Purified Anthocyanin Supplementation Reduces Dyslipidemia, Enhances Antioxidant Capacity, and Prevents Insulin Resistance in Diabetic Patients[1–3]

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Abstract

Background: Oxidative stress plays an essential role in the pathogenesis of type 2 diabetes. Anthocyanin, a natural antioxidant, has been reported to reduce oxidative stress and to attenuate insulin resistance and diabetes in animal models; however, the translation of these observations to humans has not been fully tested.

Objective: This study was designed to investigate the effects of purified anthocyanins on dyslipidemia, oxidative status, and insulin sensitivity in patients with type 2 diabetes.

Methods: A total of 58 diabetic patients were given 160 mg of anthocyanins twice daily or placebo ($n = 29$/group) for 24 wk in a randomized, placebo-controlled, double-blind trial. Participants and investigators were masked to treatment allocation.

Results: Anthocyanin supplementation significantly decreased serum LDL cholesterol (by 7.9%; $P < 0.05$), triglycerides (by 23.0%; $P < 0.01$), apolipoprotein (apo) B-48 (by 16.5%; $P < 0.05$), and apo C-III (by 11.0%; $P < 0.01$) and increased HDL cholesterol (by 19.4%; $P < 0.05$) compared with placebo after the 24-wk intervention. In addition, patients in the anthocyanin group showed higher total radical-trapping antioxidant parameter and ferric ion reducing antioxidant power values than did patients in the placebo group (both $P < 0.05$). Serum concentrations of 8-iso-prostaglandin F2α, 13-hydroxyoctadecadienoic acid, and carbonylated proteins in patients in the anthocyanin group were significantly less than in patients in the placebo group (23.4%, 25.8%; $P < 0.01$ and 20%; $P = 0.022$, respectively). Furthermore, supplementation with anthocyanin lowered fasting plasma glucose (by 8.5%; $P < 0.05$) and homeostasis model assessment for insulin resistance index (by 13%; $P < 0.05$), and elevated serum adiponectin (by 23.4%; $P < 0.01$) and β-hydroxybutyrate (by 42.4%; $P = 0.01$) concentrations compared with placebo supplementation.

Conclusion: These findings demonstrate that anthocyanin supplementation exerts beneficial metabolic effects in subjects with type 2 diabetes by improving dyslipidemia, enhancing antioxidant capacity, and preventing insulin resistance. This trial was registered at www.clinicaltrials.gov as NCT02317211. *J Nutr* 2015;145:742–8.

Keywords: anthocyanin, antioxidant capacity, dyslipidemia, insulin resistance, type 2 diabetes

Introduction

The worldwide epidemic of type 2 diabetes is a major public health problem, and recent studies estimate that >550 million people will be afflicted with diabetes by 2030 (1). Many studies indicate that in poorly controlled diabetes, altered insulin signaling, hyperglycemia, or both promote unbalanced lipid metabolism and hypertriglyceridemia (2). Furthermore, the increase in oxidative stress status is well documented to play a central role in the pathogenesis of metabolic and pathophysiologic complications associated with insulin resistance and type 2 diabetes (3–5). A limitation of currently available drug classes for the treatment of risk factors associated with type 2 diabetes is that no single agent (e.g., statin, fibrate, rosiglitazone, or metformin) is able to address more than one comorbid condition. Thus, many individual therapies are usually prescribed in combination, leading to tolerability issues, poor patient compliance, and suboptimal outcomes, all of which provide an incentive for finding new therapeutic approaches.

Anthocyanins are versatile and plentiful flavonoid pigments found in fruit and vegetables with dark color, including purple cabbage, purple grapes, blueberries, cherries, raspberries, and black rice (6–8). Accumulating evidence has shown that anthocyanins display a wide range of biological activities, including antioxidant, anti-inflammatory, antimicrobial, and anticarcinogenic activities (6–8). A higher consumption of anthocyanins and anthocyanin-rich fruit was associated with a lower risk of type 2 diabetes in large cohort studies (9, 10). Animal intervention studies specifically showed that anthocyanin-rich foods and beverages and purified anthocyanins regulated glucose metabolism, enhanced insulin sensitivity, and improved β cell dysfunction in animal models of type 2 diabetes (11–13). Results from mechanistic studies suggest that anthocyanin may decrease glycemia and exert insulin-like effects through regulating transcriptional factor Forkhead box O1 (FoxO1)[6] and nuclear receptor PPAR-γ and increasing the secretion of adiponectin, an insulin-sensitizing adipokine (14–16). However, so far, no human studies have systematically examined the metabolic effects of purified anthocyanins in subjects with type 2 diabetes. Thus, we conducted a long-term randomized trial to characterize the potential effects of anthocyanins, purified from bilberries and blackcurrants, on metabolic abnormalities commonly associated with type 2 diabetes.

Methods

Materials. Anthocyanin (Medox) and placebo capsules were provided by the Biolink Group. Each capsule of Medox contains 80 mg anthocyanin, which comprises 17 different natural anthocyanins purified from the bilberry (*Vaccinium myrtillus*) and blackcurrant (*Ribes nigrum*). The relative content of each anthocyanin was reported previously (17). In addition, anthocyanin capsules also contained pullulan, maltodextrin, and citric acid (at 4% per capsule, which helped to maintain the stability of the anthocyanins), whereas the placebo capsules contained only pullulan and maltodextrin. The anthocyanin and placebo capsules were identically packaged.

Study subjects and design. A total of 58 adult subjects with type 2 diabetes aged 56–67 y were recruited into this clinical trial between November 2008 and December 2010 from physical examination centers in one hospital in Guangzhou, Guangdong, China. We excluded recently diagnosed diabetes, pregnancy, coronary artery disease, and other known chronic diseases. The study was approved by the ethics committee of Sun Yat-sen University and was conducted in accordance with the Declaration of Helsinki. Participation was voluntary, and each participant provided written informed consent. For the intervention study, eligible participants were randomly assigned in a double-blind, placebo-controlled trial to either a placebo group (n = 29) or an anthocyanin group (n = 29). The total duration of this trial was 24 wk, and during the intervention the subjects were asked to maintain their habitual diet, lifestyle, and medications. The anthocyanin group was instructed to consume two 80-mg anthocyanin capsules twice daily (30 min after breakfast and supper) for a daily intake of 320 mg anthocyanins, which corresponds to ~100 g of a mixture of fresh blueberries and blackcurrants. The duration and dosage of treatment were according to our previous intervention studies (17–19). During each 4-wk visit, adherence of the subjects to the protocol was assessed by counting the remaining capsules and obtaining the related information, and repeated if necessary. Meanwhile, the new capsules were dispensed, and body weight, blood pressure, and waist and hip circumferences were measured. At baseline and at the end

of the trial, all patients were required to fast overnight to enable blood sample collection the next morning.

Dietary assessment. Subjects were asked to complete a 3-d (including 2 workdays and 1 weekend day) record of their food intake, which was analyzed by using CDGSS3.0 software (West China Center for Medical Science) to estimate nutrient intake at baseline and at 24 wk. A questionnaire survey was undertaken for each subject at baseline and contained questions regarding dietary habits (including how much and how often the participants consumed meat, milk, eggs, and vegetables, etc., and whether any vitamin or mineral supplements were commonly taken). Dietary flavonoid and anthocyanin intakes as well as overall dietary intake from food and beverages consumed by participants were assessed with a quantitative FFQ and calculated by using the updated USDA databases for the flavonoid and anthocyanin contents of food as primary data sources (20, 21).

Blood sampling and analysis. Serum lipid concentrations, fasting plasma glucose and insulin, and glycated hemoglobin were measured as previously described (16, 22). Serum apo C-III was determined by immunonephelometry (Siemens Healthcare Diagnostics). Serum FFAs were measured by a commercially available kit (Wako Pure Chemical Industries). Plasma β-hydroxybutyrate concentrations were determined enzymatically by using a colorimetric assay kit (ARUP Laboratories). HOMA-IR was calculated according to the equation fasting insulin (μU/mL) × fasting glucose (mmol/L)/24.

Oxidative stress markers. Plasma concentrations of 8-isoprostaglandin F2α (8-iso-PGF$_{2\alpha}$), 13-hydroxyoctadecadienoic acid (13-HODE), and protein carbonyls were quantitatively measured by using a colorimetric ELISA kit (Enzo Life Sciences International).

Antioxidant capacity. Plasma total radical-trapping antioxidant parameter (TRAP) was measured on the basis of the ability of plasma to prevent the decay of R-phycoerythrin induced by peroxyl radicals by using Northwest Life Science Specialities Total Antioxidant Capacity assay kit (catalog no. NWK-TAC01; Northwest Life Science Specialties). Plasma ferric reducing antioxidant power (FRAP) was determined on the basis of the ability of plasma to reduce ferric-tripyridyltriazine to its ferrous colored form by using a FRAP clorimetric detection kit (catalog no. K043-H1; Arbor Assays) (23, 24).

Serum adiponectin measurement. The concentrations of human total adiponectin (catalog no. DRP300) and the high-molecular-weight (HMW) form of adiponectin (catalog no. DHWAD0) were measured in stored frozen serum by using a commercially available competitive ELISA kit (R&D Systems), as previously described (16, 25).

Plasma anthocyanins. Plasma concentrations of anthocyanin were measured according to our previous study (19). The plasma anthocyanins were then analyzed by using an HP 1200 Series HPLC system equipped with an Ultimate XB-C18 column (4.6 × 250 mm) and a UV-visible detector at 520 nm.

Statistical analysis. All statistical analyses were performed by using SPSS for Windows software (version 17.0). We describe patient characteristics using means ± SEMs for continuous variables and numbers (percentages) for categorical variables. Differences (including between men and women) in these variables between placebo and anthocyanin groups at baseline were evaluated by using Student's t test for independent samples. Differences in postintervention outcome measures between the placebo and anthocyanin groups were evaluated by ANCOVA with the pretreatment values as covariates. Significance was set at $P < 0.05$.

Results

Anthropometric characteristics and dietary intake of diabetic patients. **Table 1** summarizes the physical characteristics of and medications used by the study subjects. The distribution

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[6] Abbreviations used: FoxO1, Forkhead box O1; FRAP, ferric ion reducing antioxidant power; HMW, high-molecular-weight; Sirt1, silent mating type information regulation 2 homolog 1; TRAP, total radical-trapping antioxidant parameter; 8-iso-PGF$_{2\alpha}$, 8-iso-prostaglandin F2α; 13-HODE, 13-hydroxyoctadecadienoic acid.

TABLE 1 Baseline characteristics of subjects in the placebo and anthocyanin groups[1]

	Placebo	Anthocyanin	P
Sex (M/F), n/n	17/12	17/12	—
Age, y	57.6 ± 3.4	58.1 ± 2.3	0.460
BMI, kg/m^2	23.9 ± 3.5	24.2 ± 3.1	0.66
Antihypertensive drugs, n (%)	2 (6.9)	1 (3.5)	0.05
Lipid-lowering drugs, n (%)	2 (6.9)	2 (6.9)	0.72
Oral glucose-lowering therapy, n (%)	15 (51.7)	14 (48.3)	0.19
Insulin, n (%)	4 (13.8)	3 (10.4)	0.12
Vitamin supplement (yes), n (%)	2 (6.9)	2 (6.9)	—

[1] Values are means ± SEMs unless otherwise indicated. There were no significant differences between the placebo and anthocyanin groups at baseline for any variable by independent-samples t test.

of age, sex, BMI, and medication usage was uniform between the 2 groups. There were also no significant differences in daily mean energy and energy-producing nutrient intakes of diabetic patients between the placebo and anthocyanin groups (**Supplemental Table 1**). No subjects reported any adverse events resulting from the consumption of either the placebo or anthocyanin capsules throughout the intervention.

Compliance. According to the count of the recalled capsules at every visit, compliance was very good. The rates of capsule intake were 98.1% and 98.5% in the placebo and anthocyanin groups, respectively. Compliance in the anthocyanin group was also confirmed by the increase in plasma anthocyanin concentrations, including cyanidin-3-O-β-glucoside and delphinidin-3-O-β-glucoside.

Effects of anthocyanin on blood lipids and lipoproteins. At baseline, anthocyanin could not be detected in the circulation. Plasma concentrations of anthocyanin were higher in subjects who received the anthocyanin capsules after the 24-wk intervention. Serum concentrations of lipids did not differ between the placebo and anthocyanin groups at baseline. Serum HDL cholesterol concentrations increased by 19.4% ($P < 0.05$) in the

anthocyanin group between baseline and after the 24-wk intervention. Serum concentrations of LDL cholesterol and TGs decreased significantly by 7.9% ($P < 0.05$) and 23.0% ($P < 0.01$), respectively, in the anthocyanin group but did not change significantly in the placebo group by the end of the intervention. Furthermore, serum apo B-48 and apo C-III concentrations were reduced by 16.5% ($P < 0.05$) and 11.0% ($P < 0.01$), respectively, in the anthocyanin group but not in the placebo group. However, no significant differences in serum concentrations of apo A-I, apo B-100, and FFAs were observed between the placebo and anthocyanin groups at baseline and after the 24-wk intervention (**Table 2**).

Effects of anthocyanin on antioxidant capacity. At baseline, plasma TRAP and FRAP did not differ between the placebo and anthocyanin groups. After the 24-wk intervention, patients in the anthocyanin group had higher TRAP and FRAP values than did patients in the placebo group (**Table 3**). Baseline serum 8-iso-PGF$_{2\alpha}$ and 13-HODE concentrations did not differ between the placebo and anthocyanin groups. Anthocyanin supplementation for 24 wk decreased serum 8-iso-PGF$_{2\alpha}$ and 13-HODE compared with placebo supplementation (Table 3). Plasma protein carbonyl contents did not differ at baseline between the placebo and anthocyanin groups but decreased significantly (22.4%; $P < 0.05$) in the anthocyanin group. This reduction was not observed in the placebo group (Table 3).

Effects of anthocyanin on insulin resistance. At baseline, fasting plasma glucose was not significantly different in the placebo group over the 24-wk treatment period. HOMA-IR, serum IL-6, and TNF-α were significantly lower between 24 wk and baseline in the anthocyanin group but not in the placebo group (**Table 4**). Furthermore, plasma concentrations of adiponectin and β-hydroxybutyrate and the proportion of HMW adiponectin were higher between 24 wk and baseline in the anthocyanin group but not in the placebo group (Table 4).

Discussion

Anthocyanins, which were discovered to be a potent antioxidant, have been extensively studied in animal and cellular

TABLE 2 Anthropometric data and lipid profiles of diabetic patients in the placebo and anthocyanin groups at baseline and after the 24-wk intervention[1]

	Placebo		Anthocyanin		P[2]
	Baseline	24 wk	Baseline	24 wk	
Plasma anthocyanin (Cy3g and Dp3g), nmol/L	Not detectable	Not detectable	Not detectable	9.37 ± 1.06*	<0.01
Systolic blood pressure, mm Hg	128 ± 10	129 ± 9	130 ± 13	126 ± 11	0.034
Diastolic blood pressure, mm Hg	81 ± 9	82 ± 7	82 ± 8	80 ± 10	0.16
Serum total cholesterol, mmol/L	5.03 ± 0.78	4.99 ± 0.86	5.07 ± 0.89	4.88 ± 0.94*	0.041
Serum LDL cholesterol, mmol/L	3.19 ± 0.42	3.21 ± 0.48	3.17 ± 0.35	2.92 ± 0.54*	0.030
Serum HDL cholesterol, mmol/L	0.98 ± 0.08	0.95 ± 0.07	1.03 ± 0.11	1.23 ± 0.12*	0.012
Serum TGs, mmol/L	2.02 ± 0.36	1.96 ± 0.45	2.04 ± 0.41	1.57 ± 0.72**	<0.01
Serum apo A-I, g/L	1.35 ± 0.24	1.32 ± 0.36	1.33 ± 0.32	1.39 ± 0.43*	0.13
Serum apo B-48, mg/L	0.95 ± 0.17	0.93 ± 0.22	0.97 ± 0.20	0.81 ± 0.27*	0.017
Serum apo B-100, g/L	5.93 ± 1.44	5.85 ± 1.08	5.88 ± 1.37	5.66 ± 1.22	0.09
Serum apo C-III, mg/L	134 ± 15	136 ± 14	137 ± 18	122 ± 13*	<0.01
Serum FFAs, mmol/L	0.75 ± 0.19	0.77 ± 0.28	0.77 ± 0.16	0.73 ± 0.22	0.15

[1] Values are means ± SEMs, n = 29/group. No significant differences were found for any variable between the placebo and anthocyanin groups at baseline by unpaired Student's t test. *,**Different from baseline: *$P < 0.05$, **$P < 0.01$. Cy3g, cyanidin-3-O-β-glucoside; Dp3g, delphinidin-3-O-β-glucoside.
[2] P values for differences between placebo and anthocyanin groups after the 24-wk intervention.

TABLE 3 Antioxidant capacity of diabetic patients in the placebo and anthocyanin groups at baseline and after the 24-wk intervention[1]

	Placebo		Anthocyanin		
	Baseline	24 wk	Baseline	24 wk	P[2]
Plasma FRAP, mmol Fe^{2+}/L	1.02 ± 0.13	1.04 ± 0.11	1.04 ± 0.08	1.35 ± 0.14*	0.013
Plasma TRAP, mmol/L	1.09 ± 0.06	1.12 ± 0.08	1.07 ± 0.09	1.33 ± 0.10*	0.017
Plasma 8-iso-PGF$_{2\alpha}$, pmol/mL	11.6 ± 2.78	11.4 ± 3.13	11.5 ± 3.55	8.73 ± 2.86**	<0.01
Plasma 13-HODE, pmol/mL	28.8 ± 4.87	27.9 ± 5.38	29.0 ± 6.25	20.7 ± 5.93**	<0.01
Plasma carbonylated protein, nmol/mg	0.68 ± 0.05	0.65 ± 0.03	0.67 ± 0.07	0.52 ± 0.03*	0.022

[1] Values are means ± SEMs, n = 29/group. No significant differences were found for any variable between placebo and anthocyanin groups at baseline by unpaired Student's t test. *,**Different from baseline: *$P < 0.05$, **$P < 0.01$. FRAP, ferric ion reducing antioxidant power; TRAP, total radical-trapping antioxidant parameter; 8-iso-PGF$_{2\alpha}$, 8-iso-prostaglandin F2α; 13-HODE, 13-hydroxyoctadecadienoic acid.

[2] P values for differences between placebo and anthocyanin groups at 24 wk.

studies and have shown promising results on energy metabolism and metabolic profile (11, 14, 15). Here, we show that anthocyanin supplementation in diabetic subjects exerted favorable metabolic adaptations by improving adverse pro-atherogenic lipid changes, elevating systematic antioxidant capacity, and increasing insulin sensitivity. Because no significant differences were observed between the responses of men and women to anthocyanin supplementation, all of the results were analyzed together. These data extend findings that so far have been observed only in cell and rodent models to humans, showing that anthocyanin has the potential to improve metabolic health in subjects at risk of developing diabetes and metabolic syndrome.

Dyslipidemia is one of the major risk factors for cardiovascular disease in type 2 diabetes. The characteristic features of diabetic dyslipidemia include elevated plasma concentrations of TGs and apo B–containing lipoproteins, low HDL-cholesterol concentrations, and increased concentrations of small, dense LDL-cholesterol particles (26–30). Thus, the potential of polyphenols in controlling dyslipidemia is currently under intensive study. However, although indications for positive effects of anthocyanin-rich foods and anthocyanins on diabetic lipid profiles have been obtained in vitro and in animal studies (11, 14, 31–34), definitive conclusions in diabetic patients are still lacking. In this study, we extended our findings to diabetic

patients and found that berry food derived anthocyanin intervention resulted in a dual beneficial effect in diabetic patients by increasing HDL-cholesterol and lowering LDL-cholesterol concentrations, resulting in a significant improvement of lipid profiles. Changes in these risk factors, which are of the same magnitude as those observed in this study, are clinically relevant. To put this into perspective, it was reported that the rate of cardiovascular disease events is reduced by nearly 1% for each 1% reduction in LDL cholesterol and by ≥1% for each 1% increase in HDL cholesterol (35). Therefore, the 7.9% decrease in LDL cholesterol and the 19.4% increase in HDL cholesterol observed in the present study would result in a nearly 27.3% reduction in coronary artery disease risk. Thus, our findings may provide a novel rationale to the potential clinical impact of anthocyanins for protection against vascular complications in diabetes. It is generally known that the bioavailability of anthocyanins is lower and rapidly biotransformed into the phenolic acid metabolites after ingestion. Indeed, the native forms of anthocyanins are poorly present in the bloodstream (15, 36), and they might be in the form of metabolites such as protocatechuic acid, which may reach tissues and exert biological effects.

Patients with type 2 diabetes often display hypertriglyceridemia associated with increased plasma apo C-III concentrations,

TABLE 4 Serum adipokine and proinflammatory molecules in diabetic patients in the placebo and anthocyanin groups at baseline and after the 24-wk intervention[1]

	Placebo		Anthocyanin		
	Baseline	24 wk	Baseline	24 wk	P[2]
BMI, kg/m^2	25.3 ± 2.5	25.4 ± 2.9	25.1 ± 2.7	25.0 ± 3.2	0.19
Fat mass, % (body weight)	35.2 ± 5.9	34.8 ± 5.3	35.4 ± 6.1	34.6 ± 6.5	0.13
Fasting plasma glucose, mmol/L	7.3 ± 1.7	7.1 ± 1.5	7.1 ± 2.2	6.5 ± 1.8*	0.042
Plasma insulin, mU/L	11.6 ± 4.13	11.7 ± 3.76	11.9 ± 4.30	11.1 ± 3.98	0.14
Plasma Hb A$_{1c}$, %	6.6 ± 1.5	6.5 ± 1.4	6.5 ± 1.7	6.2 ± 1.9	0.06
HOMA-IR	3.76 ± 0.53	3.69 ± 0.64	3.74 ± 0.55	3.21 ± 0.76*	0.035
Serum adiponectin, μg/mL	5.05 ± 0.79	5.09 ± 0.84	5.08 ± 0.92	6.28 ± 0.96**	<0.01
Serum HMW adiponectin, μg/mL	2.23 ± 0.56	2.16 ± 0.52	2.21 ± 0.67	3.26 ± 0.73**	<0.01
HMW:total adiponectin ratio, %	44.2 ± 6.52	42.6 ± 5.93	43.6 ± 6.79	51.9 ± 7.08*	0.024
Serum IL-6, pg/mL	3.26 ± 0.57	3.18 ± 0.63	3.23 ± 0.49	2.21 ± 0.42**	0.021
Serum TNF-α, pg/mL	16.2 ± 2.35	15.9 ± 2.67	16.2 ± 2.58	14.8 ± 2.13*	0.045
Plasma β-hydroxybutyrate, mg/dL	1.14 ± 0.37	1.18 ± 0.46	1.17 ± 0.42	1.68 ± 0.51**	0.010

[1] Values are means ± SEMs, n = 29/group. No significant differences were found for any variable between placebo and anthocyanin groups at baseline by unpaired Student's t test. *,**Different from baseline: *$P < 0.05$, **$P < 0.01$. Hb A$_{1c}$, glycated hemoglobin; HMW, high-molecular-weight.

[2] P values for differences between placebo and anthocyanin groups after the 24-wk intervention.

and modulating apo C-III per se is a novel and potent therapeutic approach to managing diabetic dyslipidemia (37). Our findings showed that anthocyanin treatment caused a significant reduction in serum TG concentrations. The TG-lowering effect observed in anthocyanin-treated subjects resulted primarily from reductions in serum apo B– and apo C-III–containing TG-rich particles because anthocyanin intervention also markedly decreased serum apo B and apo C-III concentrations. Thus, we demonstrate, for the first time to our knowledge, that anthocyanin supplementation was able to reduce potentially pro-atherogenic remnant particles, a prominent component of diabetic dyslipidemia.

A growing body of evidence suggests that oxidative stress due to excessive production of reactive oxygen species plays a pivotal role in the pathogenesis of diabetic complications under hyperglycemic conditions (38–40). Strategies to decrease intracellular reactive oxygen species concentrations have therapeutic potential in treating diabetes and its complications. The antioxidant and free radical quenching properties of anthocyanins have been highlighted in recent years (41–43). Therefore, we hypothesized that supplementation with anthocyanin in diabetic patients would affect oxidative stress status. We noted that diabetic patients in the anthocyanin group had a marked increase in serum antioxidant capacity, assessed by TRAP and FRAP assays, and a decrease in oxidative stress, assessed by measuring serum 8-iso PGF$_{2\alpha}$, 13-HODE, and protein carbonylation compared with the placebo group. These data suggest that the endogenous antioxidant defense system was activated by anthocyanin to scavenge excessive free radicals and to attenuate oxidative damage products in diabetic subjects.

Our data also point toward favorable effects on glucose homeostasis after 24 wk of anthocyanin supplementation in diabetic subjects. Indeed, HOMA-IR was improved after anthocyanin supplementation, suggesting favorable effects on insulin sensitivity. These beneficial effects of anthocyanin on metabolism are in concordance with animal studies. Because consumption of anthocyanin supplements has increased, interest in their possible interactions with drug-metabolizing enzymes has increased. In this regard, anthocyanin supplementation may affect the clearance of medications with lipid-lowering and/or glucose-lowering activities in our study patients. However, a recent study reported that anthocyanin-rich food caused only mild changes in some activities of drug-metabolizing enzymes in rat liver, whereas those in the small intestine were not affected. Thus, the consumption of anthocyanin-rich food in reasonable amounts seems to be safe, and serious supplement-drug interactions do not seem probable (44).

Adiponectin was shown to have some insulin-sensitizing properties (45) and to be decreased in the serum of insulin-resistant, diabetic, and obese subjects (46). Thus, adiponectin has often been considered as a critical target for developing therapeutic strategies for diabetes (47). The induction of adiponectin expression by the anthocyanin cyanidin-3-O-β-glucoside was reported in previous cellular and animal studies (48–50). Our recent studies extended these findings to human subjects (16), demonstrating that 12 wk of anthocyanin supplementation increased serum adiponectin concentrations in patients with type 2 diabetes. In accordance with these findings, our current study showed that diabetic subjects in the anthocyanin group had higher serum adiponectin concentrations than did those in the placebo group after the 24-wk intervention. Correct oligomerization of adiponectin protein was essential for biological activity, and a previous study showed that the HMW complex was the most active form of adiponectin in lowering blood glucose concentrations in mice (51). The present study also showed that anthocyanin supplementation could increase the HMW form of adiponectin, demonstrating the elevated, biologically active adiponectin. This effect was not observed in subjects from the placebo group. The underlying molecular mechanism for increased serum adiponectin concentrations was mainly attributed to the ability of anthocyanin to significantly stimulate the expression and secretion of adiponectin in adipocytes via silent mating type information regulation 2 homolog 1 (Sirt1)–dependent FoxO1 acetylation (16). β-Hydroxybutyrate is a "ketone body" that is produced in the liver, mainly from the oxidation of FAs, and is exported to peripheral tissues for use as an energy source (52). A significant increase in β-hydroxybutyrate was observed in the anthocyanin-supplemented group, thus implying enhanced FA oxidation and an increase in total body energy expenditure. Furthermore, the concentration of β-hydroxybutyrate was in the normal range (0.2~3.5 mg/dL), indicating that anthocyanin supplementation did not increase the risk of ketoacidosis in diabetic patients.

Several limitations should be discussed. First, although we found that anthocyanin supplementation ameliorated the insulin resistance, we could not determine whether these effects were attributed to the improvement in whole-body insulin sensitivity. Second, the intervention was conducted in lean patients with apparently well-controlled diabetes. However, the results need to be carefully extended to the general population. Additional randomized controlled studies are still needed to assess the potential benefits of anthocyanin supplementation in metabolically normal individuals. Finally, the dose-dependent effects of anthocyanin on metabolic disorders are still unknown and should be established in future studies.

In conclusion, we demonstrate beneficial effects of anthocyanin supplementation on metabolic disorders in subjects with type 2 diabetes. The protective mechanism by anthocyanin on diabetes was closely correlated with prevention of dyslipidemia, lower systemic oxidative damage, and an increase in insulin sensitivity. Furthermore, there were no effects and no adverse events were reported. Therefore, anthocyanin supplementation has the potential to overcome the metabolic aberrations that are associated with diabetes in humans.

References

1. Whiting DR, Guariguata L, Weil C, Shaw J. IDF diabetes atlas: global estimates of the prevalence of diabetes for 2011 and 2030. Diabetes Res Clin Pract 2011;94:311–21.

2. Sacks FM, Hermans MP, Fioretto P, Valensi P, Davis T, Horton E, Wanner C, Al-Rubeaan K, Aronson R, Barzon I, et al. Association between plasma triglycerides and high-density lipoprotein cholesterol and microvascular kidney disease and retinopathy in type 2 diabetes mellitus: a global case-control study in 13 countries. Circulation 2014;129:999–1008.

3. Furukawa S, Fujita T, Shimabukuro M, Iwaki M, Yamada Y, Nakajima Y, Nakayama O, Makishima M, Matsuda M, Shimomura I. Increased oxidative stress in obesity and its impact on metabolic syndrome. J Clin Invest 2004;114:1752–61.

4. Pereira S, Park E, Mori Y, Haber CA, Han P, Uchida T, Stavar L, Oprescu AI, Koulajian K, Ivovic A, et al. FFA-induced hepatic insulin resistance in vivo is mediated by PKCδ, NADPH oxidase, and oxidative stress. Am J Physiol Endocrinol Metab 2014;307:E34–46.

5. Rains JL, Jain SK. Oxidative stress, insulin signaling, and diabetes. Free Radic Biol Med 2011;50:567–75.

6. He J, Giusti MM. Anthocyanins: natural colorants with health-promoting properties. Annu Rev Food Sci Technol 2010;1:163–87.

7. Kong JM, Chia LS, Goh NK, Chia TF, Brouillard R. Analysis and biological activities of anthocyanins. Phytochemistry 2003;64:923–33.

8. Williams CA, Grayer RJ. Anthocyanins and other flavonoids. Nat Prod Rep 2004;21:539–73.

9. Wedick NM, Pan A, Cassidy A, Rimm EB, Sampson L, Rosner B, Willett W, Hu FB, Sun Q, van Dam RM. Dietary flavonoid intakes and risk of type 2 diabetes in US men and women. Am J Clin Nutr 2012;95:925–33.

10. Jennings A, Welch AA, Spector T, Macgregor A, Cassidy A. Intakes of anthocyanins and flavones are associated with biomarkers of insulin resistance and inflammation in women. J Nutr 2014;144:202–8.

11. Guo H, Xia M, Zou T, Ling W, Zhong R, Zhang W. Cyanidin 3-glucoside attenuates obesity-associated insulin resistance and hepatic steatosis in high-fat diet-fed and db/db mice via the transcription factor FoxO1. J Nutr Biochem 2012;23:349–60.

12. Takikawa M, Inoue S, Horio F, Tsuda T. Dietary anthocyanin-rich bilberry extract ameliorates hyperglycemia and insulin sensitivity via activation of AMP-activated protein kinase in diabetic mice. J Nutr 2010;140:527–33.

13. Sasaki R, Nishimura N, Hoshino H, Isa Y, Kadowaki M, Ichi T, Tanaka A, Nishiumi S, Fukuda I, Ashida H, et al. Cyanidin 3-glucoside ameliorates hyperglycemia and insulin sensitivity due to downregulation of retinol binding protein 4 expression in diabetic mice. Biochem Pharmacol 2007;74:1619–27.

14. Guo H, Guo J, Jiang X, Li Z, Ling W. Cyanidin-3-O-β-glucoside, a typical anthocyanin, exhibits antilipolytic effects in 3T3-L1 adipocytes during hyperglycemia: involvement of FoxO1-mediated transcription of adipose triglyceride lipase. Food Chem Toxicol 2012;50:3040–7.

15. Scazzocchio B, Varì R, Filesi C, D'Archivio M, Santangelo C, Giovannini C, Iacovelli A, Silecchia G, Li Volti G, Galvano F, et al. Cyanidin-3-O-β- glucoside and protocatechuic acid exert insulin-like effects by upregulating PPARγ activity in human omental adipocytes. Diabetes 2011;60:2234–44.

16. Liu Y, Li D, Zhang Y, Sun R, Xia M. Anthocyanin increases adiponectin secretion and protects against diabetes-related endothelial dysfunction. Am J Physiol Endocrinol Metab 2014;306:E975–88.

17. Qin Y, Xia M, Ma J, Hao Y, Liu J, Mou H, Cao L, Ling W. Anthocyanin supplementation improves serum LDL- and HDL-cholesterol concentrations associated with the inhibition of cholesteryl ester transfer protein in dyslipidemic subjects. Am J Clin Nutr 2009;90:485–92.

18. Zhu Y, Huang X, Zhang Y, Wang Y, Liu Y, Sun R, Xia M. Anthocyanin supplementation improves HDL-associated paraoxonase 1 activity and enhances cholesterol efflux capacity in subjects with hypercholesterolemia. J Clin Endocrinol Metab 2014;99:561–9.

19. Zhu Y, Xia M, Yang Y, Liu F, Li Z, Hao Y, Mi M, Jin T, Ling W. Purified anthocyanin supplementation improves endothelial function via NO-cGMP activation in hypercholesterolemic individuals. Clin Chem 2011;57:1524–33.

20. US Department of Agriculture. USDA database for the flavonoid content of selected foods. Release 2.1. Washington (DC): USDA; 2007.

21. Jennings A, Welch AA, Fairweather-Tait SJ, Kay C, Minihane AM, Chowienczyk P, Jiang B, Cecelja M, Spector T, Macgregor A, et al. Higher anthocyanin intake is associated with lower arterial stiffness and central blood pressure in women. Am J Clin Nutr 2012;96:781–8.

22. Meyer KA, Conigrave KM, Chu NF, Rifai N, Spiegelman D, Stampfer MJ, Rimm EB. Alcohol consumption patterns and HbA1c, C-peptide and insulin concentrations in men. J Am Coll Nutr 2003;22:185–94.

23. Fabbrini E, Serafini M, Colic Baric I, Hazen SL, Klein S. Effect of plasma uric acid on antioxidant capacity, oxidative stress, and insulin sensitivity in obese subjects. Diabetes 2014;63:976–81.

24. Lotito SB, Frei B. The increase in human plasma antioxidant capacity after apple consumption is due to the metabolic effect of fructose on urate, not apple-derived antioxidant flavonoids. Free Radic Biol Med 2004;37:251–8.

25. Pajvani UB, Hawkins M, Combs TP, Rajala MW, Doebber T, Berger JP, Wagner JA, Wu M, Knopps A, Xiang AH, et al. Complex distribution, not absolute amount of adiponectin, correlates with thiazolidinedione-mediated improvement in insulin sensitivity. J Biol Chem 2004;279: 12152–62.

26. Mooradian AD. Dyslipidemia in type 2 diabetes mellitus. Nat Clin Pract Endocrinol Metab 2009;5:150–9.

27. Taskinen MR. Diabetic dyslipidemia: from basic research to clinical practice. Diabetologia 2003;46:733–49.

28. Nicholls SJ, Lundman P, Tardif JC. Diabetic dyslipidemia: extending the target beyond LDL cholesterol. Eur J Cardiovasc Prev Rehabil 2010; 17(Suppl 1):S20–4.

29. Shepherd J. Dyslipidaemia in diabetic patients: time for a rethink. Diabetes Obes Metab 2007;9:609–16.

30. American Diabetes Association. Standards of medical care in diabetes-dyslipidemia/lipid management. Diabetes Care 2010;33 (Suppl 1):S11–61.

31. Brader L, Overgaard A, Christensen LP, Jeppesen PB, Hermansen K. Polyphenol- rich bilberry ameliorates total cholesterol and LDL-cholesterol when implemented in the diet of Zucker diabetic fatty rats. Rev Diabet Stud 2013;10:270–82.

32. Roopchand DE, Kuhn P, Rojo LE, Lila MA, Raskin I. Blueberry polyphenol- enriched soybean flour reduces hyperglycemia, body weight gain and serum cholesterol in mice. Pharmacol Res 2013;68:59–67.

33. Valcheva-Kuzmanova S, Kuzmanov K, Tancheva S, Belcheva A. Hypoglycemic and hypolipidemic effects of Aronia melanocarpa fruit juice in streptozotocin-induced diabetic rats. Methods Find Exp Clin Pharmacol 2007;29:101–5.

34. Guo H, Li D, Ling W, Feng X, Xia M. Anthocyanin inhibits high glucose-induced hepatic mtGPAT1 activation and prevents fatty acid synthesis through PKCζ. J Lipid Res 2011;52:908–22.

35. Brown BG, Stukovsky KH, Zhao XQ. Simultaneous low-density lipoprotein-C lowering and high-density lipoprotein-C elevation for optimum cardiovascular disease prevention with various drug classes, and their combinations: a meta-analysis of 23 randomized lipid trials. Curr Opin Lipidol 2006;17:631–6.

36. Vitaglione P, Donnarumma G, Napolitano A, Galvano F, Gallo A, Scalfi L, Fogliano V. Protocatechuic acid is the major human metabolite of cyanidin-glucosides. J Nutr 2007;137:2043–8.

37. Florez H, Mendez A, Casanova-Romero P, Larreal-Urdaneta C, Castillo-Florez S, Lee D, Goldberg R. Increased apolipoprotein C-III levels associated with insulin resistance contribute to dyslipidemia in normoglycemic and diabetic subjects from a triethnic population. Atherosclerosis 2006;188:134–41.

38. Nishikawa T, Edelstein D, Du XL, Yamagishi S, Matsumura T, Kaneda Y, Yorek MA, Beebe D, Oates PJ, Hammes HP, et al. Normalizing mitochondrial superoxide production blocks three pathways of hyperglycaemic damage. Nature 2000;404:787–90.

39. Yan SF, Ramasamy R, Schmidt AM. Mechanisms of disease: advanced glycation end-products and their receptor in inflammation and diabetes complications. Nat Clin Pract Endocrinol Metab 2008;4:285–93.

40. Brownlee M. Biochemistry and molecular cell biology of diabetic complications. Nature 2001;414:813–20.

41. Wu X, Gu L, Prior RL, McKay S. Characterization of anthocyanins and proanthocyanidins in some cultivars of Ribes, Aronia, and Sambucus and their antioxidant capacity. J Agric Food Chem 2004;52:7846–56.

42. Shih PH, Yeh CT, Yen GC. Anthocyanins induce the activation of phase II enzymes through the antioxidant response element pathway against oxidative stress-induced apoptosis. J Agric Food Chem 2007;55: 9427–35.

43. Amorini AM, Lazzarino G, Galvano F, Fazzina G, Tavazzi B, Galvano G. Cyanidin-3-O-beta-glucopyranoside protects myocardium and erythrocytes from oxygen radical-mediated damages. Free Radic Res 2003; 37:453–60.

44. Bártíková H, Boušová I, Jedličková P, Lněničková K, Skálová L, Szotáková B. Effect of standardized cranberry extract on the activity and expression of selected biotransformation enzymes in rat liver and intestine. Molecules 2014;19:14948–60.

45. Berg AH, Combs TP, Scherer PE. ACRP30/adiponectin: an adipokine regulating glucose and lipid metabolism. Trends Endocrinol Metab 2002;13:84–9.

46. Tan KC, Xu A, Chow WS, Lam MC, Ai VH, Tam SC, Lam KS. Hypoadiponectinemia is associated with impaired endothelium-dependent vasodilation. J Clin Endocrinol Metab 2004;89:765–9.

47. Yamauchi T, Kamon J, Waki H, Terauchi Y, Kubota N, Hara K, Mori Y, Ide T, Murakami K, Tsuboyama-Kasaoka N, et al. The fat-derived hormone adiponectin reverses insulin resistance associated with both lipoatrophy and obesity. Nat Med 2001;7:941–6.

48. Qin B, Anderson RA. An extract of chokeberry attenuates weight gain and modulates insulin, adipogenic and inflammatory signalling pathways in epididymal adipose tissue of rats fed a fructose-rich diet. Br J Nutr 2012;108:581–7.

49. Tsuda T, Ueno Y, Aoki H, Koda T, Horio F, Takahashi N, Kawada T, Osawa T. Anthocyanin enhances adipocytokine secretion and adipocyte-specific gene expression in isolated rat adipocytes. Biochem Biophys Res Commun 2004;316:149–57.

50. Tsuda T, Ueno Y, Yoshikawa T, Kojo H, Osawa T. Microarray profiling of gene expression in human adipocytes in response to anthocyanins. Biochem Pharmacol 2006;71:1184–97.

51. Liu Y, Chewchuk S, Lavigne C, Brûlé S, Pilon G, Houde V, Xu A, Marette A, Sweeney G. Functional significance of skeletal muscle adiponectin production, changes in animal models of obesity and diabetes, and regulation by rosiglitazone treatment. Am J Physiol Endocrinol Metab 2009;297:E657–64.

52. Blázquez C, Woods A, de Ceballos ML, Carling D, Guzman M. The AMP-activated protein kinase is involved in the regulation of ketone body production by astrocytes. J Neurochem 1999;73:1674–82.