Nutrition 66 (2019) 192–202

Contents lists available at ScienceDirect

Nutrition

journal homepage: www.nutritionjrnl.com

Review article

Antiobesity effects of anthocyanins on mitochondrial biogenesis, inflammation, and oxidative stress: A systematic review

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ARTICLE INFO

Article History:
Received 7 January 2019
Received in revised form 11 April 2019
Accepted 27 May 2019

Keywords:
Anthocyanidins
Lipogenesis
Cell signaling and gene expression

ABSTRACT

Studies have shown that anthocyanins attenuate obesity. In this review, we confirm these effects and explain the possible mechanisms underlying them. A systematic search was conducted in electronic databases using *obesity* as the main term along with *anthocyanins* and the main anthocyanidins, including articles in Portuguese, English, and Spanish without any restriction as to year. The review was carried out by peers following PRISMA recommendations: 1980 studies were identified, and 19 articles were analyzed. The studies varied in relation to time, pathways, cells used, and anthocyanin types. The positive effects were observed in 5′ adenosine monophosphate-activated protein kinase pathways and mitochondrial biogenesis and in a reduction in inflammation and oxidative stress. Anthocyanins can improve the metabolic control involved in obesity by reducing lipogenesis, oxidative stress, and inflammation. This can boost the speed of lipolysis and thermogenesis, regulate satiety, and reduce body fat accumulation. In addition, anthocyanins have shown promising effects on controlling obesity compared with the standard of care.

Introduction

Worldwide obesity has nearly tripled since 1975. In 2016, 39% of adults or 1.9 billion people >18 y of age were overweight. Of these, >650 million were obese, representing 13% of the population [1]. Factors that trigger obesity are attributed to genetic mechanisms, endocrine, appetite disorders, and diseases such as diabetes mellitus [2].

Lipid and energetic metabolisms encompass a series of lipid biosynthesis and fatty acid oxidation reactions needed for energy supply and adipocyte differentiation. Lipogenesis triggers metabolic reactions such as mitochondrial biogenesis, oxidative stress, and inflammatory processes that are closely linked to obesity [3–6].

The research topics in obesity are causes and mechanisms, physiologic processes, preventive measures, and possible treatment. Treatment targets include thermogenesis of brown adipose tissue (BAT), genetics and epigenetics, childhood nutrition, physical activity, and foods for prevention or treatment that are rich in bioactive compounds [2].

Anthocyanins have aroused considerable interest because of their prevalence in diets—they are ubiquitous compounds found in fruits such as açaí (*Euterpe oleracea* Mart.), plums, blackberries, cherries, raspberries, grapes, and vegetables such as purple cabbage and red potatoes. They show high antioxidant activity and act primarily in metabolic reactions such as lipogenesis, and clinical data suggest that a polyphenol-rich diet may exert health-promoting effects by reducing oxidative stress [7–12]. Davinelli et al. studied the effect of Delphinol supplementation (162 mg anthocyanins) on overweight (age 45-65 years) smokers volunteers in good health for 4 weeks. These data raise the possibility that delphinol may induce a positive and transient systemic effect on the oxidative state. Suggesting a positive nutritional influence of anthocyanin on lipid peroxidation, and this effect may be considered useful

This study was financed in part by the Coordination of Improvement of Higher Level Personnel (CAPES, Brazil) Finance Code 001 and National Council for Scientific and Technological Development (CNPq, Brazil).
The authors have no conflicts of interest to declare.
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https://doi.org/10.1016/j.nut.2019.05.005
0899-9007/

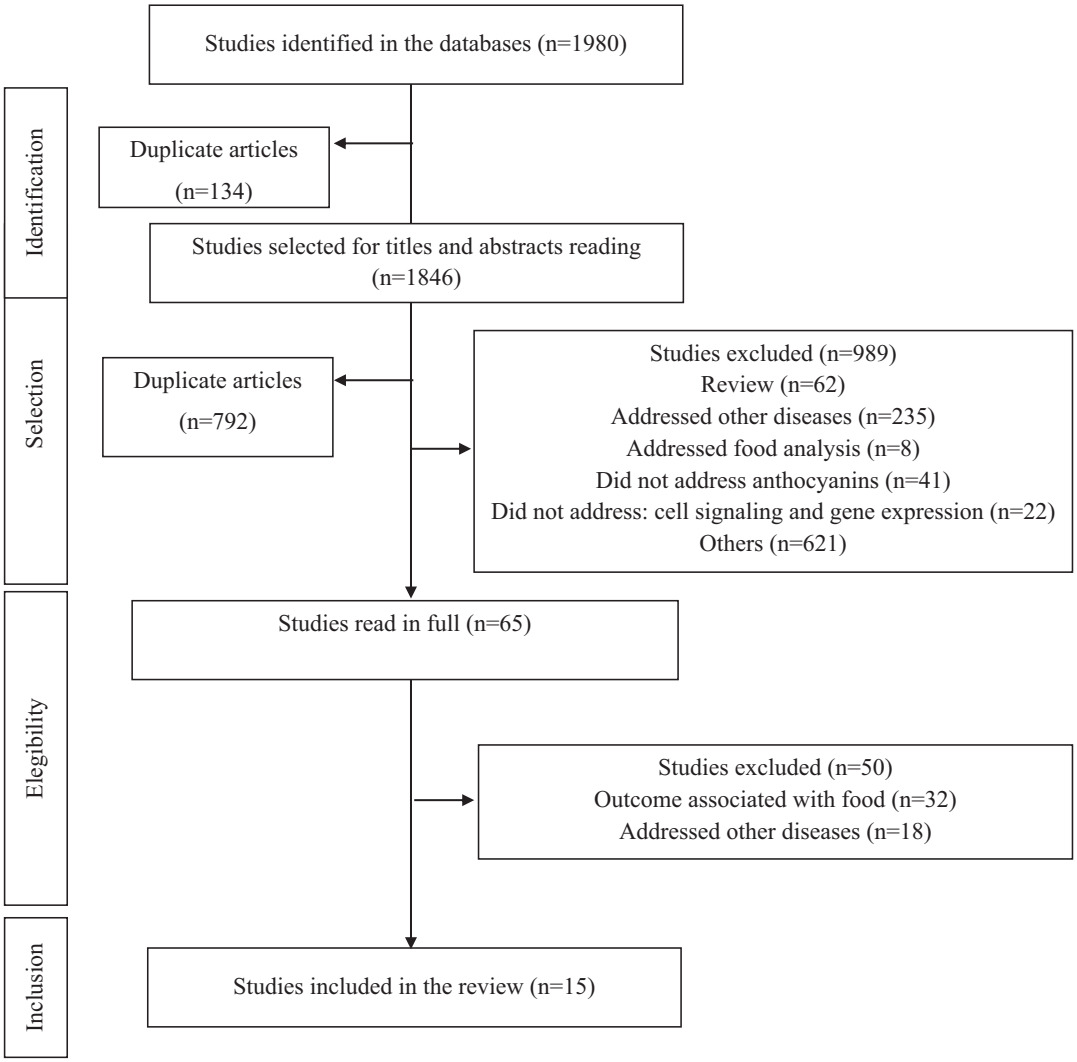

Fig. 1. Flowchart of selection of the studies included in present systematic review.

for the prevention of diseases associated with oxidative stress. However, more studies need to be performed to verify these effects through a controlled diet [13].

Composing the largest group of water-soluble pigments in the plant kingdom, anthocyanin molecules consist of two or three parts—an aglycone (anthocyanidin), a group of sugars, and generally, a group of organic acids. Approximately 22 aglycones are known, and 6 are important in foods: pelargonidin, cyanidin, delphinidin, peonidine, petunidine, and malvidin [14]. The daily intake of anthocyanins varies depending on dietary habits. In the United States, the estimated daily intake is between 180 and 215 mg [15,16].

Thus, this systematic review article verified the action of anthocyanins on glucose and lipid metabolism via 5′ adenosine monophosphate-activated protein kinase (AMPK) pathways, mitochondrial biogenesis, oxidative stress, and inflammation with a focus on anti-obesity effects.

Methods

A systematic search was carried out in the Lilacs, Medline/Pubmed, Scopus, and Science Direct databases. There was no year restriction because the purpose was to identify all articles published to date that have explored the effect of anthocyanins, covering their possible effect on cell signaling and gene expression in obesity. Original in vitro and in vivo articles were included. The main search term was *obesity* along with *anthocyanins,* and the main *anthocyanidins* (cyanidin, delphinidin, malvidin, peonidine, pelargonidine, and petunidine), including their respective terms in English and Spanish. The electronic research took place in 2019, during the months of January and February.

The titles and abstracts were examined, followed by the full articles. The studies were considered eligible regarding the following aspects: original, observational, clinical, and experimental (in vitro and in vivo) articles, and those that evaluate the possible effects of anthocyanins on obesity with an emphasis on cell signaling and gene expression.

In all, 1980 studies were identified and 19 were included. The exclusion criteria were review articles, analyses of food composition, duplicate articles, and articles that attributed the beneficial effect on obesity to food or addressed other diseases (such as diabetes, metabolic syndrome, or non-alcoholic fatty disease; Fig. 1). In every selection stage, each article was read by two researchers, following the recommendations of PRISMA to ensure the review protocol and inclusion and exclusion criteria were followed [17].

The risk for bias for animal studies was assessed using SYRCLE's risk of bias (RoB) tool [18]. We analyzed 10 types of biases according to this methodology:

- sequence generation (selection bias);
- baseline characteristics (selection bias);
- allocation concealment (selection bias);
- random housing (performance bias);
- blinding (performance bias);
- random outcome assessment (detection bias);
- blinding (detection bias);

- incomplete outcome data (attrition bias);
- selective outcome reporting (bias reporting); and
- other sources of bias (other).

For each of the biases, a categorization was performed at three levels: low risk, high risk, and unclear (when it was not clear from the text information).

Results

Nineteen experimental studies were selected for this systematic review. Of these, 13 were performed in vitro. In these studies, the following tissues and cells were used: epididymal white adipose tissue (WAT) cells [19,20], human preadipocytes [21], 3 T3-L1 cells [22–27], HepG2 and CHO-K1 [28], RAW 264.7 [29], human abdominal WAT [21,30], and BAT [31]. The other six were in vivo experimental studies with animals [32–37].

The results are summarized in Tables 1 and 2. Changes were observed in relation to anthocyanin treatments in AMPK, mitochondrial biogenesis, oxidative stress, and inflammatory cell signaling pathways, all of which are involved in obesity. Figure 2 summarizes the antiobesity effects of the anthocyanins.

According to the bias analysis of the animal studies, it was possible to verify that important information to ensure the reproducibility of the studies was not clearly described throughout the text (questions 1, 3, 5, 6, 7, and 8; Fig. 3 and Table 3). However, the results obtained were duly reported and free of selective reports (question 9; Table 3). Low risk for bias was also identified considering the basal characteristics of the animals, which allowed the formation of homogeneous groups and the comparison between them (question 2;Table 3).

Discussion

The antiobesity effect is attributed to some foods and their bioactive compounds that act in the lipolysis and β-oxidation (mitochondrial biogenesis) of fatty acids. This reduces adipogenesis and lipogenesis [38]. Anthocyanins have demonstrated this effect; however, the mechanisms of action are unclear [27].

Effect of anthocyanins on adipogenesis

Lipogenesis and adipogenesis are the main processes involved in the production and accumulation of lipids within the adipose tissues. They are promoted by transcriptional factors, and signaling molecules, such as fatty acid synthase (FAS), fatty acid-binding protein, stearoyl-coenzyme A desaturase-1 (SCD-1), lipoprotein lipase, CCAAT/α-stimulator binding protein, peroxisome proliferator-activated receptor (PPAR-γ), and sterol-1 c regulatory element binding protein (SREBP-1 c) [3,39]. Therefore, these signal molecules, transcriptional factors, and enzymes are the main targets for the control and treatment of obesity. AMPK inactivates these major enzymes involved in the synthesis of fatty acids and sterols, thus being a key factor for the control of lipid metabolism and adipogenesis [3].

Anthocyanins activated AMPK (Fig. 2) with subsequent suppression and reduced activity of liposomal enzymes FAS in vitro [23,27] and in vivo [32,35], acetyl-coenzyme A carboxylase in vitro [23,27] and in vivo [32], and SCD-1 in vitro [27], reducing lipogenesis and oxidative stress.

In addition, purified anthocyanins were efficient in suppressing SREBP-1 c, which is involved in lipid metabolism and is an additional regulator of FAS, binding proteins to the binding protein amplifier (CCAAT-C/EBPs:C/EBPα and C/EBPβ) and transcription factors such as PPAR-γ [23,24,40]. This subsequently inhibits key enzymes in hepatic cholesterol and FAS such as 3-hydroxy-3-methylglutaryl coenzyme A reductase and acetyl-coenzyme A carboxylase [3,40].

In vitro [19,21,26,27,32] and in vivo [32] are actively observing suppression of SREBP-1c of activity and reduction of the accumulation of triacylglycerols [19,21,26,27], and in vitro [32] via human preadipocyte cells, fibroblasts, and preadipocytes from 3 T3-L1 mice; epididymal WAT of Wistar rats; and HepG2 cells; and an in vivo assay [32] with animals fed a high-fat diet (HFD).

Additionally, cyanidin acted as a PPAR-α agonist—a gene involved in the activation of AMPK-induced lipolysis (Fig. 2) [28]. Anthocyanins inhibited C/EBPα-binding protein and (C/EBPβ potentiation-binding protein in vitro [22,23,25–27,31] and in vivo [35]. As a result, there is an inhibition of PPAR-γ expression (Fig. 2), resulting in the inhibition of fibroblast differentiation in adipocytes. Therefore, there is a suppression of lipogenesis because this increase will occur at intracellular levels (c-AMP), stimulating the AMPK pathway [23,24,41,42].

In three other in vitro studies [22,25,31], anthocyanins increased C/EBPβ and PPAR-γ expression by increasing intracellular (c-AMP) levels. Anthocyanins are not PPAR-γ agonists [25], thus suggesting that anthocyanins increases intracellular levels of c-AMP and regulate C/EBPβ, which then activates PPAR-γ and promotes differentiation of WAT in BAT and stimulates thermogenesis. However, further studies are needed for confirmation [43].

On the other hand, the enzyme hormone lipase-sensitive lipase, which exhibits lipolytic activity, has been shown to increase after treatment with anthocyanins in vitro, suggesting its action on lipolysis [20,30,44]. However, further studies are needed to clarify this effect.

Effect of anthocyanins on mitochondrial biogenesis, oxidative stress, and inflammation

The increase of AMPK phosphorylation in vitro [23–25,27,31] demonstrated the effect of anthocyanin on mitochondrial biogenesis (Fig. 2) by increasing the expression of peroxisome proliferator-activated gamma coactivator 1-alpha receptor (PGC-1 α), mitochondrial transcription factor A, and nuclear respiratory factor (NRF) 2. It also reduced oxidative stress in BAT cells during brown adipogenesis. Anthocyanins increased thermogenin (UCP1), which is expressed in brown fat cells and can thus be considered a BAT marker [25,27,31]. Characteristics similar to those of brown adipocytes such as increased mitochondrial content of UCP-1, proliferator-activated gamma coactivator 1-alpha receptor, cytochrome c (Cytc), and PR16 domain (PRDM16) were found in vitro [25,27,45]. These species partially contributed to the metabolism of lipids via activation of the protein kinase B (AKT) and extracellular signal-related kinases–mitogen-activated protein kinase (ERK-MAPK), in which AKT is responsible for phosphorylating NRF2 in respiratory NRF1 for performance in mitochondria biogenesis [41]. All of these genes are closely related to heat production and help maintaining the role of brown fat cells [46,47].

In these studies, we observed induction of expression of genes encoding proteins and antioxidant enzymes through the action of NRF2 [48]. The same effect was observed in vitro [25,27,31].

Oxidative stress is a process that arises from an imbalance between the antioxidant defense system and prooxidant processes. It also involves non-radical reactive species that may occur as a result of an accelerated production of the radical and non-radical species or when the antioxidant defense system is inefficient [6]. The chronic nature of this process has implications in the etiopathogenesis of diseases and chronic non-communicable diseases such as obesity [49].

Table 1
Characteristics of the in vitro studies

Author/year of publication [reference number]	Assay	Cells	Treatment	Signaling pathways studied	Observed effects
Han et al., 2018 [23]	-Western blotting	Murine 3 T3-L1 preadipocytes (ATCC, Manassas, VA, USA)	-ACY isolated fruit of *V. coignetiae* Pulliat analyzed on HPLC (Gyeongsang National University, Jinju, Korea);	-AMPK	-Suppressed the expression of leptin, FAS, ACC-1, and aP2 at 100 and 200 μg/mL;
	-MTT		- Cells treated with different concentrations of ACY (0−400 μg/mL) for 72 h (MTT solution was added 0.5 mg/mL followed by incubation at 37°C for 3 h.	-Inflammatory - Mitochondrial Biogenesis	↓ dose-dependent expression of C/EBPα, C/EBPβ, PPAR-γ, and SREBP-1 c mRNA during adipocyte differentiation.
Khan et al., 2018 [24]	-RT-PCR	Mouse 3 T3-L1 preadipocytes	-The dried Cornus kousa (leaf) and preparation ethanolic extract (lyophilized) and analyzed on HPLC (cyanidin 3-glucoside: 27,23%, delphinidin 3-glucoside: 36.54% and pelargonidin 3-glucoside: 26.32%);	-AMPK	↓ dose-dependent the lipid accumulation in 3 T3-L1 cells nearly the same as GW9662 (the positive control);
	- Western blotting		- Cells were treated with different concentrations (5, 10,15, 20, 25, 30, 50, 100, and 200 μg/mL).	- Mitochondrial Biogenesis	-↓ dose-dependent expression of C/EBPα, aP2, PPAR-γ, and SREBP-1 c mRNA during adipocyte differentiation.
Matsukawa et al., 2017 [25]	-MTT assay	Mouse 3 T3-L1 cells (JCRB Cell Bank, Osaka, Japan)	-3 T3-L1 in DMEM with 10% bovine serum and 1% penicillin (5000 μg/mL) - streptomycin (5000 IU/mL) at 37°C in a humidified atmosphere of 5% CO_2.	-AMPK	- C3 G ↑ the number of mitochondria;
	-Western Blotting		-C3 G purchased from Wako (Tokyo, Japan);	-Oxidative stress	- ↑ expression of mitochondrial genes and TFAM (CYTC, PDK4, UCP-1, UCP-2, and SOD2) were in two doses;
	-RT-PCR		-During differentiation (day 0-7), 3 T3-L1 cells were treated with or without C3 G (20, 40, 60, 80, and 100 μM).	-Mitochondrial biogenesis	- C3 G ↑ expression of the UCP-1 protein, PGC-1 α, TBX1, and CITED1 after 3 d; -3 T3-L1 treated with C3 G had a decrease in intracellular production of ROS and ATP at 50 and 100 μM; - ↑ expression of PPARγ and C/EBPβ: In the presence or absence of cocktail of differentiation at 3 d of induction.
You et al., 2017 [31]	-RT-PCR	Brown adipose tissue -BAT-cMyc(Institute of Zoology, Beijing)	-C3 G and C3 R were purchased from Sigma-Aldrich (St Louis, MO, USA);	-AMPK	- ↑ expression of the marker genes aP2 and PPAR-γ2 by C3 G and C3 R;
	-Mitochondrial copy number measurement		-C3 G or C3 R (10 μM) for 6 d during adipogenesis (BAT).	-Oxidative stress	-C3 G ↑ expression of UCP1;
	-Western Blotting			-Mitochondrial biogenesis	-C3 G and C3 R ↑ Cideα and PRDM16 and positively regulated the oxidation of fatty acids; - ↑ TFAM, NRF1 and NRF2; - AKT and ERK were regulated positively.
Mackert & McIntosh, 2016 [30]	-RT-PCR	Human abdominal subcutaneous white adipose tissue (Fisher Scientific (Norcross, GA)	-MV and PE with 95% purity Carbosynth Limited (Berkshire, UK); MM MV, PE, or a combination (50/50, w/w) of MV + PE/24 h followed by 3 μg/L of LPS treatment for 3 h, during cell differentiation.	-Inflammatory -Mitochondrial biogenesis	- CY combination ↓ gene expression of MCP-1, IL-1 β, IL-6, IL-8 and TNF; - ↑ in HSL expression.

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Table 1 (*Continued*)

Author/year of publication [reference number]	Assay	Cells	Treatment	Signaling pathways studied	Observed effects
Rahman et al., 2016 [26]	-RT-PCR	Mouse 3 T3-L1 embryo fibroblasts (Korean Cell Line Bank)	- Delphinidin chloride (purity 90%, HPLC) was purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA);	-AMPK	- Suppressed the expression of C/EBPβ, C/EBPδ, C/EBPα and PPAR-γ at 50 μM;
	- Western blotting		- Delphinidin chloride in different concentrations (25, 50, and 100 μM) was used after cell differentiation.	-Inflammatory	- Suppressed the expression of adiponectin and aP2 at 50 μM;
				-Mitochondrial biogenesis	-↑ expression Wnt1, Wnt10 b and Wnt β catenin at 100 μM.
Lee et al., 2014 [27]	-RNA isolation	Murine 3 T3-L1 preadipocytes (ATCC, Rockville, MD)	- ACY semipurified grape bark (purity 72.8%), provided by the University of Arkansas;	-AMPK	- ↓ dose-dependent expression of C/EBPα, PPAR-γ, LXRα and SREBP-1 c mRNA during adipocyte differentiation;
	-RT-PCR		-cells treated with different concentrations of ACY (2.5−20 μg/mL) for 7 d, during differentiation.	- Mitochondrial Biogenesis	- ↓ FAS mRNA, SCD-1 and ACC-1.
Lee et al., 2014 [29]	-Isolation of BMMS	Murine RAW 264.7 macrophages were purchased from ATCC (Manassas, VA, USA)	-The lyophilized BB, BK, and BC (VDF Future Ceuticals, Momence, IL, USA) ~8 g were extracted in methanol 80% containing 0.1% HCl and analyzed on HPLC.	-AMPK	- ↓ NOX1- BCA without Nrf2;
	- Enzyme-linked immunosorbent assay for TNF		-During cellular differentiation: Cells incubated with 0 to 20 μg/mL BBA, BKA, or BCA/12 h, co-incubated with the same ACY fractions and 100 ng/mL LPS/3 or 24 h;	-Inflammatory	- ↓ TNF at the 20 μg/mL dosages of BBA and BCA, but not with BKA as compared with the control. At 20 μg/mL, BBA and BCA, TNF, expression was higher than the control.
	-RT-PCR		-Control: 25 mg/mL DMSO.	-Oxidative stress	- ↓ TNF secretion in BBA, BKA, and BCA at 20 μg/mL. BBA and BCA + potent in the inhibition of TNFs at a dose of 10 μg/mL;
	- Western blotting		- BMMs treated with 20 μg/mL BBA, BKA, or BCA/12 h, co-incubated with the ACY + 100 ng/mL LPS/3 h fractions.		- ↓ levels of ROS, ↓ levels of IL-1 β mRNA;
					- Greater exposure to LPS (12 h) ↑ ROS levels in Nrf2/BMM;
					- ↓ Nrf2 +/+ BMM ROS, in the anthocyanin groups.
Jia et al., 2013 [28]	-Transfection and luciferase	CHO-K1 cells were cultured in DMEM-F/12 (Hyclone, Logan, UT, USA)	HepG2: Fatty acids (palmitic acid and oleic acid, 400 μM each) + 0.5% BSA/ 24 h, followed by:	-AMPK	- CY active PPAR: α, β, β/δ;
	-RT-PCR;		- CY (5, 10, 50, or 100 μM) and controls: FF: 10 μM; GW0742: 1 μM and 10 μM TT + 24 h, with 1% DMSO as carrier, during cell differentiation.	- Mitochondrial Biogenesis	- CY at 100 μM ↓ intracellular cholesterol and TG as the PPAR agonists: FF, GW0742, and TT;
	-TR-FRET;		-Microarrays and RT-PCR: Cells were incubated in DMEM medium with 100 μM CY; 10 μM FF or 10 μM ST/ 24 h.		- ↑ expression of PPARα, PPARγ and PPARδ/β, by CY at 50 μM and 100 μM;
	-Microarrays;		-CHO-K1: Following transfection, CY stimulus (0.5−100 μM) or 1% DMSO/ 24 h.		- Gene expression of fatty acid biosynthesis and metabolism of AG, TCA cycle was regulated by CY (100 μM) FF (10 μM).
	-SPR.				

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Table 1 (*Continued*)

Author/year of publication [reference number]	Assay	Cells	Treatment	Signaling pathways studied	Observed effects
Kim et al., 2012 [22]	-Western Blotting	3 T3-L1 (fibroblasts obtained from the ATCC, USA)	-Extraction of the ACY: Extraction with 80% ethanol. The extract (was filtered and lyophilized) analyzed on HPLC.	-AMPK	- Levels ↓ PPAR-γ1 and PPAR-γ2;
			-Tried with 12.5 and 50 μg/mL of black soybean ACY extracts, during cell differentiation.	- Mitochondrial Biogenesis	-Suppression of GPDH activity in the dosage of 50 μg/mL.
					-↑AMPK.
Tsuda et al., 2006 [21]	- RT-PCR	Human subcutaneous preadipocytes isolated from adipose tissue of healthy, non-diabetic woman (age 33 y, body mass index, 25.77) were obtained from Zen Bio, Inc.	- Cy and C3 G were obtained from Extrasynthèse (Genay, France), at purity 99%.	-Inflammatory	-C3 G ↑ the gene related to energy expenditure (UCP2, ACOX1, adiponectin) and PLN related to the degradation of TG, were regulated by CY.
	-GeneChip microarray		- 13 d after differentiation of preadipocytes in adipocytes: Treated with 100 μM C3 G, CY or vehicle (0.1% DMSO) after cell differentiation for 24 h at 37°C in a humidified atmosphere (5% CO_2).	- Mitochondrial Biogenesis	- The CCAAT/enhancer binding protein (C/EBPs), complement D component (adiposin) and cathepsin D were specifically regulated by Cy treatment, ↓ C/EBPα;
					- ↓ negative regulation in PAI-1 and IL-6 in C3 G and CY.
					- C3 G and CY/24 h ↑ adiponectin mRNA, UCP2, ACOX1, and PLN and ↓ in PAI -1.
Tsuda et al., 2005 [20]	- RT-PCR	Epididymal white adipose tissue of Wistar rats (non-purified diet for 3 d)	-Cy and C3 G were obtained from Extrasynthèse (Genay, France) at purity> 99%.	- Mitochondrial Biogenesis	-633 genes were regulated with C3 G;
	-GeneChip microarray		- Treated with 100 μM C3 G, CY or vehicle (0.1% DMSO) for 24 h at 37°C in modified atmosphere (5% CO_2), during cell differentiation.		- 427 CY-regulated genes;
					- ↑ HSL regulation.
Tsuda et al., 2004 [19]	Isolation of RNA and measurement of the level of gene expression	Epididymal white adipose tissue of Wistar rats (non-purified diet for 3 d)	-Cy and C3 G were obtained from Extrasynthèse (Genay, France) at purity >99%.	-AMPK	- CY and C3 G ↑ adipokine secretion (adiponectin and leptin)
			- Treated with 100 μM C3 G, CY, or vehicle (0.1% DMSO) for 24 h at 37°C in modified atmosphere (5% CO_2), during cell differentiation.	-Inflammatory	- ↑ the expression of adipocyte specific genes (LPL, aP2, and UCP2)
				- Mitochondrial Biogenesis	- ↓ AMP ratio: ATP

ACC-1, acetyl-coenzyme A carboxylase; ACY, anthocyanins; AGL, free fatty acid; AKT, protein kinase B; AMP, adenosine monofostafo; AMPK, 5′ adenosine monophosphate-activated protein kinase; aP2, activated protein 2; ATCC, American type culture collection; ATP, adenosine triphosphate; BAT-c-Myc, brown adipose tissue with regulatory gene that encodes transcription factor; BBA, blueberry; BCA, blackcurrant; BKA, blackberry; BMMs, macrophages derived from bone marrow; BSA, bovine serum albumin; C/EBP, CCAAT enhancer-binding protein; CY, purified cyanidin; C3 G, cyanidin-3-*O*-glycoside; Cytc, cytochrome c; C3 R, cyanidin-3-*O*-rutinoside; CHO-K1, Chinese hamster ovary cells; DE, delphinidin; DMEM, Dulbecco's modified eagle's medium; DMSO, dimethyl sulfoxide; FAS, fatty acid synthase; FF, fenofibrate; GPDH, gGlycerol-3-phosphate dehydrogenase; Gsk3 β, glycogen synthase kinase 3 beta; GW9662, antagonist of PPAR-γ; HepG2, human hepatocarcinoma cells; HPLC, high-performance liquid chromatography; HSL, hormone-sensitive lipase; IL6, interleukin; LPL, lipoprotein lipase; LPS, lipopolysaccharide; LXRα, liver X receptor α; MCP-1, monocyte chemoattractant protein-1; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; MV, malvidin; NRF, respiratory nuclear factor; NOX-1, protein coding; PAI-1, plasminogen activator inhibitor-1; PE, peonidine; PGC-1 α, peroxisome proliferator-activated gamma coactivator 1-alpha receptor; PPAR, peroxisome proliferator-activated receptor; PRDM16, PR16 domain; RAW 264.7, murine macrophages cells; ROS, reactive oxygen species; RT-PCR, real-time polymerase chain reaction; SCD-1, stearoyl coenzyme desaturase; SPR, surface plasma resonance; SREBP-1 c, sterol regulatory element binding protein-1 c; ST, lovastatin; TAC, total antioxidant capacity; TCA, tricarboxylic acid; TFAM, mMitochondrial transcription factor A; TG, triacylglycerol; TNF, tumor necrosis factor; TT, troglitazone; TR-FRET, Energy Transfer Resolved Fluorescence Resonance in Time; UCP1, thermogenin.

Table 2
Characteristics of the animal studies

Author/year of publication [reference number]	Animals	Compound used and extraction form	Duration	Intervention	Mechanism/ tissue or organ studied	Observed effects
Wu et al., 2018 [36]	36 males C57 BL/6 mice, 4 to 5 wk old	Purified raspberry anthocyanins Extraction: Methanol/ water/formic acid (90:9:1, v/v)	12-wk treatment	- LFD control group; - HFD control group; -Treated group fed with HFD plus with RA (200 mg/ kg/diet);	- Inflammation; - Oxidative stress; - Liver and adipose tissues.	- ↓ expression levels of TNF, IL-6, and NF-κB genes. - ↑ serum SOD and GPx activities.
Wu et al., 2018 [37]	60 males C57 BL/6 mice, 24 d of age	Purified blueberry and blackberry anthocyanins	7 d of acclimatation + 12-wk treatment	- LFD control group; - HFD control group; - OC group (HFD + orlistat 40 mg/kg/ diet; - BLA group (200 mg/kg/diet); -BBA group (200 mg/kg/diet); - Water and diet ad libitum.	- Inflammation; - Oxidative stress; - Serum, liver, kidney and adipose tissues.	- ↓ expression levels of TNF, IL-6, and NF-κB genes. - ↑ hepatic SOD and GPx activities.
Wu et al., 2016 [35]	72 males *C57 BL*/6 mice, 1 mo old	BA = Blueberry anthocyanin (Vaccinium ashei) Extraction: Methanol/ water/formic acid (90: 9: 1 v/v)	8-wk Obesity induction + 8-wk treatment	-LFD control group; -HFD control group; - Positive group HFD +orlistat (100 mg/kg body weight); -HFD + BA group (50 mg/kg body weight); -HFD + BA group (100 mg/kg body weight); -HFD + BA group (200 mg/kg body weight); -Water and diet, ad libitum	-AMPK; -Inflammation; - liver and white adipose epididymal tissue	-↑ expression of CPT1; - ↓ expression of the TNF, IL-6, PPARγ and FAS genes in the 4 treatments; - leptin ↓ in HFD + BA (200 mg/kg) and HFD + orlistat (100 mg/kg body weight);
Wu et al., 2014 [34]	48 males *C57 BL* /6 mice,1 mo old	- Sweet cherry isolated ACY (CACN) Extraction: Methanol/ water/formic acid (90: 9: 1 v/v)	12 wk of obesity induction and treatment	-LFD group (10% fat); -HFD group (45% fat); - HFD + CACN group (40 mg/kg/diet); -HFD + CACN group (200 mg/kg/ diet); - Water and diet ad libitum.	-Inflammation; -Oxidative stress. - Adipose tissue, liver and white adipose epididymal tissue.	↓ expression levels of TNF, IL-6 genes in the treatments; - ↑ the activity of SOD and GPx (dose-dependent effects).
Badshah et al., 2013 [33]	18 males Sprague-Dawley mice, adults	-ACY extracted from Korean black beans (Glycine max (L.) Merr.). -Extraction: 95% methanol and 1% HCl.	6-wk Treatment	Control group (1 mL of water); -group 6 mg ACY: 6 mg/kg of body weight per gavage; - ACY24 group: 24 mg/kg body weight per gavage; -Water and commercial diet, ad libitum.	-Synaptic Enhancement - Brain and white adipose epididymal tissue.	In the ACY/24 mg/kg body weight group: - ↓ NPY expression, which modulates the increase in food intake; - ↑ expression of GABAB1 R (specific involvement in the regulatory mechanism of appetite in the hypothalamus) ↓ expression of pKA-α and P-CREB;

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Table 2 (*Continued*)

Author/year of publication [reference number]	Animals	Compound used and extraction form	Duration	Intervention	Mechanism/ tissue or organ studied	Observed effects
Hwang et al., 2011 [32]	15 males Swiss mice (ICR), 1 mo old	-Antocianic extract of purple sweet potato. Lyophilized tubules: Ethanol/water solution (85:15 v/v), diluted in HCl (0.01%) and preconditioned with methanol (0.01%).	4 wk Obesity induction + 4 wk treatment	ND: Saline solution (100 μL); -HFD group: Saline solution (100 μL); -HFD + ACY group: 200 mg/kg/day, by gavage; - Water and commercial diet (ND), treatment (HFD) ad libitum.	-AMPK -Liver	- ↑ AMPK activity (dose-dependent); - ↓ SREBP-1 in the liver; - ↓ ACC-1 and FAS expression.

ACC, acetyl coenzyme carboxylase A; ACY, anthocyanins; AMPK, protein kinase-activated adenosine monophosphate; BA, anthocyanin from blueberry artificial plants; BBA, blueberry anthocyanins; BLA, blackberry anthocyanins; CPT1, camitine palmitoyl transferase; CACN, purified sweet cherry anthocyanins; FAS, fatty acid synthase; GABAB1 R, gamma-aminobutyric acid B1 receptor; GPx, glutathione peroxidase; HFD, high-fat diet; IL, interleukin; LFD, low-fat diet; ND, normal diet; NPY, neuropeptide Y; pKA-α, protein kinase α; P-CREB, cell transcription factor; PPAR, peroxisome proliferator-activated receptor; RA, purified raspberry anthocyanins; S, weeks; SOD, superoxide dismutase; SREBP-1, binding protein to the sterol regulatory elements; TNF,. umor necrosis factor.

Many studies demonstrate that obesity is associated with chronic low-grade inflammation and excessive oxidative stress. Epidemiologic studies suggest that consumption of polyphenols may reduce lipid oxidation and elevate serum antioxidant status [50,51].

The role of anthocyanins in oxidative stress reduction has been observed in some in vivo studies with animals using purified anthocyanins. According to these studies, mice fed an HFD showed the pathophysiologic condition of inflammation and excessive oxidative stress, accompanied by obesity [34,36,37]. The increase of superoxide dismutase (SOD) and glutathione peroxidase (GPx) was verified [34,36,37]. In the first study, the regulation of these markers was made through sweet cherry anthocyanins purified at the dose of 40 mg/kg per diet and 200 mg/kg per diet. In the second study, its effect was verified at a dose of 200 mg/kg per diet of purified raspberry anthocyanins. Finally, in the third study, GPx increased in blueberry anthocyanins and blackberry anthocyanins (200 mg/kg per diet). In contrast, the increase in SOD was only for blueberry anthocyanins.

Purified anthocyanins reduced the anti-inflammatory effect in vivo [34–37] and in vitro [21,29]. This led to a decline in inflammatory markers such as interleukin (IL)-6 and tumor necrosis factor (TNF). Mackert and McIntosh [30] mixed malvidin and peonidin in the same proportions (50/50, *P*/*P*) and observed a reduction in the expression of proinflammatory genes such as monocyte chemoattractant protein-1(MCP-1), IL-1β, IL-6, and IL-8.

In an in vitro study [27], anthocyanins weakened the attenuated nuclear translocation of p65, suggesting that the anti-inflammatory effects of anthocyanins are mediated, at least in part, by inhibition of nuclear factor (NF) κB translocation to the nucleus given that plays a critical role in the induction of proinflammatory gene expression in response to lipopolysaccharide [52]. Furthermore, in two in vivo studies with animals after supplementation of purified anthocyanins at 200 mg/kg of diet significantly downregulated the gene expression levels of NF-κB. One of the factors may be due to the reduction of TNF because it has a ligand that, when in contact with the cell surface, stimulates NF-κB expression.

In another in vitro study [21], a reduction was observed in plasminogen activator inhibitor-1 (PAI-1). Regulating PAI-1 expression is a major therapeutic target for metabolic syndrome including obesity-based cardiovascular disease or hyperinsulinemia [53], which can decrease the common inflammatory effects on obesity [54,55]. These findings confirm that anthocyanins can prevent and control obesity and oxidative stress while boosting fatty acid oxidation and mitochondrial gene expression [56].

Effects of anthocyanins on the canonical Wnt and neuropeptide Y pathways

The Wnt canonical signaling pathway is involved in the early differentiation of adipocytes and is considered an antiobesity target. During Wnt signaling, multifunctional β-catenin dissociates from its cytoplasmic inhibitory complexes, which consist of glycogen synthase kinase (Gsk) 3 β, casein kinase Iα, and axin. It then translocates to the nucleus where it regulates its target genes (c-Myc and cyclin D1) [57].

In this review, delphinidin [26] induced Wnt1 and Wnt10 b expression. Delphinidin could effectively activate Wnt signaling during early adipogenesis (Fig. 2) by regulating the Wnt/β-catenin pathway. This shows that treatment reduces the accumulation of intracellular lipids and promotes lipolysis without adverse effects in cell viability [26].

Badshah et al. conducted an in vivo study with animals using anthocyanins extracted from black beans (24 mg/kg of weight) for

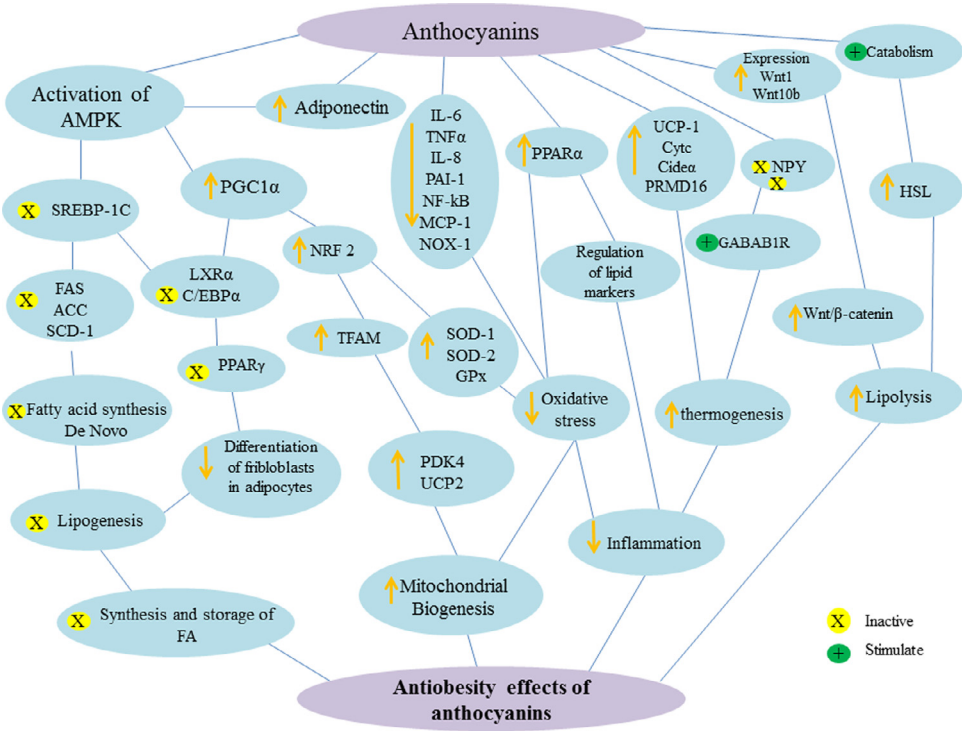

Fig. 2. Mechanisms of action of anthocyanins in the pathophysiology of obesity. Anthocyanins reduced inflammation and increased adiponectin. They activated protein kinase-activa was a decrease in the expression of inflammatory cytokines interleukin (IL)-6, IL-8, tumor necrosis factor (TNF), IL-1β, plasminogen activator inhibitor-1 (PAI-1), nuclear factor (NF)-κB, monocyte chemoattractant protein-1 (MCP-1), and protein coding (NOX-1), decreasing the production of reactive oxygen species (ROS) and reducing tissue inflammation. The enzymes superoxide dismutase, superoxide dismutase 2 mitochondrial, and glutathione peroxidase (GPx) increased their expression, having the effect of preventing oxidation resulting from the action of free radicals. Thermogenin (UCP1) is expressed in brown fat cells and can be considered as a marker of brown adipose tissue. Additional genes showing increased expression in these cells are the co-activator of PGC1α, cytochrome c (Cytc), and PR16 domain (PRDM16). All of these genes are closely related to the main role of brown fat cells, that is, the production of heat and were increased when used anthocyanins, stimulating thermogenesis. Furthermore, anthocyanins induced the expression Wnt1 and Wnt10 b, showing that it can activate *Wnt* signaling during early adipogenesis by regulating the Wnt/β-catenin pathway, decreasing the accumulation of intracellular lipids, and promoting lipolysis. The enzyme lipase sensitive hormone (HSL), has lipolytic action and has been increased with anthocyanin treatment.

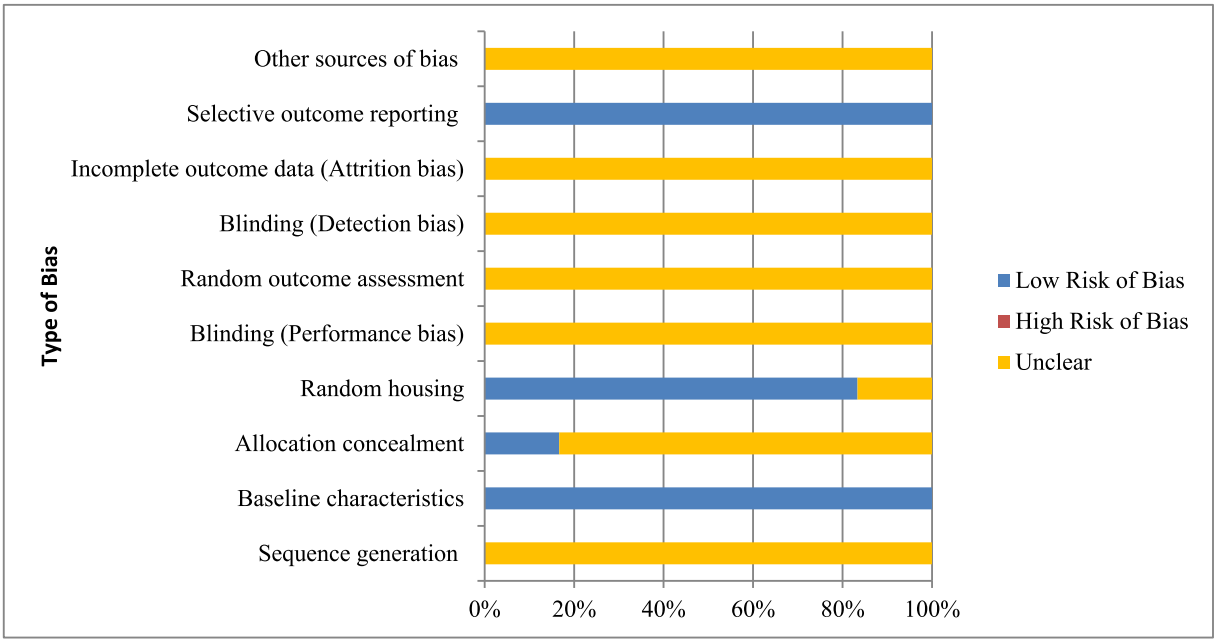

Fig. 3. Representation of risk classification by domain, according to SYRCLE's (Tool used in review studies to detect risk of bias in animal studies) risk of bias.

Table 3
Risk assessment of bias

	Hwang et al., 2011 [32]	Badshah et al., 2013 [33]	Wu et al., 2014 [34]	Wu et al., 2016 [35]	Wu et al., 2018a [36]	Wu et al., 2018b [37]
Sequence generation (selection bias)	?	?	?	?	?	?
Baseline characteristics (selection bias)	+	+	+	+	+	+
Allocation concealment (selection bias)	?	?	?	?	?	+
Random housing (performance bias)	?	+	+	+	+	+
Blinding (performance bias)	?	?	?	?	?	?
Random outcome assessment (detection bias)	?	?	?	?	?	?
Blinding (detection bias)	?	?	?	?	?	?
Incomplete outcome data (attrition bias)	?	?	?	?	?	?
Selective outcome reporting (reporting bias)	+	+	+	+	+	+
Other sources of bias (other)	?	?	?	?	?	?

+, low risk of bias; -, high risk of bias; ?, unclear.

~6 wk and found a decline in neuropeptide Y (NPY), in addition to reduced hunger and an increase in γ-aminobutyric acid B1 receptor (GABAB1 R). This reflects a decrease in food intake and weight gain in animals and a decrease in body fat accumulation [58,59].

These findings confirm that anthocyanins can prevent and control obesity and may also act in adipogenesis (AMPK activation, increased adiponectin, and inhibited lipogenesis). They also boost mitochondrial biogenesis (by reducing oxidative stress and inflammatory markers) and regulate the canonical Wnt and NPY pathways while stimulating lipolysis.

Several recent studies have evaluated the effect of anthocyanins on cell signaling and gene expression in obesity, as seen by the small number of studies using in vivo models. Therefore, research in this field is needed to encourage to better support measures aimed at preventing and treating obesity and its complications.

Antiobesity effect of anthocyanins compared with drugs

Some of the studies in this review used drugs to compare the effect of anthocyanins on obesity. The effect of blueberry anthocyanins at three dosages was compared with that of tetrahydrolipstatin in vivo, and a similar effect was observed at 200 mg/kg body weight [35]. An in vitro study [28] used purified cyanidin (CY) at 100 μM. This increased the expression of fatty acid biosynthesis genes similar to fenofibrate [28].

These two studies demonstrated an effect on the reduction of lipid absorption [28,35,60]. This can be explained by an increase in the PPAR-α subunit (Fig. 2), which is expressed in the liver, kidney, heart, skeletal muscles, and BAT. It can induce fatty acid oxidation. Its ligands activate the transcription factor including eicosanoids with the free fatty acids raising the high-density lipoprotein cholesterol (HDL-C) and lowering the blood triacylglycerols [41].

The in vivo studies showed that the study doses varied from 6 to 200 mg/kg of animal weight. Anthocyanins are an effective alternative compared with the aforementioned drugs; however, further studies are needed to define the effective dose for safe human use. Anthocyanins increased adiponectin in in vitro studies at 100 μM doses of cyanidin-3-*O*-glycoside (C3 G) and CY [19,21] and 50 μM delphinidin chloride [26]. These effects might indicate that anthocyanins have a promising effect on anti-inflammatory action by raising the uptake of fatty acids by myocytes from the blood and the β-oxidation rate in the muscle (Fig. 2) [41].

Anthocyanins may be an effective alternative in comparison to drugs; however, further studies are required for the application in humans, and especially in terms of an exact dose for the desired effect. In the selected in vivo studies, doses varied from 6 to 200 mg/kg of animal weight.

Conclusions

Anthocyanins can help improve the metabolic control dysregulated in obesity by reducing lipogenesis, oxidative stress, and inflammation. They can increase the speed of lipolysis and thermogenesis, regulate satiety, and reduce the accumulation of body fat. Moreover, anthocyanins have promising effects in controlling obesity versus other drugs used. However, their effects on glucose profiles remain unclear. Further studies are needed to clarify the effects of anthocyanins on the glucose profile related to obesity.

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