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ORIGINAL ARTICLE

Antiobesity and antidiabetic effects of biotransformed blueberry juice in KKA^{y} mice

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Aim: Biotransformation of blueberry juice by the *Serratia vaccinii* bacterium gave rise to adenosine monophosphate-activated protein kinase (AMPK) phosphorylation and glucose uptake in muscle cells and adipocytes, but inhibited adipogenesis. This study investigated the antiobesity and antidiabetic potential of biotransformed blueberry juice (BJ) in KKA^{y} mice, rodent model of leptin resistance.
Methods: BJ was incorporated in drinking water of KKA^{y} mice. Parameters of body weight, food intake, plasma glucose, insulin, leptin, and adiponectin were measured. Before and after therapy, animals were subjected to an oral glucose tolerance test. At the end of treatment, liver, muscle, kidney, epididymal fat pad, abdominal fat pad, and dorsal fat pad were collected and weighed.
Results: Incorporating BJ in drinking water protected young KKA^{y} mice from hyperphagia and significantly reduced their weight gain. Moreover, BJ protected young KKA^{y} mice against the development of glucose intolerance and diabetes mellitus. Chronic BJ administration in obese and diabetic KKA^{y} mice reduced food intake and body weight. This effect could not fully explain the associated antidiabetic effect because BJ-treated mice still showed lower blood glucose level when compared with pair-fed controls. The adipokines pathway also seems to be involved because BJ significantly increased adiponectin levels in obese mice.
Conclusions: This study shows that BJ decreases hyperglycemia in diabetic mice, at least in part by reversing adiponectin levels. BJ also protects young pre-diabetic mice from developing obesity and diabetes. Thus, BJ may represent a novel complementary therapy and a source of novel therapeutic agents against diabetes mellitus.
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Keywords: antiobesity; antidiabetic; adiponectin; KKA^{y} mice; blueberry

Introduction

Obesity and diabetes have reached epidemic proportions throughout the world.[1] Adipose tissue is now recognized as an endocrine organ that contributes to the physiopathology of type2 diabetes. Adipokines, proteins produced by adipose tissue, have been identified as potential contributors to insulin resistance in humans.[2] Adiponectin is produced by differentiated adipocytes and circulates at high levels in the bloodstream.[3] Low levels of circulating adiponectin are associated with insulin resistance and the presence of metabolic syndrome.[4,5] Increasing adiponectin expression may be integral to the therapeutic effect of antidiabetic medications such as PPARγ agonists (Thiazolidinediones)[6,7] or CB1 blocker (Rimonabant).[8,9] The best-characterized molecular mechanism mediating the metabolic and vascular actions of adiponectin involves stimulation of adenosine monophosphate-activated protein kinase (AMPK) activity.[10]

Members of the genus *Vaccinium* have been used traditionally for the treatment of diabetic symptoms and are hence reputed to be antidiabetic.[11–13] Extracts of various parts of the *V. angustifolium* plant were shown to possess insulin- and glitazone-like properties while also protecting cells against glucose toxicity.[14] The blueberry fruit is rich in phenolic compounds such as hydroxycinnamic acids, flavonoids, and proanthocyanidines.[15–17] Biotransformation of blueberry juice with a novel strain of bacteria isolated from the blueberry flora and named *Serratia vaccinii* increases its phenolic content and antioxidant activity[18] and modifies

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its biological activity.[14] Biotransformed blueberry juice (BJ) increased AMP-activated protein kinase phosphorylation and glucose uptake in muscle cells and adipocytes, but inhibited adipogenesis.[19] As BJ showed potential antiobesity and antidiabetic activities *in vitro*, the aim of this study was to assess the antiobesity and antidiabetic potential of BJ *in vivo*.

KKA^y mice are a cross between glucose-intolerant black KK female mice and yellow obese A^y male mice. They are characterized by hyperphagia because of leptin resistance. Their phenotype is characterized by obesity and the development of hyperleptinemia, insulin resistance, hyperinsulinemia, diabetes, dyslipidemia, and hypertension. Therefore, KKA^y mice are an excellent model that closely resembles obesity and obesity-linked type 2 diabetes in humans who express several disorders within a single individual.[20] This model was thus selected to evaluate the *in vivo* antiobesity and antidiabetic activity of BJ.

Materials and methods

Preparation of biotransformed blueberry juice

Mature lowbush blueberries (*V. angustifolium* Ait.) were purchased from Cherryfield Foods Inc. (Cherryfield, ME, USA) as fresh and untreated fruits. Blueberry juice was extracted by blending the fruit (100 g) with an equivalent quantity (100 g) of Minimal Broth Davis without dextrose (MM) (Difco Laboratories, Detroit, MI, USA). The fruit mixture was then centrifuged to remove insoluble particles. The resulting juice was sterilized using 0.22 μm Express Millipore filters (Millipore, Etobicoke, ON, Canada).

S. vaccinii bacteria were cultured as described earlier.[18] The juice was inoculated with a saturated culture of *Serratia* corresponding to 2% of the total juice volume. After a 4-day fermentation period, the transformed juice was sterilized by 0.22 μm filtration. Blueberry and BJ have been partially characterized elsewhere.[17,18,21] Blueberry juice contains low yet known amounts of sugars (7.9% w/w). Therefore, we prepared a sugar water (SW) solution that mimicked the sugar composition of normal blueberry juice (NJ)[22] and therefore contained 100 g of MM, 85.6 g of water, 3.1 g of glucose, 4.1 g of fructose, 0.5 g of maltose, and 0.4 g of sucrose. We specifically used this solution as a vehicle control in pair-feeding experiments (see further below).

Animals

Male non-diabetic C57BL/6J mice were obtained from Charles River Laboratories (Saint-Constant, QC, Canada). KKA^y mice were derived from an in-house colony established using breeding pairs obtained from Jackson Laboratory (Bar Harbor, ME, USA). All mice were housed individually and maintained on a 12 h light–dark cycle in a temperature-controlled animal room. All animals were allowed *ad libitum* access to solid food and water. All experimental protocols were approved by the animal experimentation ethics committee of the University of Montreal and we carried out in full respect of the guidelines from the Canadian Council for the Protection of Animals.

Antiobesity study

Four-week-old C57BL/6J and KKA^y mice were randomly divided into four groups. The C57BL/6J mice constituted the non-obese control group ($n=10$). Three KKA^y mice groups ($n=10$) received either water (obese control group), NJ, or BJ (40 ml kg^{-1} per day in drinking water). Baseline measurements of fed blood glucose levels, body weight, food intake, and water consumption were taken every 2 days at 11 am. The non-fasting blood glucose concentration was measured using an Accu-Chek glucose meter (Roche, Montreal, QC, Canada) by collecting blood from the tip of the tail vein. At the onset of experiments and after 2 weeks, an oral glucose tolerance test (OGTT) was performed (see further below). After the third week, the mice were anesthetized, killed by exsanguination, and organs such as the liver, muscle (right femoral muscle), kidney, epididymal fat pad, abdominal fat pad, and dorsal fat pad were immediately removed and weighed.

Acute study

Fifteen 7-week-old KKA^y mice were randomly divided into three groups ($n=5$) that received 5 ml kg^{-1} of either water or BJ by intragastric gavage. Metformin (0.283g kg^{-1}) was administered similarly as a positive control; this concentration was selected from a dose–effect curve.[23] The non-fasting blood glucose concentration was measured at 0, 1, 2, 3, 4, 5, 6, 24 h after the juices were administered.

Chronic treatment study

In preliminary experiments, three different doses of BJ, namely 20, 40, 80 ml kg^{-1} per day, were incorporated in the drinking water and provided to mice for 1 week. The best antidiabetic effect was obtained with the 80 ml kg^{-1} per day dose (data not shown). Therefore, this dose was used for chronic treatment studies. Forty-two 7-week-old KKA^y mice were thus randomly divided into six groups ($n=7$), which received either water, NJ (80 ml kg^{-1} per day), BJ (80 ml kg^{-1} per day), or Metformin (0.85g kg^{-1} per day)[23] in drinking water. Each mouse in the BJ group had a matched 'twin', which constituted the paired-fed group (BJ'); animals in this group received SW (80 ml kg^{-1} per day—to control for the sugar intake associated with BJ) and the amount of food consumed by their BJ 'twin' the previous day. The sixth and final group received SW but had unrestricted access to solid food. Baseline measurements of fed blood glucose levels, body weight, food intake, and water consumption were taken daily at 11.00 am. At the onset of experiments and after 4 weeks, an OGTT was performed (see further below).

Oral glucose tolerance test
Mice were fasted for 16 h. A glucose solution was administered by intragastric gavage at a dose of 1 g kg^{-1} body weight. The blood glucose concentration was measured at 0, 30, 60, 90, 120 min after glucose administration in the blood collected from the tip of the tail vein, using an Accu-Chek glucose meter (Roche).

Biochemical assays
At the end of experiments, blood samples were collected from renal artery during exsanguinations carried out under anesthesia using heparinized tools. The samples were centrifuged at 3000 *g* for 10 min at 4 °C. The plasma samples were used for the analyses of biochemical parameters. Plasma adiponectin, insulin, and leptin were measured using radioimmuno assay kits (Millipore, Montreal, QC, Canada).

Statistical analysis
Statistical analysis of the data by one-way or two-way ANOVA (as appropriate) as well as Fisher *post hoc* tests was performed using StatView software (Cary, NC, USA). Statistical significance was set at $P<0.05$. Data are reported as mean ± s.e.m.

Results

Antiobesity effect
Figure 1 presents the distribution of food intake and body weight values for groups treated with NJ or BJ during the crucial 3-week period where the development of obesity and type 2 diabetes is observed in KKA^y mice in our laboratory (4–7 weeks of age). BJ treatment significantly reduced the cumulative food intake of KKA^y mice as compared to parallel control obese or NJ-treated groups (Figure 1a) and hence also reduced the cumulative body weight gain (Figure 1b). Correspondingly, the weight of abdominal fat pads and the relative liver weight were significantly lower in BJ-treated mice as compared to control animals, whereas NJ-treated mice exhibited values very similar to control mice (Table 1).

All groups (except the non-diabetic C57BL/6J mice) had equivalent glycemia at the onset of the studies (Figure 2). After 3 weeks, all animals in the diabetic control group and in the NJ-treated group had a non-fasting glycemia at or above 20 mM (averages of 25.9 ± 4.7 and 24.9 ± 3.8 mM, respectively, NS). In contrast, only 20% of mice in the BJ group exhibited such hyperglycemia (average of 13.9 ± 5.3 mM, $P<0.05$). As expected, the glycemia of non-obese mice remained stable over time. Moreover, the glycemic response to an OGTT was found to be significantly lower in BJ-treated mice as compared to control or NJ-treated mice (reduction of 24% in AUC of BJ group compared to control, $P<0.05$, Figure 3). In contrast, BJ treatment did not induce significant changes of plasma insulin, leptin, or adiponectin values (Table 1).

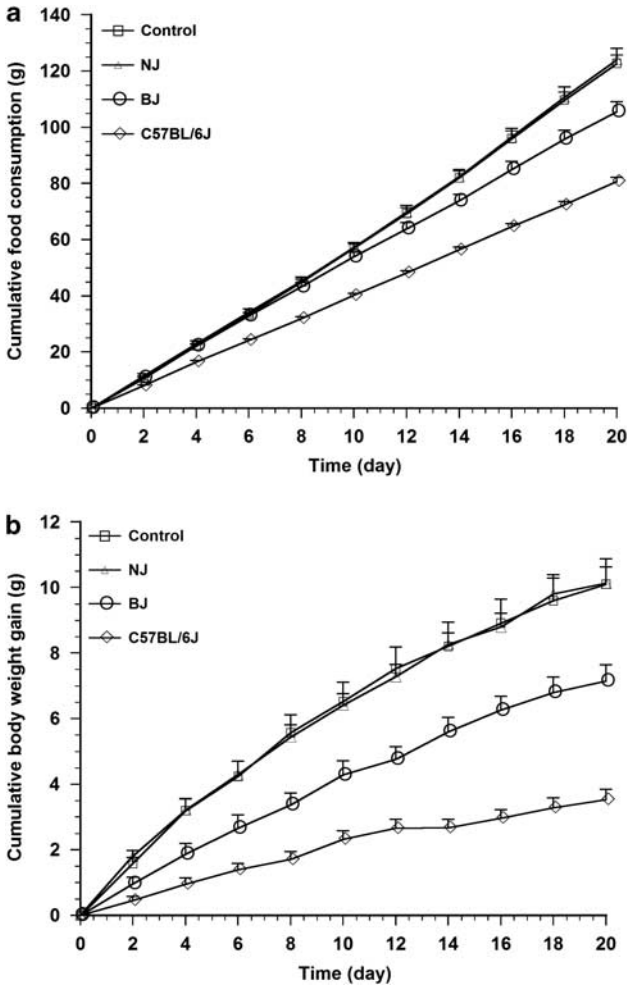

Figure 1 Cumulative food intake (**a**) and body weight gain (**b**) of young KKA^y mice treated with either water (Control), NJ (40 ml kg^{-1} per day), or BJ (40 ml kg^{-1} per day) and C57BL/6J mice that received water (no treatment). All treatments lasted 3 weeks. All values are means ± s.e.m. ($n=10$ for each experimented group).

Biotransformed blueberry juice acute effect
Administration of a single dose of BJ (5 ml kg^{-1}) did not affect food intake or body weight in KKA^y mice (data not shown). However, it triggered a decline in blood glucose that peaked 3 h after the onset of administration and declined only slowly thereafter over a 24 h period (Figure 4). The maximum effect amounted to a decrease in blood glucose by 27% as compared to controls. Metformin (0.283g kg^{-1}) administration yielded similar results.

Chronic treatment effect
Whereas NJ-treated and control mice consumed similar amounts of solid food, both Metformin- and BJ-treated groups exhibited a significant anorexic effect. Indeed, the food intake of Metformin- and BJ-treated mice was significantly reduced

Table 1 Physiological and biochemical parameters of mice in antiobesity study

	Control	*NJ*	*BJ*	*C57BL/6J*
Tissue weight (g)				
Liver	2.0 ± 0.1^a	2.0 ± 0.1^a	1.4 ± 0.1^b	1.6 ± 0.1^b
Relative liver weight	0.06^a	0.06^a	0.05^b	0.05^b
Kidney	0.4 ± 0.01^a	0.4 ± 0.02^a	0.3 ± 0.02^a	0.4 ± 0.01^a
Epididymal fat pad	1.0 ± 0.04^a	1.1 ± 0.06^a	0.9 ± 0.06^a	0.9 ± 0.05^b
Abdominal fat pad	3.2 ± 0.1^a	2.8 ± 0.2^a	2.2 ± 0.1^b	nd
Dorsal fat pad	1.0 ± 0.08^a	1.0 ± 0.09^a	0.9 ± 0.07^a	0.4 ± 0.04^b
Biochemical parameters				
Insulin (ng ml^{-1})	13.0 ± 2.4^a	12.7 ± 2.0^a	12.1 ± 2.0^a	1.3 ± 0.1^b
Adiponectin (îg ml^{-1})	5.1 ± 0.6^a	5.2 ± 0.7^a	5.9 ± 0.6^a	8.8 ± 0.6^b
Leptin (ng ml^{-1})	28.9 ± 2.4^a	28.3 ± 2.2^a	27.6 ± 1.4^a	13.1 ± 2.1^b

Control, normal blueberry juice (NJ), and biotransformed blueberry juice (BJ) are KKA^y mice, which received either water, NJ (40 ml kg^{-1} per day), or BJ (40 ml kg^{-1} per day) for 3 weeks. C57BL/6J mice received water (no treatment) for 3 weeks. All values are means ± s.e.m. ($n=10$ for each experimented group). Between-group differences, as determined by two way ANOVA, are illustrated by different letters (a and b) with significance defined as $P<0.05$.

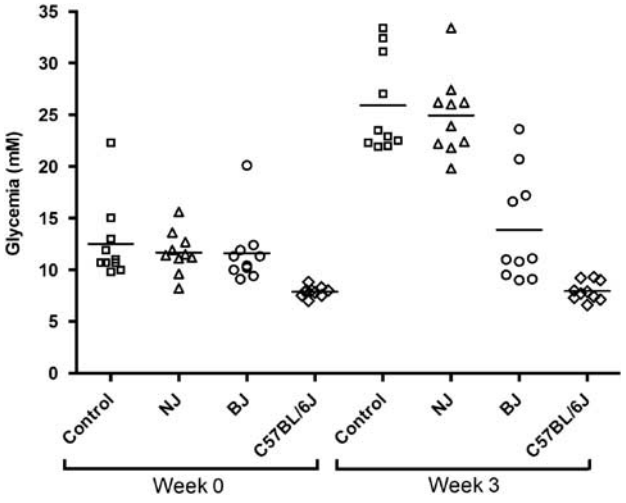

Figure 2 Glycemia of young KKA^y mice treated with either water (Control), NJ (40 ml kg^{-1} per day), or BJ (40 ml kg^{-1} per day) and C57BL/6J mice that received water (no treatment). All treatments lasted 3 weeks. Glycemia was assessed at weeks 0 and 3. All values are means ± s.e.m. ($n=10$ for each experimented group).

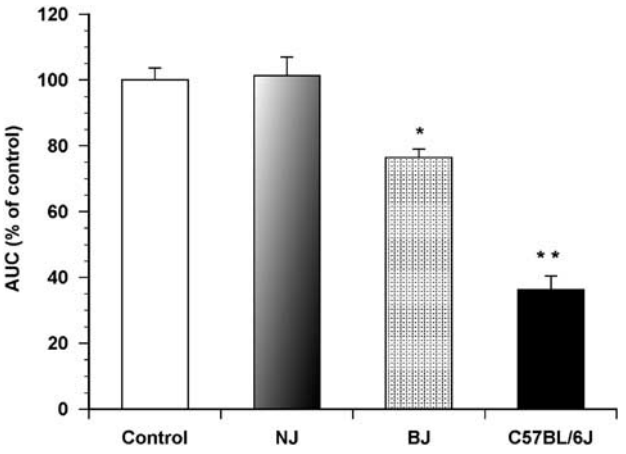

Figure 3 AUC from OGTT of KKA^y mice treated with either water (Control), NJ (40 ml kg^{-1} per day), or BJ (40 ml kg^{-1} per day) for 3 weeks. C57BL/6J mice were subjected to the same OGTT protocol but received water (no treatment) for 3 weeks. Results are expressed as % of control at week 3. All values are means ± s.e.m. ($n=10$ for each experimented group). Asterisks indicate a significant difference ($P<0.05$) from control.

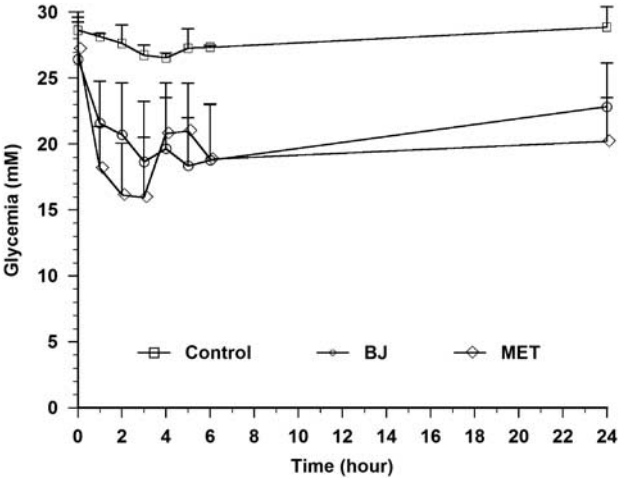

Figure 4 Effect of a single dose of BJ (5 ml kg^{-1}) and Metformin (MET) (0.283g kg^{-1}) on glycemia of diabetic obese KKA^y mice. All values are means ± s.e.m. ($n=5$ for each experimented group).

by 30 and 17%, respectively, as compared to controls (Figure 5). However, only Metformin-treated mice showed a slight but significant body weight loss, which amounted to 4% by the end of the 4-week-treatment period, as compared to controls, whereas no significant difference was observed in BJ-treated mice (Figure 6a).

As illustrated in Figure 7a, when administered chronically over a 4-week period, NJ did not significantly influence blood glucose in diabetic KKA^y mice, which oscillated in the 25–30 mM range as in control animals. In contrast, the positive control Metformin significantly reduced then maintained the glycemia of diabetic KKA^y mice at a normal level (10 mM in the fed state) within 4 days. BJ gradually and significantly reduced the high blood glucose level of diabetic mice and the maximum effect was reached after 3 days. BJ then maintained KKA^y mice glycemia level at a level 35% lower than that of control animals until the end of the study.

Moreover, both BJ and Metformin improved glucose tolerance in these diabetic mice. Indeed, the glycemic response to an OGTT, assessed by the area under the glycemia-versus-time curve (AUC), dropped by 38% between the onset and the end of the 4-week treatment in both groups (Figure 8, $P<0.05$ by two-way ANOVA), suggesting an improvement in insulin sensitivity. In comparison, control and NJ-treated

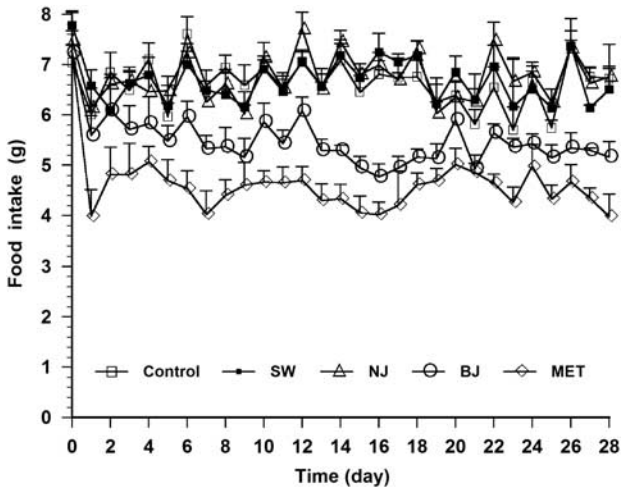

Figure 5 Food intake of diabetic KKA^{y} mice treated with MET (0.85g kg^{-1} per day), NJ (80 ml kg^{-1} per day), BJ (80 ml kg^{-1} per day), and SW (80 ml kg^{-1} per day) during 4 weeks. All values are means ± s.e.m. ($n=7$ for each experimented group).

animals had similar responses to an OGTT at weeks 0 and 4 (N.S. by two-way ANOVA). The positive impact of Metformin on insulin sensitivity was reflected in plasma insulin levels that were normalized by the end of the 4-week treatment as compared to the hyperinsulinemic values of controls (Table 2, $P<0.05$ by ANOVA). Chronic BJ treatment also had a tendency to reduce circulating insulin levels by nearly 50%, but this effect failed to reach statistical significance.

Table 2 also presents plasma leptin and adiponectin values in all experimental groups. None of the treatments were able to affect the expected hyperleptinemia observed in KKA^{y} mice. However, though neither NJ nor Metformin treatments could modify the low level of adiponectin measured in KKA^{y} mice, animals consuming BJ over a 4-week period showed a significant increase in circulating adiponectin level, which amounted to a 65% rise above values of control animals (Table 2, $P<0.05$).

Pair-feeding effect

To control for the effect of BJ on food intake, pair-feeding studies were carried out in parallel with BJ treatment. Moreover, to control for the slight sugar content of NJ, SW, having the same composition of sugars and diluted in MM as was done for NJ, was used as a vehicle control. Restricting food intake to the amount consumed by BJ-treated animals (BJ' group) did not have any significant effect on the body weight of KKA^{y} mice (Figure 6b). Similarly, supplying SW in drinking water without pair feeding did not significantly alter body weight. In contrast, pair feeding reduced the glycemia of diabetic mice by 13% by the end of the 4-week-treatment period, as compared to SW- or water-fed controls (Figure 7b). This antidiabetic effect was roughly half as important as observed in the BJ-treated group. Despite the

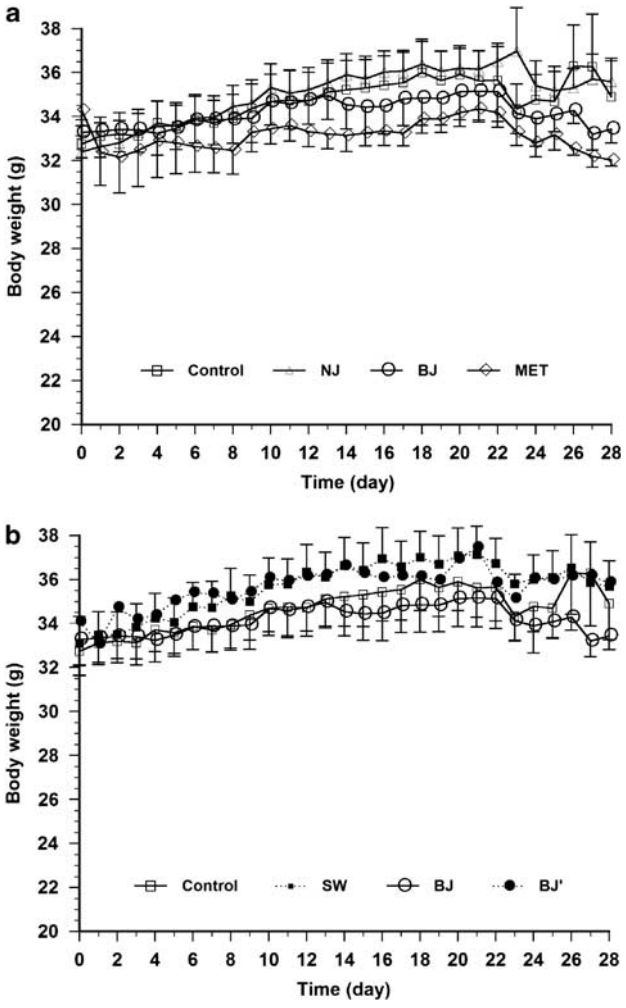

Figure 6 (**a**) Body weight of diabetic KKA^{y} mice treated with NJ (80 ml kg^{-1} per day), BJ (80 ml kg^{-1} per day), and MET (0.85g kg^{-1} per day) during 4 weeks. (**b**) Each mouse in the BJ group had a matched 'twin', which constituted the paired-fed group (BJ'); animals in this group received SW (80 ml kg^{-1} per day—to control for the sugar intake associated with BJ) and the amount of food consumed by their BJ 'twin' the previous day. The sixth and final group received SW but had unrestricted access to solid food. All values are means ± s.e.m. ($n=7$ for each experimented group).

reduction in glycemia induced by pair feeding, such treatment failed to improve the response to an OGTT as observed in BJ-treated animals (Figure 8). Similarly, pair feeding failed to increase circulating adiponectin levels in contrast to BJ treatment (Table 2).

Discussion

In earlier studies, BJ was found to inhibit adipogenesis and increase glucose uptake in muscle cells and adipocytes.[19] We therefore conducted this study to confirm its *in vivo* antiobesity and antidiabetic potential in KKA^{y} mice, a model

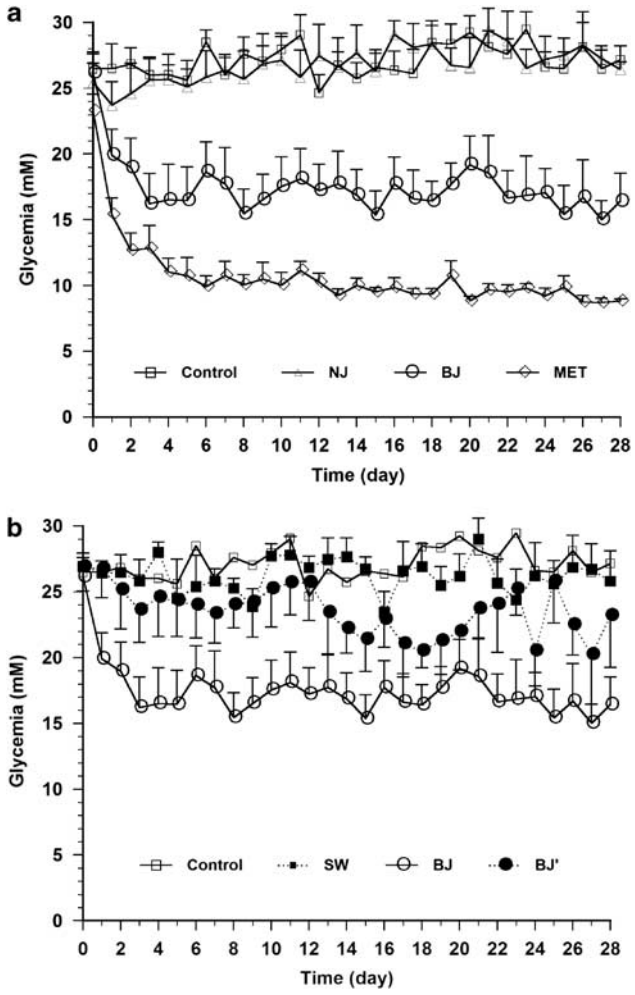

Figure 7 (**a**) Glycemia of diabetic KKA^y mice treated with NJ (80 ml kg^{-1} per day), BJ (80 ml kg^{-1} per day), and MET (0.85g kg^{-1} per day) during 4 weeks. (**b**) Each mouse in the BJ group had a matched 'twin', which constituted the paired-fed group (BJ'); animals in this group received SW (80 ml kg^{-1} per day—to control for the sugar intake associated with BJ) and the amount of food consumed by their BJ 'twin' the previous day. The sixth and final group received SW but had unrestricted access to solid food. All values are means ± s.e.m. ($n=7$ for each experimented group).

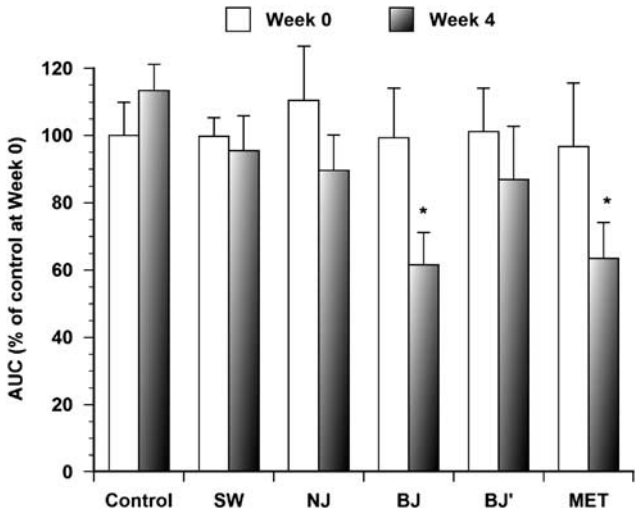

Figure 8 AUC from OGTT of diabetic KKA^y mice treated with MET (0.85g kg^{-1} per day), NJ (80 ml kg^{-1} per day), BJ (80 ml kg^{-1} per day), and SW, BJ (80 ml kg^{-1} per day of SW) after 4 weeks. Results are expressed as % of control at week 0. All values are means ± s.e.m. ($n=7$ for each experimented group). * indicates a significant difference ($P<0.05$) from control.

that closely resembles obesity and obesity-linked type 2 diabetes in humans. The results convincingly show that BJ as apposed to NJ indeed holds great promise as an antiobesity and antidiabetic agent.

In our hands, between 4 and 7 weeks of age, KKA^y mice develop hyperphagia, hyperleptinemia, hypo-adiponectinemia, hyperinsulinemia, hyperglycemia, and reduced glucose tolerance as compared to non-diabetic control C57BL/6 mice. We therefore further assessed the antiobesity potential of BJ by administering it chronically during this crucial life period of KKA^y mice. Such administration was found to prevent the development of obesity and diabetes mellitus. Indeed, BJ significantly reduced hyperphagia and, consequently, the development of obesity as seen by a significantly reduced cumulative body weight gain. Moreover, only 20% of mice reached the diabetic levels of glycemia, as compared to 100% of control or NJ-fed animals. In parallel, the relative liver weight and abdominal fat pad weight were significantly lower than those of control animals. Both increased visceral fat and steatotic liver (a probable cause of increased relative liver weight in this model—unpublished observation) have been implicated in the development of insulin resistance in the context of obesity.[24–26] It was thus not surprising that the response to an OGTT was reduced by preventive BJ administration, indicating an improved glucose tolerance possibly related to enhanced insulin sensitivity. In contrast, it was less expected that BJ could not prevent the increased insulinemia or reduced adiponectinemia that develops during this 3-week crucial period in KKA^y mice. Future experiments should attempt to detail the mechanisms underlying the beneficial effects of BJ to prevent the development of obesity and diabetes.

When administered acutely at a single oral dose of 5 ml kg^{-1}, BJ was essentially as powerful as Metformin to reduce hyperglycemia in diabetic KKA^y mice. In contrast, when given in a chronic manner at a dose of 80 ml kg^{-1}, BJ also significantly reduced glycemia in these diabetic mice, but the effect was roughly half as powerful as the oral hypoglycemic drug Metformin.

Part of the metabolic effects of chronic BJ treatment could be attributed to the significant anorexic effect it exerted, despite the fact that this effect failed to induce a change in body weight. Indeed, glycemia was also lowered in pair-fed BJ' animals, but the intensity of the effect was significantly lower than that of BJ, indicating that other factors additional to reduced caloric intake were necessarily involved. Similarly, the normalization of insulin sensitivity induced by

Table 2 Biochemical parameters of KKA^y mice after chronic treatment

Treatment	*Insulin (ng ml^{-1})*	*Adiponectin (μg ml^{-1})*	*Leptin (ng ml^{-1})*
Control	21.9 ± 6.5^a	4.3 ± 0.4^a	29.1 ± 1.9^a
SW	18.7 ± 5.1^a	5.2 ± 0.4^a	30.7 ± 2.9^a
NJ	39.9 ± 14.8^a	5.4 ± 0.8^a	33.2 ± 4.0^a
BJ	12.7 ± 2.6^a	7.9 ± 0.5^b	33.9 ± 2.7^a
BJ′	21.6 ± 7.4^a	5.9 ± 0.9^{a,b}	31.3 ± 2.4^a
MET	1.7 ± 0.5^b	5.9 ± 0.6^a	27.6 ± 2.9^a

All animals are KKAy mice treated with normal blueberry juice (NJ) (80 ml kg^{-1} per day), biotransformed blueberry juice (BJ) (80 ml kg^{-1} per day), and Metformin (MET) (0.85g kg^{-1} per day) during 4 weeks. Each mouse in the BJ group had a matched 'twin', which constituted the paired-fed group (BJ′); animals in this group received sugar water (SW) (80 ml kg^{-1} per day—to control for the sugar intake associated with BJ) and the amount of food consumed by their BJ 'twin' the previous day. The sixth and final group received SW but had unrestricted access to solid food. All values are means ± s.e.m, ($n=7$ for each experiment group). Between-group differences, as determined by two way ANOVA, are illustrated by different letters (a and b) with significance defined as $P<0.05$.

chronic BJ treatment, as assessed by the AUC of the glycemic response to an OGTT, was not because of the simple slight caloric restriction associated with the reduction of food intake as BJ′ animals failed to show this effect. Likewise, BJ but not BJ′ treatment was able to significantly increase the low circulating levels of adiponectin observed in diabetic KKA^y mice.

In fact, the reversal of low circulating levels of adiponectin provides, at least in part, an explanation for the correction of insulin resistance by BJ in the diabetic obese mice. The same mechanism has indeed been observed with PPARγ agonists (Thiazolidinediones)[6,7] and CB1 receptor antagonists (Rimonabant).[8,9] Adiponectin normally enhances lipid catabolism, leading to reduced tissue triglyceride content and improved insulin sensitivity.[27] It also reduces hepatic glucose production.[28] AMPK has been confirmed to be a major downstream component of adiponectin signaling.[10] These published data are consistent with our earlier observations that BJ stimulated AMPK in muscle cells and adipocytes.[19] Further studies will be necessary to unravel the possible causal relationship of these observations.

The mechanism by which adiponectin was increased by BJ is unclear. Adiponectin production by adipocytes is a multistep process that can be regulated at the level of gene expression, secretion, and/or formation of the multimeric forms of the protein. The molecular mechanisms involved in adiponectin secretion and multimerization as well as their regulation have not been deciphered.[10] However, it is clear that reactive oxygen species[29] and pro-inflammatory cytokines[30] are potent inhibitors of adiponectin gene expression. As BJ has a very high anti-oxidant activity, more than four times greater than NJ,[18,31] it could reduce oxidative stress in obese diabetic mice. This, in turn, could contribute to increasing adiponectin release by adipose tissue. Further studies are needed to address the involvement of antioxidant activity, adiponectin content, and AMPK stimulation in the *in vivo* metabolic effects of biotransformed juice.

Nonetheless, anthocyanins present in abundance in blueberry fruits may have been implicated. Indeed, feeding purified anthocyanins from blueberries has been shown to reduce obesity in mice.[32] However, feeding similar amounts of anthocyanins as whole blueberries did not prevent and may have actually increased obesity.[32] These results are consistent with the lack of beneficial effects of NJ in this study. In contrast, the process of preparing BJ greatly increases the content in total phenolic compounds and this was associated with greatly enhanced biological activity to treat obesity and related diabetes, as well as to protect pre-diabetic mice from the same. It thus seems worthwhile and imperative to explore the novel compounds and/or the changes in the content of known phenolics induced by the biotransformation of NJ with *S. vaccinii* and to test their effectiveness as antiobesity and antidiabetic agents.

In summary, the results of this study clearly show that BJ has strong antiobesity and antidiabetic potential. BJ protected young pre-diabetic mice against obesity and diabetes. It potently reduced the high glucose blood level in adult diabetic mice. The mechanisms of action involve, at least in part, the anorexic effect and the reversal of low circulating levels of adiponectin in obese and diabetic animals. Although the active principles and their precise mechanisms of action remain to be identified, BJ may represent a novel complementary therapy against obesity and diabetes mellitus. Moreover, the identification of active compounds in BJ may result in the discovery of promising new antiobesity and antidiabetic molecules.

Conflict of interest

The authors declare no conflict of interest.

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