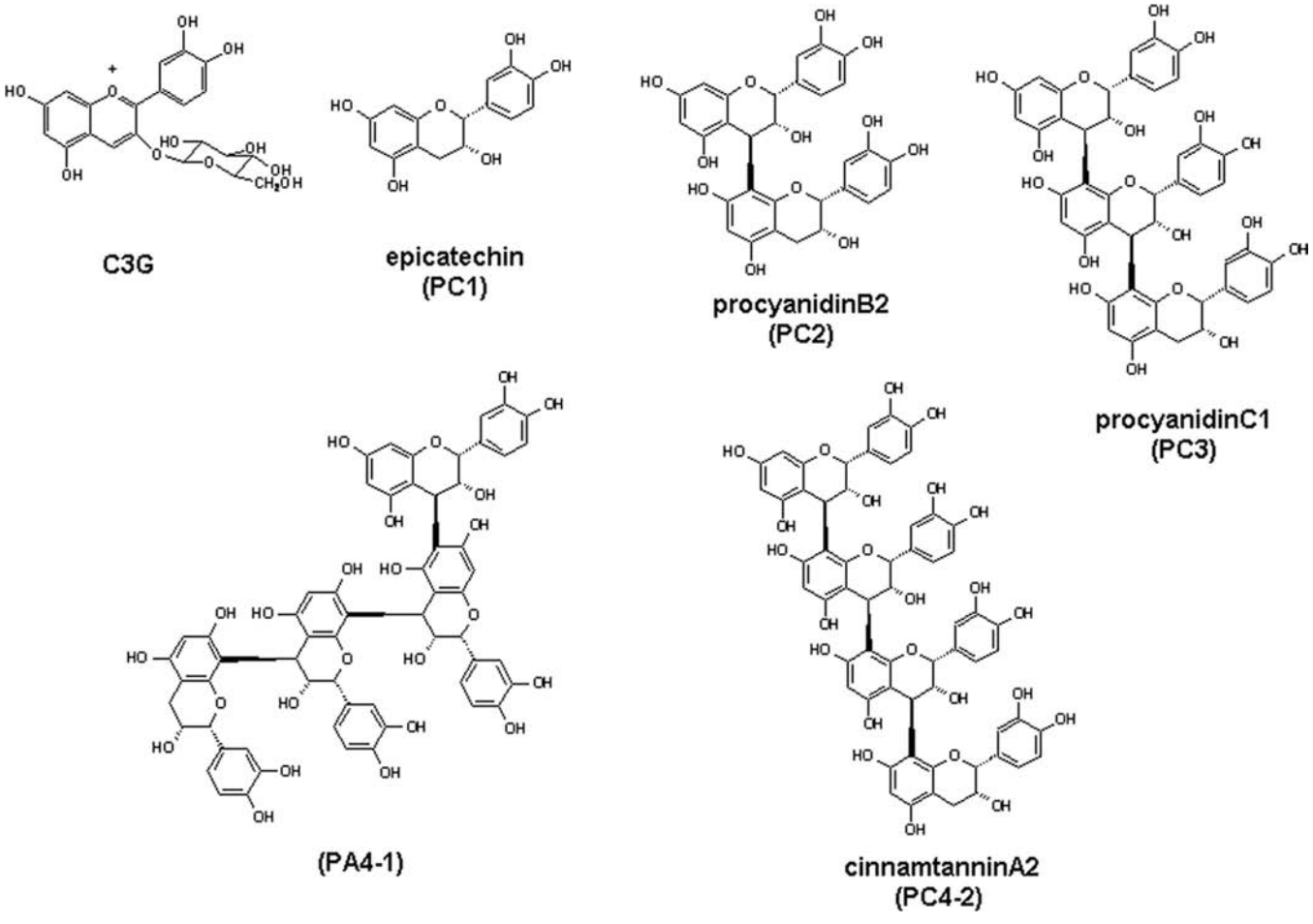

Figure 1. Chemical structures of C3G and PCs contained in BE.

dx.doi.org/10.1021/jf401190y | J. Agric. Food Chem. 2013, 61, 5558−5564

manufacturer's directions (Glucose CII-test; Wako Pure Chemical Industries, Ltd., Osaka, Japan).

Collection of Serum, Liver, Skeletal Muscle, and Adipose Tissue. After 6 weeks of receiving the experimental diets, the diets were withdrawn from all mice at 8:00 a.m. The mice were killed by decapitation 2 h later, and blood was collected. The liver, skeletal muscle, and adipose tissues [epididymal, mesenteric white adipose tissue (WAT), and interscapular brown adipose tissue (BAT)] were removed. The tissue samples were frozen immediately using liquid nitrogen and kept at −80 °C until use. The collected blood was kept at room temperature for 5 min for coagulation. Serum was isolated from the coagulated blood described above and stored immediately at −80 °C prior to use.

Measurement of Serum Insulin and Adiponectin Levels. Serum insulin and adiponectin levels were measured by ELISA using commercial assay kits according to the manufacturers' directions (Mouse Insulin ELISA Kit; Morinaga & Co., Ltd., Yokohama, Japan; Mouse/Rat Adiponectin ELISA Kit; Otsuka Pharmaceutical Co., Ltd., Tokyo, Japan).

Insulin Tolerance Test. After 5 weeks of receiving the diets, the mice were deprived of food for 14 h, and then 0.8 U/kg human insulin (Humulin R; Eli Lilly, Japan K.K., Tokyo, Japan) was injected intraperitoneally into the mice.[16] Blood samples were collected from the tail vein at set times after insulin injection (0, 30, 60, 90, and 120 min), and whole blood glucose levels were measured using an automatic blood glucose meter (GR-102; TERUMO, Tokyo, Japan).[16]

Isolation of Total RNA and Measurement of Gene Expression Levels. Total RNA from the tissues was isolated using the QIAzol reagent (QIAGEN, Tokyo, Japan) according to the manufacturer's directions. Total RNA (1.0 μg) was reverse transcribed to cDNA in a final reaction volume of 20 μL using a High Capacity cDNA Reverse Transcription Kit (Life Technologies, Tokyo, Japan) according to the manufacturer's directions. Gene expression was quantified using a real-time PCR system (ABI PRISM 7300 Sequence Detection System; Applied Biosystems, Tokyo, Japan). Amplification was performed in a final volume of 25 μL containing 50 ng of cDNA, optimized specific primers and probes (TaqMan Gene Expression Assays; Life Technologies), and Takara Premix Ex Taq (Takara Bio Inc., Shiga, Japan) according to the manufacturer's directions. The assay ID numbers of the TaqMan Gene Expression Assays were as follows: glucose-6-phosphatase (G6 Pase), Mm00839363_m1; phosphoenolpyruvate carboxykinase: (PEPCK), Mm00440636_m1; and β-2 microglobulin, Mm00437762_m1. The results were expressed as fold increase relative to the controls (=1.0) after normalization using β-2 microglobulin gene expression levels.

Immunoblot Analysis of Tissue Samples. The tissue samples were homogenized in a solution containing 50 mM Tris-HCl (pH 7.4), 150 mM NaCl, 1 mM EDTA, 50 mM NaF, 5 mM sodium pyrophosphate, 10% (w/v) glycerol, 1% (w/v) Triton X-100, 1 mM sodium metavanadate, 0.5 mM phenylmethanesulfonyl fluoride, 1 mM dithiothreitol, and protease inhibitor cocktail (P8340; Sigma-Aldrich, St. Louis, MO, USA) at 4 °C.[22] The homogenate was centrifuged at 12000g for 15 min at 4 °C. The protein concentration of the obtained supernatant was determined using a Protein Assay System (Bio-Rad, Richmond, CA, USA) with bovine γ-globulin employed as a standard. Aliquots of the supernatant were treated with Laemmli sample buffer for 5 min at 100 °C.[23] The samples were then loaded onto an SDS-PAGE system. The resulting gel was transblotted onto a nitrocellulose membrane (Hybond ECL; GE Healthcare Bioscience, Tokyo, Japan), which was blocked with 5% skim milk for 1 h at room temperature. After a washing with 20 mM Tris-HCl-buffered saline containing 0.05% (w/v) Tween 20 (TTBS), the membrane sheets were reacted with various antibodies (1:1000 dilutions) for 16 h at 4 °C. After a washing with TTBS, the membranes were reacted with horseradish peroxidase-conjugated anti-rabbit IgG secondary antibodies (1:2000 dilution; Cell Signaling) for 1 h at room temperature. After a washing, immunoreactivity was visualized using the ECL reagent (GE Healthcare Bioscience), and the relative signal intensity was evaluated with Multi Gauge ver. 3.0 Densitograph software (Fujifilm, Tokyo, Japan).

Preparation of the Plasma Membrane (PM) Fraction. The PM fraction was prepared according to the method of Nishiumi and Ashida.[24]

Cell Culture. Rat myoblast L6 cells (JCRB9081) from the Health Science Research Resources Bank (Osaka, Japan) were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% (v/v) fetal bovine serum at 37 °C in a humidified atmosphere with 5% CO_2. For the differentiation of myoblasts into myotubes, 80% confluent myoblast cells were placed in DMEM containing 2% (v/v) horse serum. The medium was changed every 2 days, and the cells were cultured for an additional 7 days to obtain mature myotubes.

Treatment of Myotubes and Immunoblot Analysis of AMPK and Related Proteins. Myotubes (differentiated for 7 days) were placed in serum-free DMEM containing 1% (w/v) BSA for 3 h. After incubation, the myotubes were treated with AICAR (1 mM), C3G, various types of PCs, or vehicle (0.1%, v/v DMSO) for the indicated time period or condition. After treatment, the cells were washed in ice-cold PBS and then lysed in a solution containing 50 mM HEPES (pH 7.5), 150 mM NaCl, 1 mM EDTA, 100 mM NaF, 10 mM sodium pyrophosphate, 1% (w/v) Triton X-100, 2 mM sodium metavanadate, and a protease inhibitor cocktail (P8340; Sigma-Aldrich) on ice for 1 min.[25] The obtained proteins from the cell lysates were used for immunoblot analysis using various antibodies described above. The experiments were repeated four times, and representative results are shown.

Glucose Uptake Assay. Glucose uptake was measured using [^{3}H]-2-deoxyglucose (2-DG).[26] Myotubes were placed in serum-free medium for 16 h. After incubation, the cells were treated with insulin (100 nM), various concentrations of C3G or PCs, or vehicle (0.1%, v/v DMSO) for 15 min in Krebs−Ringer phosphate−HEPES buffer (KRH; 50 mM HEPES, pH 7.4, 137 mM NaCl, 4.8 mM KCl, 1.85 mM $CaCl_2$, and 1.3 mM $MgSO_4$). The cells were then incubated with 6.5 mM [^{3}H]-2-DG (0.5 μCi) for 5 min at 37 °C. Uptake was terminated by washing the myotubes immediately four times with ice-cold KRH, and the cells were solubilized with a 0.5% (w/v) SDS solution. Radioactivity was measured using a liquid scintillation counter with a scintillation cocktail.

Statistical Analysis. The differences among the means of two groups were analyzed by Student's t test. Dunnett's test with repeated-measures analysis of the variance was applied to examine the results of the insulin tolerance test (Figure 2B). In Figure 5, the differences among the means were analyzed by the Tukey−Kramer test. For all tests, differences with P values of <0.05 were considered to be significant.

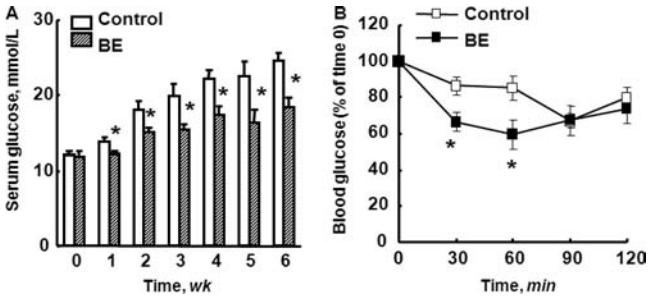

Figure 2. Serum glucose (A) and insulin tolerance test (B) in KK-A^y mice fed the control or BE diet. All data are indicated as mean ± SEM values (n = 8). (*) Mean values are significantly different from those of the control group (P < 0.05).

RESULTS

Body Weight, Food and Energy Intake, and Tissue Weight. Body weight gain between the control and BE groups did not differ and was accompanied by no significant difference in food or energy intake during the experimental period (Table 2). The weight of adipose tissue (epididymal and mesenteric

dx.doi.org/10.1021/jf401190y | J. Agric. Food Chem. 2013, 61, 5558−5564

Table 2. Body Weight, Food Intake, Relative Tissue Weights, Serum Insulin, and Adiponectin Concentrations in KK-A^y Mice Fed the Control or BE Diet for 6 Weeks[a]

	control	BE
initial body weight, g	25.2 ± 0.4	25.2 ± 0.4
final body weight, g	43.8 ± 1.0	42.4 ± 0.8
food intake, g/(6 weeks·mouse)	291.7 ± 9.0	290.0 ± 5.1
total calorie intake, MJ/(6 weeks·mouse)	4.4 ± 0.1	4.4 ± 0.1
epididymal WAT, g/100 g body	3.64 ± 0.03	3.57 ± 0.13
mesenteric WAT, g/100 g body	1.82 ± 0.08	1.98 ± 0.07
interscapular BAT, g/100 g body	0.48 ± 0.01	0.44 ± 0.03
liver, g/100 g body	6.44 ± 0.13	5.75 ± 0.19*
serum insulin, nmol/L	5.03 ± 0.79	2.43 ± 0.45*
serum adiponectin, mmol/L	0.44 ± 0.02	0.42 ± 0.02

[a]Values are means ± SEM, n = 8. *, mean values are significantly different from those of the control group ($P < 0.05$).

WAT and interscapular BAT) did not differ between the control and BE groups (Table 2). However, liver weight was significantly lower in the BE group than in the control group (Table 2).

Serum Glucose Concentration and Insulin Sensitivity. The serum glucose concentration was significantly suppressed in the BE group compared to the control group from 1 to 6 weeks after the commencement of the diet (Figure 2A). The insulin tolerance test clearly showed that dietary BE ameliorates insulin resistance; the glucose-lowering effect was significantly greater in the BE group at 30 and 60 min after insulin injection (Figure 2B).

Serum Insulin and Adiponectin Concentration. The serum insulin concentration was significantly suppressed in the BE group compared to the control group at 6 weeks (Table 2). However, the serum adiponectin concentration did not differ between the two groups at 6 weeks. The gene expression levels of adiponectin in mesenteric WAT were also not affected by the administration of BE (data not shown).

AMPK and GLUT4 Expression in the Skeletal Muscle. AMPK is known as a metabolic sensor; it up-regulates catabolic pathways that generate ATP and down-regulates anabolic pathways that consume ATP.[27] AMPK is recognized as one of the crucial targets for the prevention and treatment of obesity and type 2 diabetes.[27] Increased GLUT4 expression or translocation to the PM can be regulated by the activation of AMPK through an insulin-independent mechanism.[27] In the present study, dietary BE significantly increased the phosphorylation of AMPKα at Thr172 and the phosphorylation/total AMPKα ratio in skeletal muscle compared to that in the control group (Figure 3A). GLUT4 protein expression in the PM of the BE group was significantly higher than that of the control group (Figure 3B); however, the total GLUT4 protein expression level in skeletal muscle did not differ between the groups.

AMPK Activation and Gluconeogenesis in the Liver. The level of AMPKα phosphorylation in the liver of the BE group was significantly higher than that of the control group (Figure 4A). Activation of AMPK in the liver results in the down-regulation of the expression of gluconeogenic enzymes that are involved in hepatic glucose production. PEPCK and G6 Pase are rate-limiting gluconeogenic enzymes showing increased expression in the diabetic state. The activation of AMPK in the liver, caused by eating BE, could down-regulate

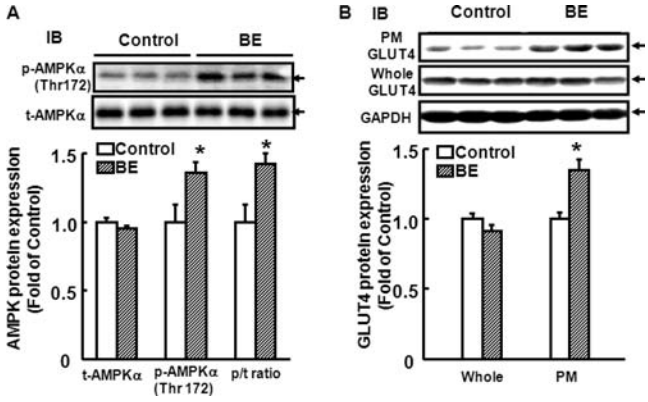

Figure 3. Immunoblot analysis of AMPKα (A) and GLUT4 protein (B) levels in the skeletal muscle of KK-A^y mice fed the control or BE diet for 6 weeks. In (A), the levels of phosphorylated (p-AMPKα) at Thr172 and total (t-AMPKα) AMPK protein were measured, and their ratio (p/t ratio) was determined. The protein levels are expressed as fold of control. In (B), Glut4 protein levels in PM and whole tissue lysates were measured. The protein levels are expressed as fold of control (=1). All data are indicated as mean ± SEM values (n = 8). (*) Mean values are significantly different from those of the control group ($P < 0.05$).

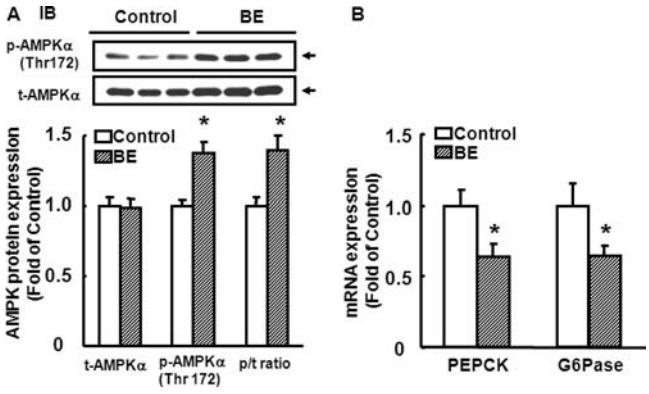

Figure 4. Immunoblot analysis of liver AMPKα protein levels (A) and gene expression levels of PEPCK and G6 Pase in the liver (B) of KK-A^y mice fed the control or BE diet. In (A), the levels of phosphorylated (p-AMPKα) at Thr172 and total (t-AMPKα) AMPK protein were measured, and their ratio (p/t ratio) was determined. The protein levels are expressed as fold of control. In (B), the gene expression levels are expressed as fold of control (=1) after normalization using the expression levels of the β-2 microglobulin gene. All data are indicated as mean ± SEM values (n = 8). (*) Mean values are significantly different from those of the control group ($P < 0.05$).

the expression of gluconeogenic enzymes and contribute to reducing the blood glucose concentration. The gene expression levels of PEPCK and G6 Pase were significantly lower in the BE group than in the control group: 36% lower for PEPCK and 37% lower for G6 Pase (Figure 4B).

Activation of AMPK and Enhancement of Glucose Uptake by BE-Derived Components in L6 Myotubes. Dietary BE significantly activated AMPK and induced GLUT4 translocation in skeletal muscle. BE contains C3G and PCs (PC1, PC2, PC3, PC4-1, and PC4-2; Figure 1), and these components can contribute to the activation of AMPK, resulting in enhanced glucose uptake. The administration of C3G or PCs significantly activated AMPK and increased GLUT4 protein expression in PM (Figure 5A). However, GLUT4 protein expression in whole lysate was not affected by

dx.doi.org/10.1021/jf401190y | J. Agric. Food Chem. 2013, 61, 5558−5564

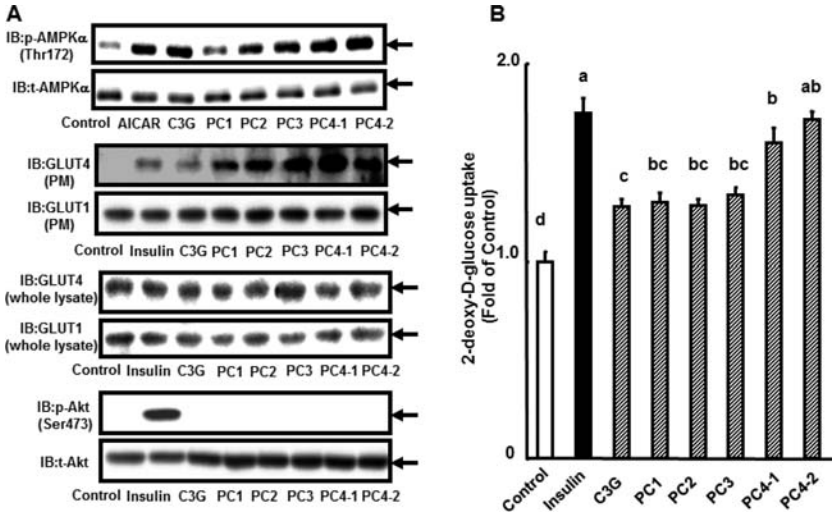

Figure 5. Immunoblot analysis of AMPKα, GLUT4, GLUT1, Akt proteins (A) and 2-deoxy-D-glucose (2-DG) uptake (B) in L6 myotubes treated with BE-derived components. In (A), the cells were treated with vehicle (0.1% DMSO) or various BE-derived components (10 μM) for 15 min. AICAR (1 mM; p-AMPK and t-AMPK) or insulin (100 nM; GLUT4, GLUT1, p-Akt, and t-Akt) was used as a positive control. The cells were lysed, and immunoblot analysis was performed. In (B), the cells were treated with vehicle (0.1% DMSO), insulin (100 nM), or various BE-derived components (10 μM) for 15 min. After treatment, 2-DG uptake into the cells was measured. The uptake levels are expressed as fold of control. All data are indicated as mean ± SEM values (n = 4). Mean values without a common letter are significantly different (P < 0.05).

administration of the BE-derived compounds. Administration of the BE-derived compounds did not induce phosphoryalation of Akt. Glucose uptake was significantly increased by the administration of C3G and PCs, whereas the tetramers (PC4-1 and PC4-2) were the strongest enhancers of glucose uptake (Figure 5B).

DISCUSSION

Anthocyanins and PCs have several biological properties: they serve as antioxidants and have anticancer and anti-inflammatory properties.[28,29] BE contains large quantities and various kinds of PCs and C3G. Previously, we and others have shown that C3G is absorbed into the blood in an intact form and metabolized to methoxyl derivatives in the liver and kidney.[30,31] It was also reported that oligomeric PCs are absorbed into the plasma and detected as an intact or conjugated form, suggesting that the intact form or metabolites (including colonic metabolites) of PCs can also modulate metabolism.[32−35] These studies suggest that BE-derived C3G, PCs, and/or their metabolites can be distributed to various tissues via the blood and are therefore expected to modulate metabolism in the body.

The present study demonstrates that dietary BE reduces blood glucose levels and enhances insulin sensitivity in type 2 diabetic mice. Food intake and calculated energy intake did not differ between the groups. These results suggest that food and energy intake did not contribute to the significant antidiabetic effect of dietary BE.

These findings raised the question of how BE reduces blood glucose levels and enhances insulin sensitivity in type 2 diabetic mice. Three possible molecular mechanisms are that BE (1) up-regulates adponectin expression, and this up-regulation significantly contributes to amelioration of hyperglycemia and insulin sensitivity; (2) induces UCP 1 expression in BAT and enhances energy expenditure, resulting in antidiabetic effects;[36] and (3) activates AMPK and results in insulin-independent enhanced glucose utilization and suppression of gluconeogenesis. However, the expression of adiponectin did not differ in response to the administration of dietary BE. Also, UCP1 expression did not differ between the control and BE groups (Supporting Information, Figure S1).

AMPK is one of the most important factors for cellular energy balance and is recognized as a potential therapeutic target for the prevention and treatment of type 2 diabetes.[27] Some drugs or food factors can activate AMPK, and this activation may potentially have antidiabetic effects.[2,3,17,37] The activation of AMPK via exercise is one of the most effective therapies for type 2 diabetic patients.[38]

The present study showed that dietary BE significantly activated AMPK in skeletal muscle and liver. This activation of AMPK in skeletal muscle resulted in a significant increase in GLUT4 protein expression in PM, which enhances glucose uptake into this tissue via an insulin-independent mechanism. This change can contribute to the amelioration of hyperglycemia.

The activation of AMPK in the liver leads to the inhibition of gluconeogenesis and lipogenesis and stimulation of fatty acid oxidation.[39] The suppression of gluconeogenesis via the down-regulation of the rate-limiting enzymes is one of the most important methods to improve hyperglycemia. It has been demonstrated that the activation of hepatic AMPK abolishes hyperglycemia in diabetic *obese* (*ob*/*ob*) mice by suppressing gluconeogenesis.[40] We and others have shown that bilberry extract or tea polyphenols suppress gluconeogenesis via the activation of AMPK in mice.[17,37] In the present study, dietary BE significantly activated AMPK and down-regulated PEPCK and G6 Pase in the liver. This suppression via AMPK activation in the liver is also an efficient mechanism that explains the antidiabetic effect of BE. Although the data were not shown, the levels of phospho (Tyr)-insulin signaling proteins (insulin receptor substrate and Akt) in skeletal muscle and liver did not differ between the dietary groups. Therefore, the AMPK activation-induced antidiabetic effect of BE is not accompanied with modulation of insulin signaling.

These findings led us to investigate what the most effective activator of AMPK and enhancer of glucose uptake is in BE. To investigate this, we assayed glucose uptake following AMPK

activation in L6 myotubes. These compounds significantly activated AMPK, translocating GLUT4 protein to PM and resulting increase glucose uptake. C3G, PCs, or combination of C3G and PCs can contribute to the BE-induced antidiabetes effect.

In conclusion, the present study demonstrates that BE reduces blood glucose levels and enhances insulin sensitivity in type 2 diabetic mice. BE modulates GLUT4 in skeletal muscle and gluconeogenesis via AMPK activation, potentially causing the amelioration of hyperglycemia. Our findings provide a biochemical basis for the use of black soybean and also have important implications for the prevention and treatment of type 2 diabetes.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Additional information (Figure S1). This material is available free of charge via the Internet at http://pubs.acs.org.

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Funding

This study was supported in part by Grants-in-Aid for Scientific Research (Nos. 23580187 and 22248014) from the Japan Society for Promotion of Science.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We thank Dr. Tatsuo Watanabe and Yusaku Iwasaki for useful discussions about UCP1 expression.

■ ABBREVIATIONS USED

AMPK, AMP-activated protein kinase; BAT, brown adipose tissue; BE, black soybean seed coat extract; C3G, cyanidin 3-glucoside; 2-DG, 2-deoxy-D-glucose; Dulbecco's modified Eagle's medium, DMEM; G6 Pase, glucose-6-phosphatase; GLUT, glucose transporter; KRH, Krebs–Ringer phosphate–HEPES buffer; PC, procyanidin; PEPCK, phosphoenolpyruvate carboxykinase; PPAR, peroxisome proliferator-activated receptor; PM, plasma membrane; TTBS, Tris-HCl-buffered saline containing 0.05% Tween 20; UCP, uncoupling protein; WAT, white adipose tissue

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