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 PPARα, 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 anti-diabetic 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 leads inactivation of ACC, AC, and carnitine palmitoyltransferase-1A. Carnitine palmitoyltransferase Cpt1a, 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.

1 Supported by a Grant-in-Aid for Scientific Research (No. 20580146 to T.T.) from the Japanese Ministry of Education, Culture, Sports, Science and Technology, Chubu University Grant A (to T.T.), and by The Japan Food Chemical Research Foundation (T.T.).
2 Author disclosures: M. Takikawa, S. Inoue, F. Horio, and T. Tsuda, no conflicts of interest.
3 Supplemental Figures 1–5 and Supplemental Table 1 are available with the online posting of this paper at jn.nutrition.org.
4 Abbreviations used: ACC, acetyl-CoA carboxylase; ACO, acyl-CoA oxidase; AMPK, AMP-activated protein kinase; BBE, bilberry extract; BAT, brown adipose tissue; CPT1A, carnitine palmitoyltransferase-1A; C3G, cyanidin-3-glucoside; GPase, glucose-6-phosphatase; Glut4, glucose transporter 4; IRS-1, insulin receptor substrate-1; PEPCK, phosphoenolpyruvate carboxykinase; RBP4, retinol binding protein 4; TORC2, transducer of regulated CRE-binding protein activity 2; WAT, white adipose tissue.
5 To whom correspondence should be addressed. E-mail: tsudat@isc.chubu.ac.jp.
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, antineurodegenerative, and 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. 

A glucose transporter 4 (Glut4), anti-β-actin, anti-phospho-AMPKα (Thr172), anti-AMPKα, anti-acetyl-CoA carboxylase (ACC), anti-phospho-ACC (Ser79), anti-Akt, anti-phospho-Akt (Ser473), and anti-insulin receptor substrate-1 (IRS-1) antibodies were obtained from Cell Signaling Technology. Anti-phospho-IRS-1 (Tyr608) was obtained from Life Technologies, Japan.

Mice and diets. Male KK-A' mice, age 4 wk (CLEA Japan) were used (17) and maintained at 23 ± 3°C under an automatic lighting schedule (0800–2000 h light). The mice were allowed free access to water and a laboratory diet (CE-2, CLEA Japan) containing: protein, 254 g/kg; fat, 67 g/kg; ash, 67 g/kg; energy, 15.2 MJ/kg; and sufficient minerals and vitamins to maintain the health of the mice for 1 wk. After 1 wk of breeding, 5-wk-old mice were then divided into 2 groups and assigned to the control (CE-2) or the BBE diet (CE-2 supplemented with BBE) containing: protein, 244 g/kg; fat, 49 g/kg; non-nitrogenous substances, 521 g/kg; crude fiber, 34 g/kg; crude ash, 65 g/kg; energy, 15.2 MJ/kg; and a total anthocyanin concentration of 10 g/kg diet (27 g of BBE/kg diet). The BBE diet was replaced every day to avoid depletion of the anthocyanins. The dose of BBE was based on a preliminary experiment to show that the dose for knockdown of insulin sensitivity is not yet fully understood in vivo.

Measurement of serum glucose concentration. The results of the experiments 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).

Collection of serum, liver, skeletal muscle, and adipose tissue. After 5 wk of feeding the experimental diets (control or BBE diet), the mice were then killed by decapitation at 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 was immediately stored at −80°C prior to use.

Measurement of serum and 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 Fielch 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).

Insulin tolerance test. After 4 wk of the diets, mice were deprived of food for 14 h, then 0.33 U/kg human insulin (Humulin R; Eli Lilly, Japan K.K.) was intraperitoneally injected into the mice (11). 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 (MR-102, TERUMO) (11).

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 injection (0, 30, 60, 90, 120, and 150 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 QIAzol 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_m1; AdipoR2, Mm01184030_m1; RBP4, Mm00803266_m1; glucose-6-phosphatase (G6Pase), Mm00839363_m1; phosphoenol pyruvate carboxykinase (PEPCK), Mm00440636_m1; PPARα, Mm00409359_m1; ACO, Mm00443759_m1; CPT1A, Mm00350438_m1; β2 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.
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).

![FIGURE 1](image) 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.

**TABLE 1** Body weight, food intake, and relative tissue weights in KK-Ay mice fed the control or BBE diet for 5 wk

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>BBE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial body weight, g</td>
<td>21.2 ± 0.4</td>
<td>21.2 ± 0.3</td>
</tr>
<tr>
<td>Final body weight, g</td>
<td>40.0 ± 0.6</td>
<td>39.2 ± 0.6</td>
</tr>
<tr>
<td>Food intake, g/5 wk · mouse</td>
<td>204.5 ± 2.9</td>
<td>194.0 ± 4.2</td>
</tr>
<tr>
<td>Energy intake, MJ/5 wk · mouse</td>
<td>3.11 ± 0.04</td>
<td>2.95 ± 0.06</td>
</tr>
<tr>
<td>Epididymal WAT, g/100 g body</td>
<td>3.42 ± 0.04</td>
<td>3.54 ± 0.10</td>
</tr>
<tr>
<td>Mesenteric WAT, g/100 g body</td>
<td>1.97 ± 0.10</td>
<td>1.81 ± 0.08</td>
</tr>
<tr>
<td>Interscapular BAT, g/100 g body</td>
<td>0.50 ± 0.03</td>
<td>0.48 ± 0.02</td>
</tr>
<tr>
<td>Liver, g/100 g body</td>
<td>6.47 ± 0.22</td>
<td>5.43 ± 0.13*</td>
</tr>
</tbody>
</table>

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 greater 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 in WAT (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 gluconeogenic 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 inhibition 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...
metabolic effects (28–31). Administered anthocyanins have also been detected in ocular tissues (32). These results suggest that bilberry anthocyanins and/or their metabolites can be distributed to various tissues via blood and are therefore expected to modulate metabolic changes in the body.

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

Expression of adiponectin and the adiponectin receptors (AdipoR1 and R2) did not differ in skeletal muscle and liver in response to the administration of dietary BBE. Although dietary BBE significantly decreased the gene expression level of RBP4, the RBP4 protein concentration did not differ in either the serum or WAT between the control and BBE groups, suggesting that the antidiabetic effect may be only partially due to modulation of RBP4 expression. Also, IRS-1 and Akt levels (ratio of insulin-induced phospho-protein:total protein) in the WAT, skeletal muscle, and liver did not differ between the dietary groups. The antidiabetic effect of BBE is therefore due to an insulin-independent mechanism.

Recent studies show that AMPK is one of the most important factors for cellular energy balance and is recognized as a potential therapeutic target in the prevention and treatment of type 2 diabetes (24,33,34). Some drugs for the treatment of type 2 diabetes (Metformin and thiazolidinediones) activate AMPK (1,2). Other drugs or food factors can activate AMPK and this activation may potentially have antidiabetic effects (35–36). The present study demonstrates that dietary BBE reduces blood glucose levels and enhances insulin sensitivity in type 2 diabetic mice by 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

### TABLE 2
Liver and serum lipid concentrations in the liver of KK-A mice fed the control or BBE diet for 5 wk

<table>
<thead>
<tr>
<th>Group</th>
<th>Total lipids (mg/g)</th>
<th>Triglyceride (μmol/g)</th>
<th>Cholesterol (mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>74.2 ± 3.2</td>
<td>29.0 ± 1.7</td>
<td>7.33 ± 0.18</td>
</tr>
<tr>
<td>BBE</td>
<td>60.3 ± 3.0*</td>
<td>22.8 ± 2.3*</td>
<td>6.82 ± 0.16*</td>
</tr>
</tbody>
</table>

*Different from control, P < 0.05.

### FIGURE 3
Immunoblot (IB) analysis of liver AMPK-α protein levels (A) and gene expression levels of PEPCK and G6Pase (B), and pyruvate tolerance test (C) for KK-A mice fed the control or BBE diet. In A, levels of phosphorylated (p-AMPKα) at Thr172 and total (t-AMPKα) AMPK protein were measured and the ratio (p/t ratio) of these determined. The protein level is expressed as fold of control. In B, the gene expression levels are expressed as fold of control (=1) after normalization using the β-2 microglobulin gene expression level. Values are the means ± SEM, n = 6–8. *Different from control, P < 0.05.

### FIGURE 4
Immunoblot (IB) analysis of ACC protein levels (A) and gene expression levels of PPARα, ACO, and CPT1A (B) in the liver of KK-A mice fed the control or BBE diet. In A, levels of phosphorylated (p-ACC) at Ser79 and total (t-ACC) ACC protein were measured and the ratio (p/t ratio) of these determined. The protein level is expressed as fold of control. In B, the gene expression levels are expressed as fold of control (=1) after normalization using the β-2 microglobulin gene expression level. Values are the means ± SEM, n = 6–8. *Different from control, P < 0.05.
activity 2 (TORC2) has emerged as a critical regulator of
gluconeogenesis in mice (40). Activation of AMPK attenuates
the gluconeogenic program by promoting TORC2 phosphor-
ylation 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 choles-
terol 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 this upregulation
results in enhancement of glucose uptake and utilization in these
tissues. In the liver, dietary BBE clearly reduced glucose
production via AMPK activation. This reduction efficiently
ameliorates hyperglycemia in type 2 diabetic mice. Furthermore,
BBE-induced AMPK activation in the liver results in significantly
decreased liver and serum lipid content via upregulation of
PPARα and ACO. The upregulation of CPT1A by dietary BBE
could enhance the decrease in lipid content via enhancement of
fatty acid oxidation. These changes may also contribute to the
antidiabetic effect of BBE (Fig. 5).

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
We thank Tsuyoshi Watanabe for kindly providing BBE and for
useful discussions. T.T. designed this research; M.T., S.I., and F.
H. conducted research; M.T. and T.T. wrote the paper; and T.T.
had primary responsibility for final content. All authors read
and approved the final manuscript.

Literature Cited
1. Yamauchi T, Kamon J, Waki H, Murakami K, Motojima K, Komeda K,
Ide T, Kubota N, Terauchi Y, et al. The mechanisms by which both
heterozygous peroxisome proliferator-activated receptor (PPARγ)
2001;276:41245–54.
2. Lehrke M, Lazar MA. The many faces of PPARγ. Cell. 2005;123:
993–9.
Ventre J, Doebber T, et al. Role of AMP-activated protein kinase in
4. Hawley SA, Gadalla AE, Olsen GS, Hardie DG. The anti-diabetic drug
metformin activates the AMP-activated protein kinase cascade via
an adenosine nucleotide-independent mechanism. Diabetes. 2002;51:
2420–5.
Concentrations of anthocyanins in common foods in the United States


533