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The update of anthocyanins on obesity and type 2 diabetes: Experimental evidence and clinical perspectives

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Abstract With the dramatically increasing prevalence of obesity and type 2 diabetes mellitus (T2DM) worldwide, there is an urgent need for new strategies to combat the growing epidemic of these metabolic diseases. Diet is an essential factor affecting the development of and risk for obesity and T2DM and it can either help or hurt. In searching for preventative and therapeutic strategies, it is therefore advantageous to consider the potential of certain foods and their bioactive compounds to reverse or prevent the pathogenic processes associated with metabolic disease. Anthocyanins are naturally occurring polyphenolic compounds abundant in dark-colored fruits, vegetables and grains. Epidemiological studies suggest that increased consumption of anthocyanins lowers the risk of T2DM. Many *in vitro* and *in vivo* studies also reveal an array of mechanisms through which anthocyanins could prevent or reverse obesity- and T2DM-related pathologies including promotion of antioxidant and anti-inflammatory activities, improvement of insulin resistance, and hypolipidemic and hypoglycemic actions. Here, we summarize the data on anthocyanin-mediated protection against obesity and T2DM and the underlying mechanisms. Further population-based and long-term human intervention studies are necessary to ultimately evaluate the use of anthocyanins for protection/prevention against the development of obesity and T2DM.

Keywords Anthocyanin · Inflammation · Obesity · Oxidative stress · Type 2 diabetes mellitus

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Abbreviations

CAN	Anthocyanin
ACD	Anthocyanidin
AMPK	AMP-activated protein kinase
BMI	Body mass index
C3G	Cyanidin-3-*O*-β-glucoside
CETP	Cholesteryl ester transfer protein
CVD	Cardiovascular disease
GLUT4	Glucose transporter 4
GSH	Glutathione
HDL	High-density lipoproteins
HFD	High fat diet
hs-CRP	High-sensitivity C-reactive protein
IL-8	Interleukin-8
LDL	Low-density lipoproteins
LPS	Lipopolysaccharide
MAPK	Mitogen-activated protein kinase
MCP-1	Monocyte chemotactic protein 1
MMD	Monocyte to macrophage differentiation associator
NF-Kb	Nuclear factor κB
PPARγ	Peroxisome proliferator-activated receptor γ
ROS	Reactive oxygen species
SOD	Superoxide dismutase
T2DM	Type 2 diabetes mellitus
TNFα	Tumor necrosis factor α

1 Introduction

In recent years, the overweight and obese population has reached pandemic levels, leading to a dramatic rise in the incidence of type 2 diabetes mellitus (T2DM) and its associated complications, such as diabetic nephropathy, ischemic heart disease, and stroke. As a result, these comorbidities have collectively become the leading healthcare burden and cause of mortality worldwide [1–3] and have necessitated

development of effective population-wide interventions and policy recommendations to solve these urgent public health problems.

Lifestyle and dietary habits are major factors determining the development and progression of obesity and T2DM. Mounting evidence indicates that dietary modifications that increase fruit and vegetable intake could prevent these chronic degenerative diseases [4]. Fruits and vegetables contain a high amount of water, fiber, vitamins and minerals, but are low in calories. When substituted for high calorie foods, they can aid in controlling body weight [5]. Furthermore, studies have reported the presence of many bioactive compounds called phytonutrients or phytochemicals in fruits and vegetables and their mechanisms of action have been characterized in both cell culture and animal models [6]. Among them, polyphenols, such as resveratrol, catechins and anthocyanins, are of great interest due to their wide distribution in plant foods and potent health-promoting activities [7–9].

Anthocyanins naturally occur in the vacuolar sap of the epidermal tissues of flowers and fruits, imparting a pink, red, blue, or purple color. They belong to the family of compounds known as flavonoids, and are distinguished from other flavonoids as a separate class by their flavylium cation structures [10]. Anthocyanins are present in nature mainly in the form of heterosides. The aglycon form of anthocyanins, also called anthocyanidin, is structurally based on the flavilium ion or 2-phenylbenzopyrilium, and presents hydroxyl and methoxyl groups in different positions. The physical and chemical properties of anthocyanins have been systematically reviewed in several recent articles [10–12]. The aim of this article is to summarize recent studies exploring the relationship between anthocyanins and development of obesity and T2DM, including epidemiological studies, randomized trials, animal models and *in vitro* studies. This review will focus on the putative biological mechanisms through which anthocyanins prevent obesity and diabetes.

2 Anthocyanins intake and their major food source

Anthocyanins are of great nutritional interest, because they are found in a wide variety of plant foods. They are especially abundant in dark-colored fruits such as berries, cherries, hawthorn, peaches, grapes, apples, and plums as well as some dark-colored vegetables, such as red onion, red radish, black beans, eggplant, red cabbage, and purple potatoes [13, 14]. In addition to the fruits and vegetables listed above, anthocyanins also accumulate in pigmented grains, such as black rice, red sorghum, and purple maize [11]. The relative abundance of anthocyanins varies due to different external and internal factors, such as genetic and agronomic variation, light intensity and type, temperature, harvest time, storage and processing technique. For example, the red grape (*Vitis vinifera* L. cv Cabernet Sauvignon) contains anthocyanin at different concentrations depending on the stage of maturity at which it is harvested, with values reaching as high as 1.87 mg/g fresh weight, while in red wines anthocyanin concentrations vary according to grape maturity as well as the type of vinification, sugar concentration and yeast metabolism, with values ranging from 411 to 728 mg/L in young wines [15].

Until recently, assessment of anthocyanin intake was hampered by the limited data on anthocyanins in food composition tables. In 2003, the United States Department of Agriculture (USDA) established the first database for the flavonoid content of 225 kinds of foods. Now, the average anthocyanin content of common foods can be easily retrieved from the new version of the USDA Database (506 food items) and the Phenol-Explorer database (452 food items) [16, 17]. In addition to their natural occurrence in fruits, vegetables and grains, anthocyanins can also be used as colorants in beverages, fruit fillings, snacks and dairy products, accounting for a considerable portion of anthocyanins in the average diet.

Based on the anthocyanin content database and dietary survey assessment, several estimates of anthocyanin intake have been published. In 1976, Kuhnau reported that the average daily intake of anthocyanins in the United States is around 215 mg during the summer and 180 mg during the winter [18]. However, recent investigations by Wu and colleagues that take into consideration more than 100 kinds of common foods estimate values of anthocyanin consumption of only 12.5 mg/d in the United States [13]. In ten countries participating in the European Prospective Investigation into Cancer and Nutrition (EPIC) study, the mean anthocyanidin (aglycone of anthocyanin) intake for each country ranged from 19.8 to 64.9 mg/d [19]. In south China, the average intake of anthocyanidin was estimated at 27.6 mg/d [20], similar to those of Germany (35.1 mg/d), the United Kingdom (26.1 mg/d), Denmark (28.2 mg/d) and the Netherlands (21.9 mg/d) [19]. As one can imagine, dietary habits and choices have a significant impact on anthocyanin consumption. For instance, the traditional Fijian diet contains a lot of meat from sea mammals and land animals, but few fruits and vegetables, and therefore their anthocyanin intake (0.04 mg/d) is far lower than that of Europeans, who eat a mixture of food items [21]. Recently, new intake estimates were published for children and adolescents, allowing a comparison of anthocyanin intake across different ages and countries (Table 1) [22, 23]. It is important to note that deviations may occur due to the older, less complete versions of the USDA database and differences in food items ascertained on dietary questionnaires. It is also plausible that relying solely on dietary questionnaires may have introduced misclassification, as dietary habits are dynamic, frequently changing and evolving.

Table 1 Estimated intake of anthocyanin/anthocyanidin in different ages and countries

Country	Subjects	ACN/ACD intake (mg/d)	References
United States	Adults	ACN: 180 (winter), 215 (summer)	Kuhnau, J., 1976 [18]
United States	Participants of NHANES 2001-2002	ACN: 12.5	Wu et al., 2006 [13]
Fiji	140 households in Fiji Food Choice Survey	ACN: 0.04	Lako et al., 2006 [21]
Australia	Participants of the National Nutrition Survey 1995	*n*=13858, ACN: 2.9	Johannot, et al., 2006 [24]
China	Residents of Guangzhou	Men, *n*=446, ACD: 26.6±0.6[a]; Women, *n*=947, ACD: 27.1±0.4	Li et al., 2013 [20]
France	Participants of the SU.VI.MAX study	Men, *n*=2596, Women, *n*=2364, ACD: 35, ACN: 57	Perez-Jimenez, et aL., 2011[25]
Greece	Participants of EPIC study	Men, *n*=1314, ACD: 37.8±1.3[b]; Women, *n*=1373, ACD: 25.8±1.3	Zamora-Ros et al., 2011 [19]
Spain	Participants of EPIC study	Men, *n*=1377, ACD: 41.7±2.6; Women, *n*=1443, ACD: 22.7±2.8	Zamora-Ros et al., 2011 [19]
Italy	Participants of EPIC study	Men, *n*=1442, ACD:56.5±2.5; Women, *n*=2511, ACD: 35.2±2.1	Zamora-Ros et al., 2011 [19]
Germany	Participants of EPIC study	Men, *n*=2267, ACD: 31.7±1.5; Women, *n*=2148, ACD: 38.4±1.5	Zamora-Ros et al., 2011 [19]
Netherlands	Participants of EPIC study	Men, *n*=1024, ACD:19.8±1.5; Women, *n*=2956, ACD: 23.9±1.3	Zamora-Ros et al., 2011 [19]
United Kingdom	Participants of EPIC study	Men, *n*=516, ACD: 24.8±3.5; Women, *n*=767, ACD: 27.4±2.7	Zamora-Ros et al., 2011 [19]
Denmark	Participants of EPIC study	Men, *n*=1923, ACD: 29.8±1.8; Women, *n*=1994, ACD: 26.6±1.2	Zamora-Ros et al., 2011 [19]
Sweden	Participants of EPIC study	Men, *n*=2700, ACD: 20.7±1.3; Women, *n*=3285, ACD: 20.7±1.2	Zamora-Ros et al., 2011 [19]
Finland	Adults in the FINDIET 2002 Study	Men, *n*=912, ACD: 53 Women, *n*=1095, ACD: 43	Ovaskainen et al., 2008 [26]
Germany	12 months old infants in DONALD Study	*N*=738, ACD: 6.24 (1.29, 7.42)	Drossard et al., 2011 [23]
Germany	3 years old toddlers in DONALD Study	*N*=701, ACD: 8.69 (2.03, 9.94)	Drossard et al., 2011 [23]
Germany	7–12 years old children in DONALD Study	Boys, *n*=599, ACD: 12.06 (5.57, 14.37) Girls, *n*=582, ACD: 12.41 (6.04, 14.47)	Drossard et al., 2013 [22]
Germany	13–18 years old adolescents in DONALD Study	Boys, *n*=368, ACD: 15.23 (2.33, 17.46) Girls, *n*=355, ACD: 12.19 (2.40, 14.54)	Drossard et al., 2013 [22]

[a] Expressed as mean±SEM (all such values); [b] Geometric mean with upper and lower quartiles in parentheses (all such values); *ACN* anthocyanin; *ACD* anthocyanidin; *DONALD* Dortmund Nutritional and Anthropometric Longitudinally Designed, *NHANES* National Health and Nutrition Examination Survey; *EPIC* European Prospective Investigation into Cancer and Nutrition; *SU.VI.MAX* Supplementation of Vitamins, Minerals and Antioxidants

3 Epidemiology and clinical trials of anthocyanins on obesity and T2DM

3.1 Prospective and cross-sectional studies

Several large, prospective and cross-sectional studies assessing the relationship between anthocyanin intake and the incidence of T2DM are summarized in Table 2. In a prospective, population-based cohort study in Finland, dietary questionnaires were collected from 10,054 men and women and an association was found between higher consumption of apples and berries, foods rich in anthocyanins, and a lower risk for T2DM [27]. Similarly, Mursu et al. reported that a higher intake of berries correlated with a significantly reduced (35 % lower) risk of T2DM in middle-aged and older Finnish men, whereas no associations were shown with other fruits and vegetables [28]. In line with these findings, three, large, prospective, cohort studies from the United States that included 200,994 health professionals revealed that consumption of foods rich in anthocyanins, particularly strawberries, blueberries and grapes/raisins, was inversely associated with the risk of hypertension and T2DM [29, 30]. In addition, data from studies assessing individual flavonoid consumption suggest that a mean intake of 22.3 mg/d of anthocyanins correlates with reduced risk of T2DM, whereas no significant associations were found for other flavonoid subclasses or total flavonoid intake [31]. In contrast, a longitudinal analysis within the Framingham Offspring cohort of 2,915 members found an inverse association between higher flavonol intake and risk of T2DM, but there was no specific association with anthocyanins [32]. These

Table 2 Principal prospective and cross-sectional studies of the association between anthocyanin intake and the incidence of T2DM

Study population	Length of follow-up (years)	Adjustments considered	Main results and risk for incident T2DM between the highest and lowest intake– hazard ratios (95 % CI)	References
Finnish Mobile Clinic Health Examination Survey; *n*=10,154	28	Sex and age	A lower risk of T2DM tended to be associated with higher berries intake. HR: 0.74 (0.58, 0.95), *P*=0.03	Knekt et al., 2002 [27]
Middle-aged men in the Kuopio Ischaemic Heart Disease Risk Factor Study in Finland; *n*=2,682	20	Age, examination years, BMI, smoking, physical activity, education, family history of T2DM, energy and alcohol intake	A higher intake of berries was related with significant reduced risk of T2DM. HR: 0.65 (0.49, 0.88), *P*=0.003	Mursu et al., 2014 [28]
Nurses' Health Study in United States; *n*=70,359	24	BMI, ethnicity, physical activity, smoking, family history of T2DM, energy and alcohol intake, menopausal status	Blueberry consumption was inversely associated with risk of T2DM. HR: 0.82 (0.69 to 0.98) Higher intakes of anthocyanins were significantly associated with a lower risk of T2DM. Quintile 5 (Q5)=22.3 mg/d, Q1=2.2 mg/d; HR: 0.83 (0.77, 0.90)	Muraki et al., 2013 [29] Wedick et al., 2012 [31]
Nurses' Health Study II in United States; *n*=89,201	16	BMI, ethnicity, physical activity, smoking, family history of T2DM, energy and alcohol intake, menopausal status	Blueberry consumption was inversely associated with risk of T2DM. HR: 0.69 (0.55 to 0.87) Higher intakes of anthocyanins were significantly associated with a lower risk of T2DM. Q5=24.3 mg/d, Q1=2.0 mg/d; HR: 0.83 (0.73, 0.94)	Muraki et al., 2013 [29] Wedick et al., 2012 [31]
Health Professionals Follow-Up Study in United States, *n*=41,334	20	BMI, ethnicity, physical activity, smoking, family history of T2DM, energy and alcohol intake	Blueberry consumption was inversely associated with risk of T2DM. HR: 0.74 (0.55 to 1.00) Higher intakes of anthocyanins were significantly associated with a lower risk of T2DM. Q5=24.2 mg/d, Q1=2.3 mg/d; HR: 0.93 (0.81, 1.05)	Muraki et al., 2013 [29] Wedick et al., 2012 [31]
Members of the Framingham Offspring cohort in United States, *n*=2,915	17	Age, sex, BMI, energy intake, prevalence of CVD, current smoking	No significant associations between intake of anthocyanins and risk of T2DM. HR: 0.98 (0.88, 1.10)	Jacques et al., 2013 [32]
Women in the TwinsUK registry in United Kingdom, *n*=1,997	Cross-sectional study	Age, BMI, physical activity, smoking, drugs, dietary supplements, energy and alcohol intake, menopausal status	Higher anthocyanins intake were associated with significantly lower index of peripheral insulin resistance. Q 5 (39.9 mg/d) to Q1(3.5 mg/d)=−0.1, *P*-trend=0.04. Higher anthocyanin intake was associated with significantly lower cSBP. Q5 to Q1=−3.0, *P*-trend=0.02	Jennings et al., 2012 [33] Jennings et al., 2014 [34]
Residents of Guangzhou, China, *n*=1,393	Cross-sectional study	Age, energy intake, carbohydrate, total fat, total cholesterol, protein, fiber, vitamin E and vitamin C intake, BMI, smoking, and drinking	Higher daily consumption of anthocyanidins was associated with elevated serum HDL-cholesterol concentrations. Q3 (31.1 mg/d) to Q1 (20.0 mg/d)=0.18, *P*-trend=0.001	Li, et al., 2013 [20]

HR hazard ratios, 95 % confidence interval in parentheses (all such values); *BMI* body mass index; *cSBP* central systolic blood pressure; *CVD* cardiovascular disease; *HDL* high-density lipoproteins

prospective studies are based on observational data and cannot rule out the potential for residual bias related to lifestyle differences between individuals consuming higher or lower amounts of anthocyanins and other flavonoids.

Several cross-sectional studies confirm the inverse association between anthocyanin intake and markers of metabolic disorders from obesity and T2DM. In a cross-sectional study of 1,997 females from the United Kingdom, higher intake of anthocyanins was associated with significantly lower central systolic blood pressure [33], and lower peripheral insulin resistance resulting from decreased insulin concentrations in blood [34]. The authors also observed that increased anthocyanin consumption correlated with lower concentrations of high-sensitivity C-reactive protein (hs-CRP), a marker of systemic inflammation in obesity and T2DM [35]. In our recent cross-sectional study, residents of south China who consumed higher daily amounts of anthocyanidins had elevated serum high-density lipoprotein (HDL)-cholesterol concentrations, reflecting better lipid profiles [20].

Collectively, the epidemiological data provide clear evidence that increased anthocyanin intake is associated with a lower incidence of T2DM, and suggest that overweight/obese subjects should consider consuming more anthocyanin-rich foods to prevent development and progression of T2DM.

3.2 Intervention studies

To date, the anti-diabetic potentials of anthocyanin-rich foods have been well documented in the context of their antioxidant, anti-inflammatory, and hypolipidemic properties (Table 3). However, it is possible that these findings reflect other dietary components that co-exist in anthocyanin-rich foods, and randomized trials are needed to establish the effects that can be specifically attributed to anthocyanins. Therefore, recent efforts have been directed toward elucidating the impact of consuming purified anthocyanins on the development and progression of obesity and T2DM. A randomized double-blind trial of 32 obese men and women with insulin resistance examined the effect of consuming bioactives extracted from blueberries over the course of 6 weeks. This intervention resulted in a significant improvement of whole-body insulin sensitivity [36]. Recently, we found that in overweight, dyslipidemic patients, consumption of anthocyanins isolated from berries produced favorable effects on blood lipoprotein profiles, including increased HDL-cholesterol and decreased low-density lipoproteins (LDL)-cholesterol [37, 38]. Purified anthocyanin supplementation also decreased the activity of plasma cholesteryl ester transfer protein (CETP) and enhanced the activity of HDL-associated paraoxonase-1, leading to an increase in cholesterol efflux capacity and promotion of the antioxidant effects on HDL, respectively [39]. Furthermore, anthocyanin consumption significantly decreased the levels of serum hs-CRP, soluble vascular cell adhesion molecule-1 (sVCAM-1), and interleukin-1β (IL-1β), indicating an attenuated inflammatory response in overweight subjects with hyperlipidemia [40]. The findings were similar in a study of healthy adults consuming 300 mg anthocyanins per day for 3 weeks [41]. Most recently, Liu et al. reported that purified anthocyanin supplementation for 12 weeks significantly reduced fasting blood glucose and increased serum adiponectin concentrations in patients with T2DM [42]. These promising results warrant further long-term clinical trials assessing the effects of anthocyanin consumption on metabolic and cardiovascular health in overweight, obese and diabetic people.

4 Laboratory studies of anthocyanins on obesity and T2DM

In recent years, many *in vitro* and *in vivo* studies have been conducted to investigate the biological effects of anthocyanins and their mechanisms of action. Using both genetic and dietary models of obesity, animal studies have examined the effect of anthocyanins (either anthocyanin extracts from different plants or pure anthocyanin) on obesity- and T2DM-related pathologies, including oxidative stress, chronic inflammation, insulin resistance, hyperlipidemia and hyperglycemia. Mechanistic studies using cell lines and purified enzymes have focused on the ability of anthocyanins to inhibit free radical generation, improve insulin signaling, alter the expression of genes involved in inflammatory response, or directly modulate the activities of key enzymes.

4.1 Inhibition of body weight gain

Controlling weight gain is the first step in treating and preventing obesity and type 2 diabetes. In 2003, Tsuda et al. published the first report on the preventive effects of anthocyanins against obesity [52]. C57BL/6 J mice were fed a high fat diet (HFD) with or without anthocyanin-rich purple corn color (2 g/kg of diet) for 12 weeks. Anthocyanin supplementation was found to significantly reduce HFD-induced body weight gain and fat accumulation in white and brown adipose tissue. Subsequently, anthocyanin extracts from blackberries (*Rubus* sp.), blueberries (*Vaccinium angustifolium*), mulberries (*Morus australis* Poir) and blood oranges (*Citrus sinensis* L. Osbeck) were shown to prevent obesity in mice fed a HFD [53–56]. Wu and colleagues recently reported further evidence supporting a role for anthocyanins in controlling weight gain [57]. They fed mice a HFD for 8 weeks and then the HFD was continued but supplemented with different doses (50, 100, or 200 mg/kg diet) of anthocyanins from honeysuckle (*Lonicera Caerulea* L.) for another 8 weeks. Supplementation of the HFD with 100 or 200 mg/kg suppressed body weight gain by 24 % and 17 %, respectively. In a study of ovariectomized rats, Kaume et al. reported that

Table 3 Anti-diabetic potentials of anthocyanins in clinical trials

Subjects (numbers)	Study design	Intervention duration	Administered form and dosage of anthocyanins	Main results	References
Diabetic patients (n=33); Healthy volunteers (n=28)	Case–control study	2 months	Red orange extract; 6.2 mg/d	↓Blood levels of thiol groups on proteins and free radicals in diabetic patients	Bonina et al., 2002 [43]
Diabetic patients (n=19)	Cross reference	6 weeks	Concentrated sour cherry juice; 720 mg/d	↓Body weight, blood pressure and HbA1c; ↓Plasma total cholesterol and LDL-cholesterol	Ataie-Jafari et al., 2008 [44]
Women with metabolic syndrome (n=16)	Cross reference	4 weeks	Freeze-dried strawberry powder; 154 mg/d	↓Plasma LDL-cholesterol; ↓Plasma oxidized LDL and malondialdehyde	Basu et al., 2009 [45]
Adults with metabolic syndrome (n=44)	Randomized, controlled study	8 weeks	Freeze-dried blueberries beverage; 742 mg/d	↓Plasma oxidized LDL; ↓Serum malondialdehyde and hydroxynonenal concentrations	Basu et al., 2010 [46]
Obese adults (n=16)	Randomized, controlled study	4 weeks	Dried purple or orange carrot; 39.5 mg/d	No significant changes in body mass, blood pressure, plasma lipids or hs-CRP	Wright et al., 2013 [47]
Adults with metabolic syndrome (n=44)	Randomized, controlled study	8 weeks	Fresh bilberries plus dried bilberries; 374 mg/d	↓Serum hs-CRP, IL-6 and IL-12; ↓Expression of *MMD* and *CCR2* in peripheral blood mononuclear cells	Kolehmainen et al., 2012 [48]
Overweight men (n=18)	Randomized, controlled, crossover study	6 weeks	Freeze-dried blueberries powder; 375 mg/d	↓Oxidatively induced DNA damage in blood mononuclear cells	Riso et al., 2013 [49]
Young adults with features of non-alcoholic fatty liver disease (n=44)	Randomized, controlled, crossover study	4 weeks	Bayberry juice; 415 mg/d	↓Plasma levels of protein carbonyl groups, TNFα and IL-8; ↓Plasma apoptotic markers tissue polypeptide-specific antigen and cytokeratin-18 fragment M30	Guo et al., 2014 [50]
Obese adults with hyperlipidemia (n=80)	Randomized, controlled study	2 months	Anthocyanin-rich extract from whortleberry; 1050 mg/d	↓Plasma total cholesterol, triglyceride and LDL- cholesterol; ↑Plasma HDL-cholesterol	Kianbakht et al., 2013 [51]
Overweight adults with hyperlipidemia (n=120)	Randomized, double-blind, controlled study	12 weeks	Anthocyanin-rich extract from bilberry and black currant; 320 mg/d	↑Plasma HDL-cholesterol; ↓Plasma LDL-cholesterol and CETP activity	Qin et al., 2009 [37]
Overweight adults with hypercholesterolemia (n=150)	Randomized, double-blind, controlled study	24 weeks	Anthocyanin-rich extract from bilberry and black currant; 320 mg/d	↑Plasma HDL-cholesterol and paraoxonase-1 activity; ↑Plasma antioxidant and cholesterol efflux capacity; ↓Plasma LDL-cholesterol; ↓Plasma hs-CRP, sVCAM-1, and IL-1β	Zhu et al., 2011 [38] Zhu et al., 2013 [40] Zhu et al., 2014 [39]
T2DM patients (n=58)	Randomized, double-blind, controlled study	12 weeks	Anthocyanin-rich extract from bilberry and black currant; 320 mg/d	↓Fasting blood glucose ↑Serum adiponectin	Liu et al. 2014 [42]

CCR2 chemokine (C-C motif) receptor; *CETP* cholesteryl ester transfer protein; *HDL* high-density lipoproteins; *hs-CRP* high-sensitivity C-reactive protein; *IL-8* interleukin-8; *LDL* low-density lipoproteins; *MMD* monocyte to macrophage differentiation associator; *TNFα* tumor necrosis factor α

treatment with 10 % anthocyanin-rich blackberries protected against body weight gain and hepatic lipid accumulation and this likely resulted from suppression of lipid synthesis in liver and in adipose tissue [58]. Interestingly, Badshah et al. demonstrated that anthocyanins from black soybean can efficiently prevent obesity in rats by inhibiting expression of neuropeptide Y and activating the γ-amino butyric acid (GABA) receptor in the hypothalamus [59]. In summary, studies in rodent models suggest that anthocyanins protect against weight gain and fat accumulation induced by high fat diet or estrogen loss. However, the effects of administering anthocyanins in a powdered mixture remain controversial. The majority of studies evaluating the biological effects of anthocyanins have relied on anthocyanin-rich crude extracts from plant sources, and it is unclear which particular anthocyanin molecular structure is responsible for the anti-obesity activity. Furthermore, based on average weight and daily food intake, the animals in these studies would have consumed approximately 50–300 mg anthocyanins per kilogram body weight per day; such an oral dose would be impractical in humans.

4.2 Relief of oxidative stress

In obesity and prediabetes, chronically elevated insulin levels lead to increased mitochondrial respiration and ultimately increased generation of reactive oxygen species (ROS). Elevated ROS production saturates the neutralizing capacity of antioxidant defenses, resulting in oxidative stress [60]. Prolonged oxidative stress impairs glucose uptake in muscle and adipose tissue and decreases insulin secretion from pancreatic β cells, thereby accelerating the pathologic process of T2DM [61].

The antioxidant properties of anthocyanins are well characterized. Anthocyanins may serve as free radical scavengers, hydrogen-donating compounds, singlet oxygen quenchers, and metal ion chelators, and therefore they rank highly in *in vitro* analyses of antioxidant characteristics such as oxygen radical absorbance capacity (ORAC) and ferric reducing antioxidant potential (FRAP) [62]. The potent antioxidant activities of anthocyanins have been attributed to their molecular structure, particularly the phenolic hydroxyl groups attached to ring structures [63]. However, there is emerging evidence that anthocyanins and their aglycones exert modulatory actions on antioxidant signaling molecules, enzyme activity, and gene expression in both cellular and animal models. Chiang et al. reported that the antioxidant effects of anthocyanin-rich black rice extract are mediated through decreases in free-radical generation as well as increases in hepatic superoxide dismutase (SOD) and catalase activities in C57BL/6 mice [64]. Roy et al. examined the effect of pure anthocyanidin on hyperglycemia-related oxidative damage in rats. Rats with streptozotocin (STZ)-induced diabetes received an intraperitoneal injection of the anthocyanin pelargonidin (3 mg/kg body weight, BW). The injection of pelargonidin resulted in increased serum levels of SOD and catalase, and decreased levels of malondialdehyde and fructosamine [65]. Recently, Zhu et al. observed that treatment of human HepG2 cells with cyanidin-3-*O*-β-glucoside (C3G), the most abundant anthocyanin in plants, increased glutamate–cysteine ligase expression, which in turn mediated a reduction in ROS levels [66]. In the same study, the authors reported that C3G (200 mg/kg diet) increased glutathione (GSH) synthesis in the liver of diabetic *db/db* mice through a protein kinase A (PKA)- and cAMP-response-element binding protein (CREB)- dependent induction of the glutamate–cysteine ligase catalytic subunit [66]. These observations suggest that anthocyanins may modulate antioxidant defense by activating anti-oxidative enzymes and promoting GSH synthesis.

4.3 Regulation of inflammatory response

Inflammation is a key component of obesity-related metabolic disorders such as T2DM. Excess energy intake stimulates over-production of ROS, resulting in metabolic oxidative stress and cellular redox imbalance. This change in cellular redox status activates redox sensitive signaling molecules, including nuclear factor κB (NF-κB), mitogen-activated protein kinases (MAPKs) and other stress signaling molecules, resulting in increased expression of many inflammatory mediators, such as TNFα, IL-6 and monocyte chemotactic protein 1 (MCP-1), which further exacerbate the proinflammatory state.

NF-κB is an oxidative stress-sensitive transcription factor that controls the expression of numerous genes involved in the inflammatory response, and therefore inhibiting NF-κB activation is one potentially effective way to reduce inflammation. In cultured monocytes, anthocyanins isolated from bilberries and black currants efficiently suppressed lipopolysaccharide (LPS)-induced activation of NF-κB [41]. The authors also observed down-regulation of several NF-κB related inflammatory mediators, including IL-8, RANTES (regulated on activation, normal T cell expressed and secreted) and interferon-α. Examining cellular mechanisms more closely, Jeong et al. demonstrated that anthocyanins from black soybean prevented the transcriptional effects of NF-κB by inhibiting nuclear translocation in LPS-stimulated BV2 cells. The mechanisms blocking nuclear translocation of NF-κB involved reducing inhibitor of NF-κB alpha degradation as well as phosphorylation of extracellular signal-regulated kinase, c-Jun N-terminal kinase, and p38 mitogen-activated protein kinase [67]. The reproducibility of this anthocyanin-mediated inhibition of the NF-κB pathway was confirmed using pure anthocyanin C3G in human macrophage and endothelial cells [68–70].

The anti-inflammatory properties of anthocyanins have been demonstrated in animal models as well. In mice with

HFD-induced obesity, supplementation with blueberry powder (2.7 % of total energy) was associated with a ~50 % reduction in the frequency of dead adipocytes and a global downregulation of inflammatory genes (TNFα, IL-6, MCP-1, inducible nitric oxide synthase, etc.) in adipose tissue [71]. In studies with rats fed a fructose-rich diet, 100 and 200 mg chokeberry extract/kg BW (representing an estimated daily intake of anthocyanins about 10 and 20 mg/kg BW) significantly reduced expression of genes for inflammatory cytokines such as IL-1β, IL-6 and TNFα, and enhanced protein and gene expression of ZFP36 (zinc finger protein) in the epididymal adipose tissue [72]. To clarify whether this effect was due primarily to anthocyanins, or if it was resulted from other polyphenols in the extracts, HFD-induced obese mice and diabetic *db/db* mice received pure anthocyanin C3G (200 mg/kg diet) for 5 weeks. The results revealed that C3G decreased serum concentrations of inflammatory cytokines (TNFα, IL-6 and MCP-1). Also, examination of the white adipose tissue in C3G-treated mice showed lower mRNA levels of the above cytokines and reduced macrophage infiltration [73]. In a separate study, a mixture of anthocyanins from wild mulberry and C3G reduced mRNA and protein levels of cyclooxygenase-2 (COX2), the main enzyme responsible for generating proinflammatory prostanoids. As a result, the anthocyanin mixture effectively minimized carrageenan-induced acute inflammation in mice [74]. Interestingly, anthocyanin supplementation does not affect the systemic immune system, serum inflammation markers, or activation of NF-κB in adipose tissue, in healthy, unchallenged rats [75]. These observations suggest that anthocyanins may modulate inflammatory responses induced by a variety of stress factors, especially metabolic stress, by inhibiting NF-κB transactivation.

4.4 Improvement of insulin resistance

Obesity is strongly associated with insulin resistance, and the improvement of insulin resistance is important in preventing the development of T2DM. The efficacy of anthocyanins in preventing insulin resistance has been demonstrated in different animal models. Treatment of HFD-fed mice with 1 g/kg anthocyanins purified from Cornelian cherries (*Cornus mas*) resulted in decreased weight gain and improved glucose tolerance compared with untreated HFD-fed controls [76]. We have compared the effects of treatment with anthocyanin-rich black rice extract (5 g/kg diet) or pioglitazone (270 mg/kg diet), an insulin-sensitizing drug, in high-fructose fed rats, and found that both treatments improved glucose intolerance and hyperlipidemia. However, pioglitazone could reverse the fructose-induced hyperinsulinemia whereas the black rice extract could not [77]. In our second study, HFD-induced obese mice or genetically diabetic *db/db* mice received dietary C3G supplementation (2 g/kg diet) for 5 weeks. We found that dietary C3G lowered fasting glucose levels and markedly improved insulin sensitivity assessed by insulin tolerance tests in both HFD and *db/db* mice, as compared with unsupplemented controls [73]. In a study of KK-A^y diabetic mice, Sasaki et al. reported that treatment with 2 g/kg dietary C3G significantly up-regulated the glucose transporter 4 (GLUT4) and down-regulated retinol binding protein 4 (RBP4) in white adipose tissue, thereby improving hyperglycemia and insulin resistance [78].

A major metabolic defect associated with insulin resistance is the failure of peripheral tissues in the body to properly utilize glucose, thereby resulting in chronic hyperglycemia. GLUT4 is the primary insulin-dependent glucose transporter, which is present predominantly in skeletal muscle, myocardium, and adipose tissue. Some compelling studies have reported that anthocyanins achieve their hypoglycemic effects by promoting GLUT4-dependent glucose uptake in peripheral cells and tissues. Nizamutdinova et al. reported that administration of black soybean anthocyanins to STZ-induced diabetic rats markedly enhanced GLUT4 membrane localization in heart and skeletal muscle tissues, decreased fasting blood glucose levels, and improved heart hemodynamic function [79]. Similarly, in cultured L6 myotubes, treatment with C3G significantly increased GLUT4 protein expression in the plasma membrane fraction without affecting total GLUT4 protein expression, indicating increased localization of GLUT4 to the cell surface [80]. In H_2O_2- or TNFα-induced insulin-resistant 3 T3-L1 adipocytes, C3G reduced intracellular ROS production and increased insulin-stimulated glucose uptake in a dose-dependent manner, indicating that anthocyanin-mediated reversal of adipocyte dysfunction could be a crucial target for preventing insulin resistance [81]. Tsuda et al. demonstrated that adiponectin and leptin, which are two important adipocytokines regulating metabolism, were upregulated by C3G in primary human and rat adipocytes [82, 83]. Peroxisome proliferator-activated receptor γ (PPARγ) is a ligand-activated nuclear hormone receptor that controls glucose and lipid metabolism and the transcription of proteins involved in glucose and fatty acid uptake [84]. To further investigate the mechanism underlying the insulin-sensitizing effects of C3G, Scazzocchio et al. assessed PPARγ gene expression and transcriptional activity in human omental and murine adipocytes. The investigators found that the C3G-mediated increase in glucose uptake was associated with enhanced GLUT4 translocation to the membrane and enhanced adiponectin secretion, both caused by increased PPARγ activity [85]. Furthermore, the PPAR-promoting activity was confirmed using cyanidin, the aglycone of C3G, in lipid-loaded primary hepatocytes [86].

4.5 Alleviation of chronic diabetic complications

The aim of diabetes management is to protect patients from the acute and chronic complications associated with diabetes.

Acute complications include diabetic ketoacidosis, hyperosmolar coma, infections, hypoglycaemic episodes etc. Some of the chronic or long term complications include eye, kidney and nerve damage. Metabolic confusion has been implicated as a major contributor to these diabetic complications. AMP-activated protein kinase (AMPK) is a crucial cellular energy sensor and plays a pivotal role in regulating lipid metabolism, glucose homeostasis, and insulin sensitivity. Elevated glucose can inhibit AMPK phosphorylation and activity, thereby impairing its downstream signaling and energy-conserving effects and resulting in glycolysis and lipolysis which worsen diabetic pathology [87]. Several studies have suggested that anthocyanins impact metabolic homeostasis by targeting AMPK. Kurimoto et al. reported that dietary black soybean seed coat extract, which is rich in anthocyanins, activated AMPK in skeletal muscle and liver of KK-A^y mice. The activation of AMPK resulted in enhanced glucose utilization and suppression of gluconeogenesis in response to insulin [80]. Our study further demonstrated increased phosphorylation of AMPK in the skeletal muscle and visceral adipose of KK-A^y mice gave pure C3G. This was accompanied by the suppression of lipoprotein lipase in visceral adipose tissue and reduction of plasma triglyceride levels [88]. In diabetic apolipoprotein E-deficient mice, anthocyanin C3G supplementation effectively increased the number of circulating endothelial progenitor cells (EPC) and improved their function, including adhesion, migration, and tube formation. The restoration of EPC number and function after C3G treatment was paralleled by increased AMPK activation [89]. *In vitro* data showed that incubation with C3G increased the activity of AMPK and provided significant protection against high glucose-induced lipolysis in 3 T3-L1 adipocytes [90]. In addition, anthocyanin treatment of HepG2 hepatocytes increased AMPK and acetyl-coenzyme A carboxylase (ACC) phosphorylation, leading to stimulation of carnitine palmitoyl transferase 1 (CPT-1) expression and a significant increase in fatty acid oxidation [91, 92]. These observations suggest that anthocyanins can improve diabetes-associated pathologies and disorders by activating AMPK.

Diabetic nephropathy is one of the most common microvascular complications of diabetes and a leading cause of end-stage renal disease. In order to shed light on the effects of anthocyanins on renal injury caused by hyperglycemic conditions, diabetic *db/db* mice were orally administrated 10 mg/kg BW anthocyanin-rich purple corn extract daily for 8 weeks. This intervention resulted in a significant reduction in glomerular monocyte activation and macrophage infiltration in the kidney tissues of *db/db* mice [93]. In an earlier study, this research group reported that the same anthocyanin extract reduced inflammatory expression of connective tissue growth factor (CTGF) and secretion of collagen IV in human renal mesangial cells exposed to high glucose [94]. Anthocyanin treatment also dampened NF-κB translocation and MCP-1 transcription in high glucose-exposed mesangial cells, suggesting a potentially protective role for anthocyanins in diabetes-associated mesangial fibrosis and inflammation.

5 Conclusion and perspectives

People can ingest significant amounts of anthocyanins by consuming dark-colored plant foods. A growing body of scientific evidence indicates that higher consumption of anthocyanins is associated with lower risk of T2DM (Table 2). The results of most human intervention studies support the hypothesis that anthocyanins can positively affect markers of obesity and T2DM (Table 3). Further research on anthocyanins may lead to more specific recommendations for consumption of anthocyanin supplements or anthocyanin-rich foods, and thus aid in managing obesity and T2DM.

Despite the promising results published so far, uncertainty remains as to whether anthocyanins are capable of reversing the complex pathological processes accompanying obesity and T2DM. Though studies support this possibility, they are too few and methodologically not rigorous enough. To date, we are not aware of any long-term human intervention studies relating anthocyanin intake to T2DM incidence, and there are few clinical studies in humans that have examined the effect of

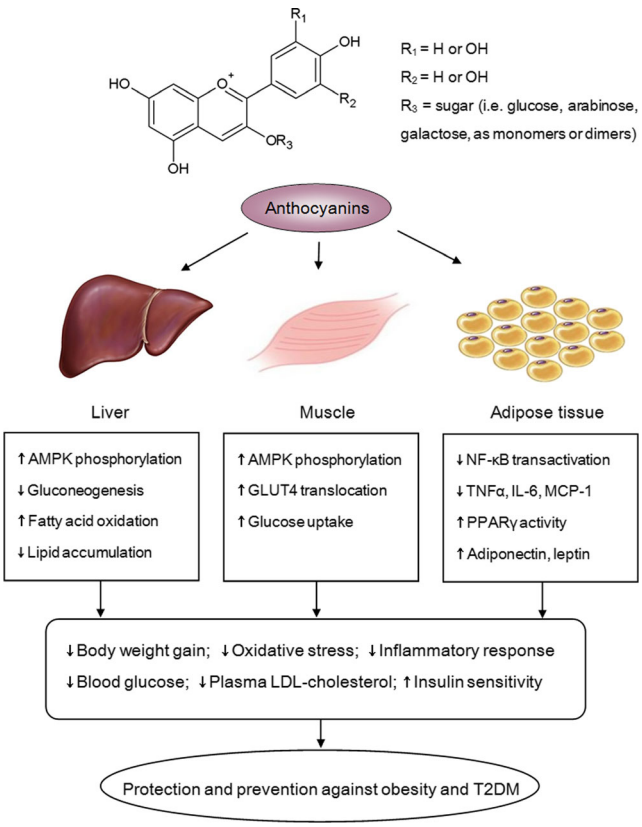

Fig. 1 Putative biological mechanisms underlying the action of anthocyanins on obesity and type 2 diabetes

interventions with anthocyanin-rich diets on intermediate risk factors for T2DM, such as glucose homeostasis and insulin resistance. In addition, the results from these published studies may not be generalizable to other ethnic populations. Thus, additional large-scale and long-term clinical trials are necessary to draw a firm conclusion about contribution of anthocyanins to the management and prevention of obesity and diabetes.

In the interim, from the perspective of clinical practice, there are at least two key questions that should be addressed. Firstly, an effective dose of anthocyanins needs to be validated in humans. Epidemiological studies indicate that intake of 22–35 mg anthocyanins per day can be readily associated with lower risk of T2DM. However, subjects were given a much higher dose (50–320 mg/d) of anthocyanins in human intervention trials. Dose–response trials are needed to ascertain optimal intakes required to reduce the risk of T2DM. The second key question concerns its safety, particularly when high doses of anthocyanins are given for long periods.

Anthocyanins are widely distributed in the human diet, and we ingest large amounts of anthocyanins daily. No incidences of anthocyanin poisoning have been reported in humans. Information from animal studies regarding the occurrence of adverse effects with anthocyanin treatment is also limited. Toxicological studies in rats treated with anthocyanins from purple corn estimated a no-observed-adverse-effect level (NOAEL) of 5.0 % of the dietary supplementation for both sexes (male: 3,542 mg/kg/d, female: 3,849 mg/kg/d) [95]. For humans, anthocyanin was well tolerated even at the highest doses (640 mg/d for 4 weeks) or longest duration (50 mg/d for 2 years) tested [96, 97]. No adverse effects were reported by any of the participants consuming anthocyanins or placebo throughout the intervention period. Therefore, the results of these studies provide strong support for the safety of conventional use of anthocyanin extract. However, caution should be exercised in the use of anthocyanins-based dietary supplements due to the source of raw materials. Further, to our knowledge no studies have been conducted to determine the upper limit of safety for anthocyanins or similar polyphenols. Such studies are also warranted.

As schematically shown in Fig. 1, the putative mechanisms underlying anti-diabetic potentials of anthocyanins might originate from the inhibition of body weight gain, prevention of free radical production and lipid peroxidation, regulation of inflammatory response, reduction of blood glucose and lipids, and improvement of insulin resistance. The overall beneficial effects of anthocyanins represent a complex interaction of multiple signaling pathways, transcription factors, and enzymes. Future studies should focus on using *in vitro* studies to identify the most promising downstream targets of anthocyanins and expanding these findings to mechanistic studies in animals.

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