Dietary Anthocyanin-Rich Bilberry Extract Ameliorates Hyperglycemia and Insulin Sensitivity via Activation of AMP-Activated Protein Kinase in Diabetic Mice

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Abstract

Blueberries or bilberries contain large amounts of anthocyanins, making them one of the richest sources of dietary anthocyanin. These berries are widely consumed as fresh and dried fruits, jams, or juices. Considerable attention has been focused on the health benefits of bilberry fruits beyond their antioxidant content or their ability to improve vision. In this study, we tested the effect of dietary bilberry extract (BBE) on hyperglycemia and insulin sensitivity in type 2 diabetic mice. We found that dietary BBE ameliorates hyperglycemia and insulin sensitivity via activation of AMP-activated protein kinase (AMPK). Dietary BBE significantly reduced the blood glucose concentration and enhanced insulin sensitivity. AMPK was activated in white adipose tissue (WAT), skeletal muscle, and the liver of diabetic mice fed BBE. This activation was accompanied by upregulation of glucose transporter 4 in WAT and skeletal muscle and suppression of glucose production and lipid content in the liver. At the same time, acetyl-CoA carboxylase was inactivated and PPAR γ, acyl-CoA oxidase, and carnitine palmitoyltransferase-1A were upregulated in the liver. These changes resulted in improved hyperglycemia and insulin sensitivity in type 2 diabetes. These findings provide a biochemical basis for the use of bilberry fruits and have important implications for the prevention and treatment of type 2 diabetes via activation of AMPK. J. Nutr. 140: 527–533, 2010.

Introduction

Therapeutic regimens that target the regulation of adipocyte or other cells’ functions are being used because of their ability to improve insulin sensitivity and glucose homeostasis. Thiazolidinediones, for example, are a class of synthetic PPAR ligands used as antidiabetic drugs because of their effects on adipocyte differentiation and adipocyte gene activation (1,2). Another drug, Metformin, ameliorates hyperglycemia without stimulating insulin secretion (3,4). The administration of Metformin increases AMPK activation in white adipose tissue (WAT), skeletal muscle, and the liver of diabetic mice fed Metformin. This activation was accompanied by upregulation of glucose transporter 4 in WAT and skeletal muscle and suppression of glucose production and lipid content in the liver. At the same time, acetyl-CoA carboxylase was inactivated and PPAR γ, acyl-CoA oxidase, and carnitine palmitoyltransferase-1A were upregulated in the liver. These changes resulted in improved hyperglycemia and insulin sensitivity in type 2 diabetes. These findings provide a biochemical basis for the use of bilberry fruits and have important implications for the prevention and treatment of type 2 diabetes via activation of AMPK, which has been implicated as a potential target for treatment of type 2 diabetes (3,4).

Recently, considerable attention has focused on dietary constituents that may be beneficial for the prevention and treatment of diabetes. Although there are some drugs that have been used as therapeutic regimens for obesity-related metabolic diseases, there is little evidence that food factors themselves can be directly beneficial for modulating insulin sensitivity.

Anthocyanins are the largest group of water-soluble pigments in the plant kingdom. They are widely available in the human diet in cereals, beans, fruits, vegetables, and red wine (5), suggesting that we ingest large amounts of anthocyanins daily from plant-based diets (6).

In general, anthocyanins are stable under acidic conditions but are unstable and rapidly broken down under neutral conditions (7). Therefore, anthocyanins have not yet been recognized as a physiologically functional food factor (7). However, we previously demonstrated that cyanidin-3-glucoside (C3G), which is a typical anthocyanin, significantly suppresses the development of obesity induced by a high-fat diet fed to C57BL/6 mice (8,9). Our prior studies also showed that C3G and cyanidin modulates the gene expression of adipokines in human adipocytes (9,10). Moreover, we demonstrated that purified dietary C3G reduces the blood glucose level and improves insulin sensitivity in type 2 diabetic mice (9,11).
Berry fruits, such as blueberries, bilberries, or blackcurrants, contain large amounts of anthocyanins, making them one of the major sources for dietary anthocyanin intake (6,12). Blueberries (Vaccinium cyanococcus) or bilberries (Vaccinium myrtillus) are widely consumed as fresh and dried fruits, jams, or juices in diets. Considerable attention has focused on the health benefits of bilberry, which include antioxidant, anticancer, antiinflammatory activities (13). Vuong et al. (14) reported that fermented blueberry juice, but not native bilberry extract (BBE) or isolated compounds, stimulated glucose uptake and AMPK activation in vitro. Recent research by Prior et al. (15) indicated that consumption of whole blueberries did not prevent obesity; however, extracted blueberry anthocyanins significantly reduced high-fat diet-induced body fat accumulation in mice.

The results of these studies raise the question of whether dietary anthocyanin-rich BBE can ameliorate hyperglycemia and insulin sensitivity. Moreover, the molecular action of the bilberry responsible for ameliorating hyperglycemia and the enhancement of insulin sensitivity is not yet fully understood in vivo. Therefore, the present study was designed to examine whether the administration of anthocyanin-rich BBE reduces the blood glucose level and improves insulin sensitivity in type 2 diabetic mice.

Materials and Methods

**Chemicals.** BBE (containing 375 g anthocyanins/kg) was a kind gift from Tama-Biochemicals. The composition of anthocyanins in the BBE was analyzed by HPLC (16). BBE contains 15 kinds of anthocyanins, including 5 types of anthocyanidin aglycons of 3 different glycosides (Supplemental Fig. 1). The composition ratio of the anthocyanins in the BBE is listed in Supplemental Table 1. The composition ratio of the anthocyanins in the BBE was optimized specific primers and probes (TaqMan Gene Expression Assays, Applied Biosystems). Amplification was performed in a 25-μL using a High Capacity cDNA Reverse Transcription kit (Applied Biosystems). 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 (Applied Biosystems) according to the manufacturer’s directions. Quantification of gene expression was measured using the real-time PCR system (ABI PRISM 7300 Sequence Detection System, Applied Biosystems). Amplification was performed in a 25-μL final volume containing 50 ng of cDNA, optimized specific primers and probes (TaqMan Gene Expression Assays, Applied Biosystems), and Takara Premix Ex Taq (Takara Bio) according to the manufacturer’s directions. The assay identification numbers of the TaqMan Gene Expression Assays were as follows: adiponectin, Mm00456425_m1; AdipoR1, Mm01291334_mH; AdipoR2, Mm01184030_m1; RBP4, Mm00803266_m1; glucose-6-phosphatase (G6Pase), Mm00839363_m1; phosphoenol pyruvate carboxykinase (PEPCK), Mm00440636_m1; PPARα, Mm00449393_m1; ACO, Mm00443797_m1; CPT1A, Mm00350438_m1; B2 microglobulin, Mm00437762_m1; and TATA box binding protein, Mm00446973_m1. Results were expressed as fold increase relative to the controls (+1.0) after normalization using β-2 microglobulin (WAT and liver) or TATA box binding protein (skeletal muscle) gene expression level.

**Measurement of serum liver lipids, serum insulin, adiponectin, and retinol binding protein 4 levels.** The triglyceride and total cholesterol levels in the liver and serum were measured using commercial assay kits according to the manufacturer’s directions (Triglyceride-E test and Cholesterol-E test; Wako Pure Chemical Industries). Total liver lipid content was measured according to the method of Fuch et al. (18). Serum insulin, adiponectin, and retinol binding protein 4 (RBP4) levels were measured by ELISA using a commercial assay kit according to the manufacturers’ directions (mouse insulin ELISA kit, Morinaga and Co.; mouse/rat adiponectin ELISA kit, Otsuka Pharmaceutical; and Dual mouse/rat RBP4 ELISA kit, AdipoGen).

**Pyruvate tolerance test.** A pyruvate tolerance test was performed according to a previous study (19). In brief, mice were deprived of food for 14 h after 4 wk on the diets, then 2 g/kg pyruvate (Sigma-Aldrich) was intraperitoneally injected into the mice (19). Blood samples were collected from the tail vein at set times after insulin injection (0, 30, 60, 90, and 120 min), and the whole-blood glucose level was measured with an automatic blood glucose meter (GR-102, TERUMO) (11).

**Isolation of total RNA and measurement of gene expression level.** Total RNA from the tissues was isolated with TRIzol reagent (QIAGEN) 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 (Applied Biosystems) according to the manufacturer’s directions. Quantification of gene expression was measured using the real-time PCR system (ABI PRISM 7300 Sequence Detection System, Applied Biosystems). Amplification was performed in a 25-μL final volume containing 50 ng of cDNA, optimized specific primers and probes (TaqMan Gene Expression Assays, Applied Biosystems), and Takara Premix Ex Taq (Takara Bio) according to the manufacturer’s directions. The assay identification numbers of the TaqMan Gene Expression Assays were as follows: adiponectin, Mm00456425_m1; AdipoR1, Mm01291334_mH; AdipoR2, Mm01184030_m1; RBP4, Mm00803266_m1; glucose-6-phosphatase (G6Pase), Mm00839363_m1; phosphoenol pyruvate carboxykinase (PEPCK), Mm00440636_m1; PPARα, Mm00449393_m1; ACO, Mm00443797_m1; CPT1A, Mm00350438_m1; B2 microglobulin, Mm00437762_m1; and TATA box binding protein, Mm00446973_m1. Results were expressed as fold increase relative to the controls (+1.0) after normalization using β-2 microglobulin (WAT and liver) or TATA box binding protein (skeletal muscle) gene expression level.

**Collection of serum, liver, skeletal muscle, and adipose tissue.** After 5 wk of feeding the experimental diets (control or BBE diet), the mice were withdrawn from all mice at 0800. Mice were then killed by decapitation 2 h after withdrawing the experimental diets from the mice and blood was collected. The liver, skeletal muscle, and adipose tissues were removed. Tissue samples were then immediately frozen using liquid nitrogen and kept at −80°C until use. The collected blood was kept at room temperature for 5 min for coagulation. The serum was then isolated from the coagulated blood by centrifugation at 1600 × g for 15 min at 4°C. The serum glucose concentration was immediately assayed using a commercial assay kit according to the manufacturer’s directions (Glucose CII-test; Wako Pure Chemical Industries).

**Measurement of serum glucose concentration.** Experimental diets were withdrawn from all mice at 0800 and blood was obtained from the tail vein 1 h after withdrawing the experimental diets from the mice. The collected blood was kept at room temperature for 5 min for coagulation. The serum was then isolated from the coagulated blood by centrifugation at 1600 × g for 15 min at 4°C. The serum glucose concentration was immediately assayed using a commercial assay kit according to the manufacturer’s directions (Glucose CII-test; Wako Pure Chemical Industries).

**Measurement of serum glucose concentration.** Experimental diets were withdrawn from all mice at 0800 and blood was obtained from the...
Immunoblot analysis. The tissue was homogenized with 50 mmol/L Tris-HCl (pH 7.4), 150 mmol/L NaCl, 1 mmol/L EDTA, 50 mmol/L NaF, 5 mmol/L sodium pyrophosphate, 10% glycerol, 1% Triton X-100, 1 mmol/L sodium metavanadate, 0.5 mmol/L phenylmethylsulfonyl fluoride, 1 mmol/L dithiothreitol, and a protease inhibitor cocktail (P8340, Sigma-Aldrich) at 4°C (20). The homogenate was centrifuged at 12,000 × g for 15 min at 4°C. The protein concentration of the obtained supernatant was determined using a Protein Assay system (Bio-Rad) with bovine γ-globulin employed as a standard. Aliquots of the supernatant were treated with Laemmli sample buffer for 5 min at 100°C (21). The samples were then loaded into the SDS-PAGE system. The resulting gel was transblotted onto a nitrocellulose membrane (Hybond ECL, GE Health Care Bioscience), which was blocked with 5% skim milk for 1 h at room temperature. After washing with 20 mmol/L Tris-HCl-buffered saline containing 0.05% Tween 20, the membrane sheets were then reacted with various antibodies (1:1000 dilutions) for 16 h at 4°C. After washing with Tris-HCl-buffered saline containing 0.05% Tween 20, membranes were reacted with horseradish peroxidase-conjugated antirabbit IgG secondary antibodies (Cell Signaling, 1:2000 dilutions) for 1 h at room temperature. After washing, the immunoreactivity was visualized using the ECL reagent (GE Health Care Bioscience) and the relative signal intensity was evaluated with a Multi Gauge version 3.0 Densitograph Software (Fuji Film).

Histological analysis of liver. Small pieces of liver were fixed with formalin (200 g/kg) in PBS and embedded in paraffin. Sections (8 µm) were cut and stained with hematoxylin and eosin. Images were captured using a CCD Camera (Olympus Optical) at a 400× magnification (8).

Statistical analysis. The differences among the means were analyzed by the Student’s t test if data in the 2 groups were normally distributed with equal variance. In other cases, the nonparametric Mann-Whitney test was used. Differences at P < 0.05 were considered significant. Dunnett’s test with repeated-measures ANOVA was applied for insulin tolerance (Fig. 1B) and pyruvate tolerance (Fig. 3C) results. All statistical analyses were performed using StatView version 5.0 software (SAS Institute).

Results

Body weight, food intake, energy intake, and tissue weight. Body weight gain between the control and BBE groups did not differ and was accompanied by no significant difference in food or energy intake during the experimental period (Table 1). Adipose tissue (epididymal and mesenteric WAT and interscapular BAT) weight did not significantly differ between the control and BBE groups (Table 1). However, liver weight was significantly lower in the BBE group than in the control group (Table 1).

![Graph A](image1.png)

**FIGURE 1** Serum glucose concentration (A) and insulin tolerance test results (B) in KK-Ay mice fed the control or BBE diet for 5 wk. Values are the means ± SEM, n = 6–8. *Different from control, P < 0.05.

Serum glucose, insulin concentration, and insulin sensitivity. The serum glucose concentration was significantly suppressed in the BBE group compared with the control group during the 3–5-wk period after commencement of the diet (Fig. 1A); however, at 5 wk, the serum insulin concentration did not differ between the control (3017.2 ± 741.3 pmol/L) and BBE (2465.5 ± 724.1 pmol/L) groups. The results of the insulin tolerance test clearly showed that dietary BBE ameliorates insulin resistance; the glucose-lowering effect was significantly greater in the BBE group at 30, 90, and 120 min after insulin injection (Fig. 1B).

Adiponectin and adiponectin receptor expression. The gene expression level of adiponectin in the mesenteric WAT did not differ between the groups (Supplemental Fig. 2A). The serum adiponectin concentration was also not affected by the administration of BBE (Supplemental Fig. 2B). The gene expression level of adiponectin receptors (AdipoR1 and R2) in both skeletal muscle and liver did not differ between the control and BBE groups (Supplemental Fig. 2C,D).

RBP4 expression. Recent studies have provided a new link between RBP4, which is recognized as an adipocytokine, and insulin resistance. RBP4 contributes to the progression of type 2 diabetes, and lowering the RBP4 level is considered a new target for preventing and treating type 2 diabetes (22). In diabetic mice with adipocyte-specific ablation of Glut4, the downregulation of Glut4 in WAT upregulates the expression and secretion of RBP4 from adipocytes (22). The gene expression level of RBP4 in the mesenteric WAT was significantly suppressed in the BBE group compared with the control group; however, the RBP4 protein concentration in the serum and WAT did not differ between the groups (Supplemental Fig. 3A–C).

Insulin signaling protein expression in WAT, skeletal muscle, and liver. Activation of the insulin signaling pathway, which involves phosphorylation of IRS-1 and Akt following insulin stimulation, is an important target for the prevention and treatment of diabetes (23). The levels of tyrosine phosphorylation of IRS-1 and serine phosphorylation of Akt (ratio of phospho-protein:total protein) in the WAT, skeletal muscle, and liver did not differ between the control and BBE groups (Supplemental Fig. 4A–F).

AMPK and Glut4 expression in the WAT and skeletal muscle. AMPK is known as a metabolic sensor; it upregulates catabolic pathways that generate ATP and downregulates anabolic pathways that consume ATP (24). AMPK is recognized

<table>
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<tr>
<th>TABLE 1</th>
<th>Body weight, food intake, and relative tissue weights in KK-Ay mice fed the control or BBE diet for 5 wk</th>
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<tr>
<td><strong>Control</strong></td>
<td><strong>BBE</strong></td>
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<tr>
<td>Initial body weight, g</td>
<td>21.2 ± 0.4</td>
</tr>
<tr>
<td>Final body weight, g</td>
<td>40.0 ± 0.6</td>
</tr>
<tr>
<td>Food intake, g/(5 wk · mouse)</td>
<td>204.5 ± 2.9</td>
</tr>
<tr>
<td>Energy intake, MJ/(5 wk · mouse)</td>
<td>3.11 ± 0.04</td>
</tr>
<tr>
<td>Epididymal WAT, g/(100 g body)</td>
<td>3.42 ± 0.04</td>
</tr>
<tr>
<td>Mesenteric WAT, g/(100 g body)</td>
<td>1.97 ± 0.10</td>
</tr>
<tr>
<td>Interscapular BAT, g/(100 g body)</td>
<td>0.50 ± 0.03</td>
</tr>
<tr>
<td>Liver, g/(100 g body)</td>
<td>6.47 ± 0.22</td>
</tr>
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1 Values are means ± SEM, n = 6–8. *Different from control, P < 0.05.
as one of the crucial targets for prevention and treatment of obesity and type 2 diabetes (24). Increased Glut4 expression or translocation to the plasma membrane can be regulated by the activation of AMPK through an insulin-independent mechanism (24). In the present study, dietary BBE significantly increased total AMPKα and phosphorylation of AMPKα at Thr172 in the WAT compared with that in the control group (Fig. 2A). In addition, phosphorylation at Thr172 of AMPKα in the skeletal muscle of the BBE group was significantly higher than that of the control group (Fig. 2B). The Glut4 protein expression level in the BBE group was significantly greater and was 2.1-fold that of the control group (Fig. 2C) and 2.3-fold that of the control group in skeletal muscle (Fig. 2D).

AMPK activation and gluconeogenesis in the liver. Phosphorylation of AMPKα in the liver of the BBE group was significantly higher than that of the control group (Fig. 3A). Activation of AMPK in the liver results in downregulation of expression of gluconeogenic enzymes that are involved in hepatic glucose production. PEPCK and G6Pase are rate-limiting gluconeogenic enzymes and their expression is increased in the diabetic state. The activation of AMPK in the liver, caused by feeding BBE, could downregulate the expression of gluco- neogenic enzymes and result in lowered blood glucose concentration. The gene expression levels of PEPCK and G6Pase were significantly lower (31 and 38%, respectively) in the BBE group than in the control group (Fig. 3B). Glucose flux into the blood, as measured by the pyruvate tolerance test, was significantly suppressed in the BBE group at 90, 120, and 150 min after pyruvate injection (Fig. 3C).

Lipid metabolism in the liver and serum. The total lipid, triglyceride, and cholesterol contents of the liver were significantly suppressed in the BBE group compared with the control group (Table 2). The histological data support the finding that lipid content significantly decreased by dietary BBE (Supplemental Fig. 5). The serum triglyceride and total cholesterol concentration was also significantly suppressed in the BBE group (Table 2).

ACC is the rate-limiting enzyme for fatty acid synthesis. Activated AMPK consisting of α2, β1, and γ1 subunits has been shown to inactivate ACC via Ser79 phosphorylation, thereby stimulating fatty acid oxidation. On the other hand, activated AMPK consisting of α2, β2, and γ1 subunits translocates to the nucleus and induces PPARα gene expression (25,26). Therefore, the activation of AMPK by BBE may be accompanied by significant phosphorylation of ACC and upregulation of PPARα and ACO, which catalyzes fatty acid oxidation. These changes can result in a reduction in lipid content in the liver and serum. Activation of AMPK by BBE was accompanied by significant inactivation of ACC in the liver (Fig. 4A). In addition, the gene expression levels of PPARα, ACO, and CPT1A were significantly upregulated by the administration of BBE (Fig. 4B).

Discussion

Anthocyanins have several biological properties: they are antioxidants and have anticancer and antiinflammatory properties (13). Bilberry fruits contain large quantities and various kinds of anthocyanins. Our previous study showed that anthocyanins (C3G) were absorbed into the blood in an intact form and metabolized to methoxy derivatives in the liver and kidney (27). Other research groups have also reported that intact bilberry anthocyanins were detected in the plasma as intact forms, with metabolites detected in the liver and kidney, suggesting that metabolites of anthocyanin may also modulate...
TABLE 2

<table>
<thead>
<tr>
<th>Group</th>
<th>Total lipids</th>
<th>Triglyceride</th>
<th>Cholesterol</th>
<th>Triglyceride</th>
<th>Cholesterol</th>
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<tbody>
<tr>
<td></td>
<td>mg/g</td>
<td>μmol/g</td>
<td>mmol/L</td>
<td>mmol/L</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>74.2 ± 3.2</td>
<td>29.0 ± 1.7</td>
<td>7.33 ± 0.18</td>
<td>5.81 ± 0.46</td>
<td>3.07 ± 0.12</td>
</tr>
<tr>
<td>BBE</td>
<td>60.3 ± 3.0*</td>
<td>22.8 ± 2.3*</td>
<td>6.82 ± 0.16*</td>
<td>4.00 ± 0.38*</td>
<td>2.61 ± 0.16*</td>
</tr>
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Values are means ± SEM, n = 6–8. *Different from control, P < 0.05.

Which enhances glucose uptake into these tissues via an insulin-independent mechanism. This change can contribute to the amelioration of hyperglycemia.

AMPK also plays an important role in the control of hepatic glucose and lipid metabolism. Activation of AMPK in the liver leads to the inhibition of glucose production, lipogenesis, and stimulation of fatty acid oxidation (37). In type 2 diabetes, hepatic glucose production is increased and this increase strongly contributes to elevate the blood glucose level. Suppression of gluconeogenesis and hepatic glucose output is one of the most important methods to improve hyperglycemia. It has been demonstrated that hepatic AMPK activation abolishes hyperglycemia in diabetic ob/ob mice by suppression of gluconeogenesis (38). Some reports have shown that tea polyphenols suppress gluconeogenesis and ameliorate lipid metabolism via activation AMPK in hepatocytes and mice (35,39).

In this study, dietary BBE significantly activated AMPK and downregulated PEPCK and G6Pase in the liver, resulting in a decrease in the glucose output into the blood. The results of the pyruvate tolerance test clearly supported the observation that BBE significantly suppressed hepatic gluconeogenesis and glucose output. This suppression via AMPK activation in the liver is an efficient mechanism that explains the antidiabetic effect of BBE. Transducer of regulated CRE-binding protein...
activity 2 (TORC2) has emerged as a critical regulator of gluconeogenesis in mice (40). Activation of AMPK attenuates the gluconeogenic program by promoting TORC2 phosphorylation and blocking its nuclear accumulation (38). Dietary BBE may modulate TORC2 phosphorylation and nuclear accumulation.

Activation of AMPK leads to phosphorylation of ACC and 3-hydroxy-3-methyl-glutaryl-CoA reductase, resulting in the inhibition of their activity (37). Phosphorylation of ACC reduces the formation of malonyl-CoA. This in turn results in the activation of CPT1A, a step required for the stimulation of fatty acid oxidation in mitochondria (37). The inhibition of cholesterol synthesis is preceded by phosphorylation of 3-hydroxy-3-methyl-glutaryl-CoA reductase (41). The activation of AMPK upregulates PPARα gene expression and downregulates fatty acid synthase, ACC, and sterol regulatory element binding protein-1c, which are involved in lipid synthesis (25,26,37). The dietary BBE-induced AMPK activation also affected lipid metabolism in the liver. Total lipid, triglyceride, and cholesterol content of the liver were significantly reduced in the BBE group and this reduction was accompanied by a significant decrease in serum triglyceride and cholesterol concentration. The AMPK activation induced significant phosphorylation of ACC and upregulated PPARα and ACO gene expression in the liver. These changes due to AMPK activation lead to a significant reduction of lipid content in the BBE group and may have contributed to the amelioration of insulin sensitivity via reduction of lipotoxicity.

Dietary BBE activates AMPK in the WAT, skeletal muscle, and liver. In the WAT and skeletal muscle, the activation of AMPK induces upregulation of Glut4 and enhancement of glucose uptake and utilization in these tissues. In the liver, dietary BBE reduces glucose production via AMPK activation, which ameliorates hyperglycemia in type 2 diabetic mice. The AMPK activation induces phosphorylation of ACC and upregulation of PPARα, ACO, and CPT 1A gene expression in the liver. These changes lead to reductions in lipid content and increases in insulin sensitivity via reduction of lipotoxicity.

The question remains, however, as to what the most effective inducer of AMPK in the BBE is and how AMPK activation is regulated by BBE. AMPK is regulated by the AMP:ATP ratio and upstream kinases (calcium-dependent protein kinase kinase or LKB1) (42–46). BBE may modulate the cellular AMP:ATP ratio or an upstream kinase of AMPK.

In conclusion, this study demonstrates that BBE reduces blood glucose level and enhances insulin sensitivity in type 2 diabetic mice. The BBE modulates Glut4 in the WAT and skeletal muscle and glucose output and lipid metabolism via AMPK activation. These potentially ameliorate hyperglycemia. Our findings provide a biochemical basis for the use of bilberry fruits and also have important implications for the prevention and treatment of type 2 diabetes.

Acknowledgments
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