Lead Article

The health benefits of anthocyanins: an umbrella review of systematic reviews and meta-analyses of observational studies and controlled clinical trials

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Anthocyanins (ACNs) are phenolic compounds present in foods and have undefined health benefits. The present umbrella review aimed to analyze the effects of ACNs on multiple aspects of human health (from systematic reviews and meta-analyses [SRMs] of randomized controlled trials [RCTs]), and the associations of ACNs with the risk of various diseases (from SRMs of observational studies [OSs]). Following the PRISMA methodology, the PubMed, SCOPUS, and Cochrane databases were searched up to November 1, 2020 for OS-SRMs and RCT-SRMs that examined the effects of ACNs on health. The risk of bias of RCT-SRMs was assessed using the AMSTAR 2, and that of OS-SRMs was assessed using the Joanna Briggs Institute methodology. Based on 5 OS-SRMs (57 studies and 2 134 336 participants), ACNs of various sources were significantly associated with a reduction in the risks of hypertension and type 2 diabetes mellitus. According to 8 RCT-SRMs (139 interventions and >4984 participants), ACNs improved plasmatic lipids, glucose metabolism, and endothelial function, without affecting blood pressure. No associations between ACNs and breast or gastric cancer risks were found. ACN intake opens new pathways for the management of glucose metabolism, the plasmatic lipid profile, and the improvement of endothelial function in humans.

INTRODUCTION

Anthocyanins (ACNs) are phenolic compounds that belong to the flavonoid subclass.[1] ACNs are natural plant pigments that are responsible for the red, pink, blue, and purple colors present in the seeds, flowers, fruits, and leaves of a large number of plants,[2] and they undergo significant chemical structure modifications as they pass through the gastrointestinal tract and encounter a variety of pH values.[3–6] These changes result in varied ACN profiles and bioavailability in diverse body fluids and tissues, suggesting the possibility of a number of potential health benefits.[3–6]

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In other research, ACNs have demonstrated potential for the prevention and treatment of various cardiometabolic diseases. For instance, observational data have demonstrated that dietary ACN intake is inversely associated with the risk of cardiovascular diseases (CVDs) in populations from Europe and the United States.[7] Additionally, ACNs have been shown to significantly decrease serum low-density lipoprotein cholesterol (LDLc),[8] to modify low-grade systemic inflammation,[9] and to reduce the damage caused by reactive oxygen species concentrations compared with placebo in humans.[2] Moreover, dietary ACN consumption seems to be beneficial for the prevention of type 2 diabetes mellitus (T2DM), some types of cancer, and hypertension.[1,6,10–13]

Interpretation of the results obtained from both randomized clinical trials (RCTs) and observational studies (OSs) can help determine the effectiveness, safety, and health properties of whole foods containing ACNs and bioactive compounds.[14] Moreover, the combination of both RCTs and OS may help researchers understand and solve questions that could not be answered through the analysis of either type of study alone.[14] Accordingly, to provide a full summary of the available data regarding the health properties of ACNs, our work integrates the information on both the associations between ACNs and diverse health outcomes (from OSs), as well as the ACN effects on diverse cardiovascular and metabolic disease risk factors (from RCTs). Moreover, the available information on ACNs from multiple systematic reviews and meta-analyses (SRMs) can be compiled into an umbrella review[15] to provide the reader with one accessible and usable document on the proven health benefits of ACNs. The present umbrella review aimed to appraise and summarize the current knowledge (from RCT-SRMs) of the effects of ACNs on, and (from OS-SRMs) of the associations of ACNs with, multiple aspects of human health.

METHODS

Search strategy and selection criteria

An umbrella review is a summary of the existing SRMs on a specific subject, such as health benefits due to ACN consumption among humans. Thus, an umbrella review is a way to provide decision-makers with all of the known information obtained from systematically performed research, therefore organizing and assessing the evidence from various health outcomes associated, in this case, with ACN consumption.[15]

For the present umbrella review, our group followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) methodology (http://www.prisma-statement.org/),[16] and followed the principles published by the Joanna Briggs Institute, an international research organization based in the Faculty of Health and Medical Sciences at the University of Adelaide, South Australia.[15]

A web-based search in 3 of the most important reference scientific libraries – PubMed (http://www.ncbi.nlm.nih.gov/pubmed), the Scopus library (www.scopus.com), and the Cochrane Library (http://www.cochranelibrary.com/) – was performed. The following search terms were used, first, to retrieve SRMs regarding the effects of ACN supplementation on various disease risk factors from RCT-SRMs in which supplementary ACNs were added to the diet of human subjects to determine their known effects, and second, to retrieve OS-SRMs regarding the associations between ACN dietary intake and the risk of death or disease in humans: ("Anthocyanin" OR "ACN" OR "anthocyanins") AND ("systematic review" OR "meta-analysis" OR "metaanalysis" OR "meta analysis") AND ("health" OR "cardiovascular" OR "cancer" OR "disease" OR "metabolic" OR "chronic" OR "diabetes" OR "illness" OR "mortality" OR "risk"). The populations, interventions, comparisons and outcomes (PICOS) are shown in Table 1.

The results were screened based on their titles and abstracts, according to the previously established inclusion criteria as follows: (1) SRMs of RCTs reporting the effects of the consumption or supplementation of ACNs on any health outcome; (2) SRMs of OS regarding the epidemiological associations between ACN consumption/supplementation and any health outcome; (3) studies published from inception up to December 1, 2019; (4) articles reporting the effect size (relative risk, odds ratio, etc.) after the confounding variables were taken into consideration; (5) articles reporting both the ACN dietary intake/dose (mg) and ACN source. The exclusion criteria were as follows: (1) non-English articles; (2) articles presenting the results from nonclinical outcomes; (3) articles not fulfilling the inclusion criteria; (4) articles reporting incomplete methodology; and (5) low-quality SRMs after risk-of-bias analysis.

Data extraction and analysis

After independent analysis of the titles and abstracts, 2 authors independently extracted the published data from the main text and tables (Ú.C. and B.-A.S.-R.), and all differences were resolved by a third author (R.S.) as recommended by the PRISMA criteria.[16] The following information was extracted from the included articles: (1) general information, including title, author, year published, and health outcome assessed; (2) type of review performed; (3) assessed population; (4) databases

Table 1 **Population, Intervention, Comparison and Outcomes (PICOS) for the present umbrella review**

Parameter	Inclusion criterion	Exclusion criterion
Population	(1) SRMs of RCTs reporting the effects from the consumption of or supplementation with ACNs on any health outcome; (2) SRMs of OSs regarding the epidemiological associations between ACN consumption/supplementation and any health outcome	(1) Non-English articles; (2) articles presenting the results from nonclinical outcomes; (3) articles not fulfilling the inclusion criteria; (4) articles reporting incomplete methodology; (5) low-quality SRMs after risk-of-bias analysis
Intervention	(1) RCT-SRMs assessing the effects of ACN supplementation on various disease risk factors; (2) OS-SRMs assessing the associations between ACN dietary intake and various health outcomes	(1) RCT-SRMs assessing the effects of the specific ACN or ACN metabolite supplementation on various health outcomes. (2) OS-SRMs assessing the associations between the dietary intake of specific ACNs or ACN metabolites on various health outcomes
Comparison	RCT-SRMs assessing the effects of ACN supplementation on various disease risk factors	
Outcomes	(1) Cardiovascular disease; (2) cancer; (3) metabolic disease; (4) chronic disease; (5) diabetes; (6) hypertension; (7) inflammation	None
Study design	(1) RCT-SRMs; (2) OS-SRMs	Not a systematic review

Abbreviations: ACN, anthocyanin; OS, observational study; RCT, randomized controlled trial; SRM, systematic review and meta-analysis.

searched; (5) date range of the database search; (6) intervention type of the reviewed articles; (7) publication date range of the included articles; (8) quality appraisal tool used for bias assessment; and (9) main conclusions. However, the extraction of the findings and results for an umbrella review ought to be limited to those results obtained directly from the results of SRMs; thus, primary study level data was not reported as a part of the present umbrella review.[15]

Since the results from the OS-SRMs are estimates obtained from food frequency questionnaires, the ACN intake of the included OS-SRMs was transcribed from the original OS-SRMs in round numbers, with the estimated quantities expressed in milligrams and using "approximately" or (in tables and in parentheses) the symbol "∼" to better reflect the imprecise nature of observational data. Moreover, in RCT-SRMs, the results are reported in milligrams (mg) of ACN from the pure ACN doses administered or from the doses of ACNs measured in extracts of ACN-rich fruits administered.

Finally, for heterogeneity (I^2) assessment, the cut-off values for low, moderate, and high heterogeneity were 25%, 50%, and 75%, respectively.[17]

SRM risk-of-bias assessment

The potential risk of bias was independently assessed by 2 authors (B.-A.S.-R. and Ú.C.) for the SRMs that met our inclusion criteria, using the critical appraisal tool for systematic reviews including randomized or non-randomized studies (AMSTAR 2).[18] AMSTAR 2 was based on the evaluation of 16 items for which simple response categories must be fulfilled. The results were used to rate the assessed SRMs as high, moderate, low, or critically low quality.[18] Moreover, the risk of bias of the meta-analysis of OSs was assessed using the Joanna Briggs Institute risk-of-bias assessment.[15] Following the PRISMA criteria, 2 authors (B.-A.S.-R. and Ú.C.) reached a consensus for the risk-of-bias evaluation scores, and all discrepancies were resolved by a third independent author (R.S.).

RESULTS

Literature search, study selection, and characteristics

From our initial screening, 68 SRMs published from inception up to November 1, 2020 were retrieved. After assessing all titles and abstracts, 54 SRMs were excluded for not meeting the inclusion criteria, while no studies were excluded as repeated entries.

The resultant 14 SRMs were further examined. One out of the 14 eligible publications was excluded due to low-quality risk-of-bias assessment (n = 1)[19] (Figure 1), leading to the final inclusion of 13 SRMs.[8,20–31]

From the 13 SRMs included in the present umbrella review, 5 OS-SRMs comprising the information from 57 OSs (2 134 336 participants) were included.[20,22,25,27,31] Moreover, as a part of the present umbrella review, 8 RCT-SRMs were included, summarizing the information from 139 RCTs (>4984 volunteers).[8,21,24–26,28–30]

Regarding the ACN-related outcomes assessed on the included SRMs:

a. T2DM risk associations with ACNs and the effects of ACNs on diverse glycemic control biomarkers were assessed in 3 SRMs. The first OS-SRM included 3 prospective cohort studies[20] assessing the risk of T2DM, 1 RCT-SRM including 32 RCTs to

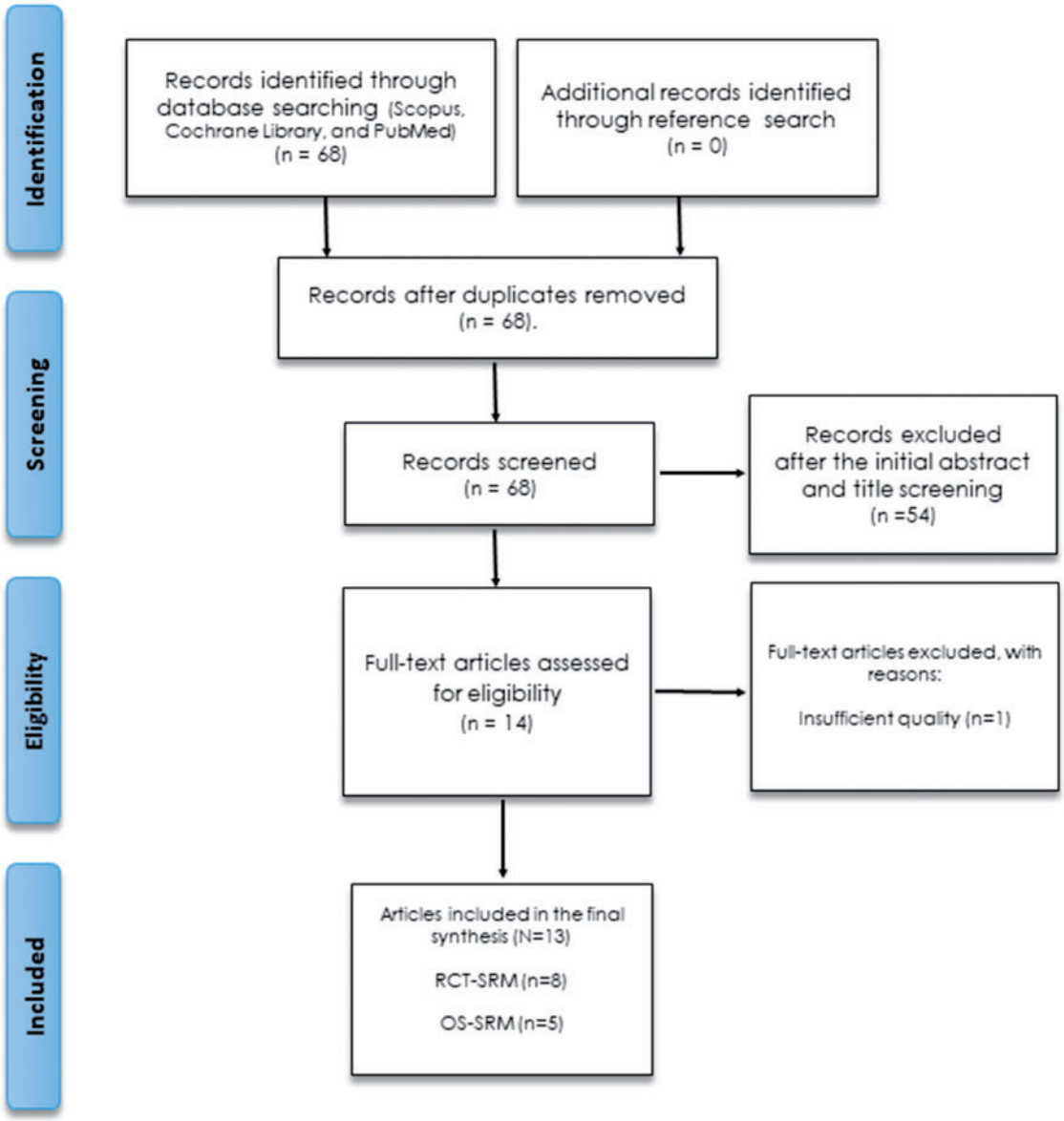

Figure 1 **PRISMA flowchart for the included studies.**

assess the effects of ACNs on diverse glycemic control biomarkers,[21] and 1 RCT-SRM including 19 interventions to evaluate the effects of ACNs on diverse CVD markers.[26]

b. The effects of ACNs on hypertension and blood pressure levels were assessed in 4 SRMs: 1 OS-SRM including 20 OSs (15 cross-sectional trials and 7 prospective OSs)[27]; 1 RCT-SRM including 6 RCTs (1 of them a crossover),[28] 1 RCT-SRM including 6 RCTs,[29] and 1 RCT-SRM including 19 interventions to evaluate the effects of ACNs on hypertension and blood pressure levels.[26]
c. The effects of ACNs on diverse CVD biomarkers, such as lipids, were evaluated from 3 RCT-SRMs: 1 RCT-SRM including 12 RCTs,[8] 1 RCT-SRM including 6 RCTs,[30] and 1 RCT-SRM including 19 interventions to evaluate the effects of ACNs on diverse CVD markers.[26]
d. The effects of ACNs on vascular function were assessed from 1 RCT-SRM including 29 RCTs (8 acute and 21 chronic interventions).[24]
e. The effects of ACNs on vascular and systemic inflammation biomarkers were assessed in 1 RCT-SRM including 32 intervention studies.[25]
f. Finally, the associations between ACN dietary intake and cancer were assessed:
   i. Breast cancer risk and its associations with ACN dietary intake were evaluated from 1 OS-SRM including 12 OSs (6 prospective cohorts, 1 nested case–control, 2 population-based case–controls, and 3 hospital-based case–controls).[31]
   ii. The gastric cancer risk and its association with dietary ACN consumption were assessed from 1 OS-SRM including 6 articles (2 cohorts and 4 case–control studies).[22]

Complete information on the general characteristics of the included OS-SRMs and RCT-SRMs is presented in Table 2.[8,20–31]

In relation to the ACN sources, due to the diverse ACN sources (purified ACNs, n = 37; fruit extracts, n = 42; freeze-dried fruits, n = 11; fruit juices, n = 13) reported in the original RCTs assessed in the SRMs included as a part of our umbrella review, the specific source for attaining the benefits of ACNs could not be identified. Accordingly, the ACN results should be interpreted as the effects or associations of a given ACN dose/dietary intake (ie, extracts or fruits), since the studies only detailed the effects or associations between ACNs and human health that have been reported specifically for ACN consumption and not for fruit intake without a report of ACN equivalence.

Methodological quality and funding of the included studies

AMSTAR 2 was used to assess the methodological quality of the included RCT-SRMs, all of which were selected from inception.[18] As a result, 6 of the RCT-SRMs included were scored as high quality,[8,21,24,28–30] and 1 systematic review was excluded from this analysis due to a critically low score.[19]

None of the RCT-SRMs included reported any relevant conflict of interest concerning their funding. Complete analysis of the AMSTAR 2 risk-of-bias assessment from the included RCT-SRMs is shown in Table S1 in the Supporting Information online. Moreover, the risk of bias of the compiled OS-SRMs[20,22,27,31] was assessed with the "Joanna Briggs Institute's Risk of Bias Assessment Checklist for Systematic Reviews"[15] (see Table S2 in the Supporting Information online).

The effects and associations of ACNs on human health

Glucose metabolism and type 2 diabetes mellitus. The associations between dietary ACN consumption and the risk of T2DM were assessed in 1 high-quality OS-SRM of 3 prospective cohort studies published in 2012 from a total sample of 200 894 participants (12 611 diagnosed with T2DM).[20] As a result, the individuals consuming the highest dietary ACN intake (~22 mg/d), estimated through food frequency questionnaires, showed a significant reduction of 15% in the risk of T2DM (relative risk [RR] = 0.85; 95% CI: 0.80, 0.91; I^2: 14.5%).[20] Moreover, it was noted that the risk of T2DM was decreased by 5% per 7.5 mg/day increase in dietary ACN intake (RR = 0.95; 95% CI: 0.93, 0.98; I^2: 0.00%).[20] Additionally, significant curvilinear associations were described for the dietary intake of ACNs (P for nonlinearity = 0.006).[20]

The effects of ACNs on various glucose metabolism biomarkers were appraised in the high-quality RCT-SRM, including 1491 volunteers (759 cardiometabolic patients/732 controls) from 32 RCTs published between 2004 and 2016.[21]

As a result, when compared against control subjects, the volunteers supplemented with 200–400 mg/day of ACNs significantly reduced their fasting glucose levels by 0.31 mmol/L, equal to 5.58 mg/dL (standard mean deviation [SMD]: −0.31; 95% CI: −0.59, −0.04; I^2 = 80.7%), and the glycated hemoglobin (HbA1c) values decreased by 0.65 units (SMD: −0.65; 95% CI: −1.00, −0.29; I^2 = 72.7%) in cardiometabolic patients receiving ACNs.[21]

Furthermore, ACN consumption in overweight-obese subjects significantly reduced the Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) by 0.65 units (SMD: −0.65; 95% CI: −1.23, −0.06; I^2 = 45.2%),[21] and the 2-h postprandial glucose value by 0.82 mg/dL (SMD: −0.82; 95% CI: −1.49, −0.15; I^2 = 77.7%) in patients supplemented with ACNs. Due to the scarce data described in the Yang et al RCT-SRM,[21] the precise ACN dose needed for reduction of the 2-h postprandial glucose response was not defined.[21]

Finally, the effects of ACN supplementation on various CVD biomarkers were assessed, with the cohort including a total of 572 volunteers from 19 RCTs that were conducted in Poland, the United Kingdom, Italy, Korea, China, Japan, Norway, Austria, Mexico, the United States, Australia, and Iran.[26] It was reported that supplementation with ACN doses between 31.45 and 1050 mg/day had no significant effects on HbA1c or serum insulin levels[26]; however, ACN supplementation with similar doses (31.45–1050 mg/d) was able to significantly reduce the HOMA-IR in treated patients (−0.21, 95% CI −0.36, −0.07; P = 0.004, I^2 = 37.9%).[26]

In short, from OSs, the ACN chronic dietary intake (mainly from fruits) was associated with a reduced risk of T2DM. This association can be explained by the effects of extracts or purified ACNs in RCTs, which cause a significant decrease in fasting glucose levels, and in HbA1c, and HOMA-IR T2DM biomarkers.

Vascular and systemic inflammation. The impact of dietary ACNs on various systemic- and vascular-inflammation biomarkers was assessed from an RCT-SRM assessing the results from 32 RCTs, including 1744 participants, with a moderate risk-of-bias assessment, published between 2007 and 2019, and conducted in Norway, Poland, the United Kingdom, the United States, Iran, Italy, the Czech Republic, China, Korea, Japan, Colombia, Sweden, Australia, and Finland.[25] In the original article, not all outcome results were

Table 2 **General characteristics of the included systematic reviews and/or meta-analyses regarding the effects of or associations between ACN dietary intake or supplementation and human health issues**

Title	Reference	Type of review	Outcome/s	Population	Databases	Data range	N=	Intervention type (n)	Participants involved in meta-analysis (total [participants/cases])	The publication date range of the included studies	Quality appraisal tool used	ACN source	Total ACN dose (mg/d)/Total dietary ACN intake estimation from FFQ (mg/d)	Reported results of ACNs on health outcomes
Type 2 diabetes mellitus and glucose metabolism														
Associations of dietary intakes of ACNs and berry fruits with risk of type 2 diabetes mellitus: a systematic review and meta-analysis of prospective cohort studies	Guo, X. *et al* (2016)	Systematic review and meta-analysis	Type 2 diabetes mellitus risk	General population/Diabetic subjects	Cochrane Library, Embase, and PubMed	Inception – January 2016	3	Cohort (n = 3)	200 894/12 611	2012	Newcastle–Ottawa Scale	Dietary berry intake from FFQ (ie, blueberry, raspberry, strawberry, etc.)	~22.0	15% reduction of T2DM risk (summary RR = 0.85; 95% CI: 0.80, 0.91) 5% T2DM extra risk decrease per each 7.5 mg/d increment of ACN intake (RR = 0.95; 95% CI: 0.93, 0.98)
Effects of ACNs on cardiometabolic health: a systematic review and meta-analysis of randomized controlled trials	Yang, L. *et al* (2017)	Systematic review and meta-analysis	Glycemic regulation and lipid profile	Healthy population/cardiometabolic patients	MEDLINE, Embase, Cochrane database, OVID EBM Reviews, and clinicaltrials.gov	Inception – February 2017	32	RCT (n = 32)	1491 (759/732)	2004–2016	Cochrane risk-of-bias tool	Purified ACNs and berry extracts (whortleberry, cranberry, raspberry, blueberry, blackberry, dried purple carrot, maqui berry, strawberry, black soybean, pomegranate juice, cranberry juice, elderberry juice, tart cherry juice)	2.2–742.0	Fasting glucose reduction (SMD: −0.31; 95% CI: −0.59, −0.04; $I^2 = 80.7\%$) 2-h postprandial glucose reduction (SMD: −0.82; 95% CI: −1.49, −0.15; $I^2 = 77.7$) Glycated hemoglobin reduction (SMD: −0.65; 95% CI: −1.00, −0.29; $I^2 = 72.7\%$)
Effect of ACN supplementation on cardiometabolic biomarkers: a systematic review and meta-analysis of randomized controlled trials	Daneshzad, E. *et al* (2019)	Systematic review and meta-analysis	Cardiovascular disease risk biomarkers	Poland, UK, Italy, Korea, China, Japan, Norway, Austria, Mexico, US, Australia, and Iran	MEDLINE and Embase	Inception – August 2017	19	RCT (n = 19)	572	2000–2016	Jadad scoring system	Purified ACNs	31.5–1050.0	HOMA-IR reduction (−0.21, 95% CI −0.36, −0.07; $P = 0.004$, $I^2 = 37.9\%$) No significant effects on HbA1c, or serum insulin
Endothelial function														
The effect of ACN-rich foods or extracts on vascular function in adults: a systematic review and meta-analysis of randomized controlled trials	Fairlie-Jones, L. *et al* (2017)	Systematic review and meta-analysis	Vascular function	UK, North America, China, Korea, Italy, Australia, Greece, and Israel Adults over 18 years	Embase, MEDLINE, Cochrane, CINAHL, and Scopus	Inception – June 2017	29	Acute RCT (n = 8); chronic RCT (n = 21)	NR	2006–2016	Jadad scoring system	Purified ACNs and berry extracts (blueberry, black currant, cranberry , branberry, cherry juice, cranberry juice, blueberry juice)	1.0–724.0	Improves the FMD after the acute (SMD: 3.92%, 95% CI: 1.47, 6.38, $P = 0.002$; $I^2 = 91.8\%$) and chronic supplementation (SMD: 0.84%, 95% CI: 0.55, 1.12, $P = 0.000$; $I^2 = 62.5\%$) Improves the pulse wave velocity (SMD = −1.27 m/s, 95% CI: −1.96, −0.58, $P = 0.000$; $I^2 = 17.8\%$)
Vascular and systemic inflammation														
Impact of dietary ACNs on systemic and vascular inflammation: systematic review and meta-analysis on randomised clinical trials	Fallah, A. *et al* (2020)	Systematic review and meta-analysis	Markers of vascular and systemic inflammation	Norway, Poland, UK, US, Iran, Italy, Czech Republic, China, Korea, Japan, Colombia, Sweden, Australia, and Finland	PubMed, ISI Web of Science, Scopus, MagIran, and Scientific Information Database	Inception – April 2019	32	RCT (n = 32)	1744	2007–2019	NutriGrade scoring system	Purified ACNs	1.6–1323.0	Decrease plasma concentrations of: CRP (−0.33 mg/L, 95% CI: −0.55, −0.11, $P = 0.003$), IL-6 (−0.41 ρg/mL , 95% CI: −0.70, −0.13, $P = 0.004$) TNF-α (−0.64 ρg/ml, 95% CI: −1.18, −0.09, $P = 0.023$) ICAM-1 (−52.4 ng/mL, 95% CI: −85.7, −19.1, $P = 0.002$)

(continued)

Table 2 **Continued**

Title	Reference	Type of review	Outcome/s	Population	Databases	Data range	N=	Intervention type (n)	Participants involved in meta-analysis (total [participants/cases])	The publication date range of the included studies	Quality appraisal tool used	ACN source	Total ACN dose (mg/d)/Total dietary ACN intake estimation from FFQ (mg/d)	Reported results of ACNs on health outcomes
														VCAM-1 (–49.6 ng/mL, 95% CI: −72.7, −26.5, $P < 0.001$) Increase plasma concentrations of: Adiponectin (0.75 μg/ml, 95% CI: 0.23, 1.26, $P = 0.004$)
Cardiovascular disease risk and risk factors														
Dietary intake of ACNs and risk of cardiovascular disease: a systematic review and meta-analysis of prospective cohort studies	Kimble, R. *et al* (2018)	Systematic review and meta-analysis	Cardiovascular disease risk	US, Europe, and Asia	Scopus, MEDLINE, CINAHL, and Cochrane Library	Inception – January 2018	19	Prospective cohort (n = 19)	602 054/22 673	1996–2016	Metaanalysis Of Observational Studies in Epidemiology (MOOSE)	Dietary berry intake from FFQ (ie, blueberry, raspberry, strawberry, etc.)	∼2.0–75.0	CHD risk reduction (RR = 0.91, 95% CI: 0.83, 0.99; $I^2 = 12.0$) CVD mortality risk reduction (RR = 0.92, 95% CI: 0.87, 0.97; $I^2 = 0\%$) No reduction for myocardial infarction or total CVD
Effect of ACN supplementation on cardiometabolic biomarkers: a systematic review and meta-analysis of randomized controlled trials	Daneshzad, E. *et al* (2019)	Systematic review and meta-analysis	Cardiovascular disease risk biomarkers	Poland, UK, Italy, Korea, China, Japan, Norway, Austria, Mexico, US, Australia, and Iran	MEDLINE and Embase	Inception – August 2017	19	RCT (n = 19)	572	2000–2016	Jadad scoring system	Purified ACNs	31.5–1050.0	No significant effects on weight, waist circumference, or body mass index
Blood pressure and hypertension														
Dietary polyphenol intake, blood pressure, and hypertension: a systematic review and meta-analysis of observational studies	Godos, J. *et al* (2019)	Systematic review and meta-analysis	Blood pressure and hypertension	General population/hypertensive subjects ranging from 40 to 70 years of age	PubMed, Embase	Inception – April 2019	20	Cross-sectional (n = 15); prospective cohort (n = 7)	200 256/45 732	2002–2018	Funnel plot analysis	Dietary berry intake from FFQ (ie, blueberry, raspberry, strawberry, etc.)	"Highest dietary intake"	8% reduction in hypertension risk (RR: 0.92, 95% CI: 0.88, 0.97)
Systematic review of ACNs and markers of cardiovascular disease	Wallace, T. *et al* (2016)	Systematic review and meta-analysis	Cardiovascular disease markers	European, Mexican, Chinese, and Iranian populations	PubMed, Web of Science, BIOSIS	Inception –July 2014	12	RCT (n = 12)	NA	2005–2014	Scottish Intercollegiate Guidelines Network (SIGN) grading system	Purified ACNs from natural berry extracts (whortleberry, bilberry, black currant), and natural berry extracts (chokeberry, red grape, whortleberry, elderberry)	19.2–640.0	No significant effects on blood pressure values ($P > 0.05$)
The effect of ACNs on blood pressure: a PRISMA-Compliant Meta-Analysis of Randomized Clinical Trials	Zhu, Y. *et al* (2016)	Systematic review and meta-analysis	Blood pressure	Chinese, English, Norwegian, and Italian populations	PubMed, Web of Science, Wanfang Database, China National Knowledge Infrastructure	Inception – 2015	6	RCTs (n = 5), crossover (n = 1)	472	2008–2015	Cochrane risk-of-bias tool	Purified ACNs	162.0–640.0	No significant associations with the systolic blood pressure (WMD: 1.15 mmHg, 95% CI: −3.17, 5.47; $I^2 = 56\%$) or the diastolic blood pressure (WMD: 1.06 mmHg, 95% CI: −0.71, 2.83; $I^2 = 0\%$)
Effects of flavonoid-rich fruits on hypertension in adults: a systematic review	Ellwood, L. *et al* (2019)	Systematic review and meta-analysis	Hypertension	Hypertensive subjects	MEDLINE, Embase, Cochrane Trials (CENTRAL), and CINAHL	Inception – September 2018	3	RCTs (n = 3)	119/119	2007–2016	Critical appraisal tools from the Joanna Briggs Institute	Flavonoid-rich fruit (blueberry, bilberry, chokeberry) or equivalent supplement (pomegranate juice, cranberry juice, grape juice, grape extract, bilberry and cranberry extract, plum juice, tart cherry concentrate, cherry juice, purple passionfruit extract)	69.0–742.0	No effects on the systolic blood pressure (MD = 0.96, 95% CI: −3.22, 1.30 ; $P = 0.41$, $I^2 = 0\%$)

(continued)

Table 2 **Continued**

Title	Reference	Type of review	Outcome/s	Population	Databases	Data range	N=	Intervention type (n)	Participants involved in meta-analysis (total [participants/cases])	The publication date range of the included studies	Quality appraisal tool used	ACN source	Total ACN dose (mg/d)/Total dietary ACN intake estimation from FFQ (mg/d)	Reported results of ACNs on health outcomes
Effects of ACNs on cardiometabolic health: a systematic review and meta-analysis of randomized controlled trials	Yang, L. *et al* (2017)	Systematic review and meta-analysis	Glycemic regulation and lipid profile	Healthy population/cardiometabolic patients	MEDLINE, Embase, Cochrane database, OVID EBM Reviews, and clinicaltrials.gov	Inception – February 2017	32	RCTs (n = 32)	1491 (759/732)	2005–2016	Cochrane risk-of-bias tool	Purified ACNs and berry extracts (whortleberry, cranberry, raspberry, blueberry, blackberry, dried purple carrot, maqui berry, strawberry, black soybean, pomegranate juice, cranberry juice, elderberry juice, tart cherry juice)	2.2–742.0	No significant effects on the systolic or diastolic blood pressure ($P > 0.05$)
Effect of ACN supplementation on cardiometabolic biomarkers: a systematic review and meta-analysis of randomized controlled trials	Daneshzad, E. *et al* (2019)	Systematic review and meta-analysis	Cardiovascular disease risk biomarkers	Poland, UK, Italy, Korea, China, Japan, Norway, Austria, Mexico, US, Australia, and Iran	MEDLINE and Embase	Inception – August 2017	19	RCT (n = 19)	572	2000–2016	Jadad scoring system	Purified ACNs	31.5–1050.0	No significant effects on the systolic or diastolic blood pressure
Plasmatic lipid profile Systematic review of ACNs and markers of cardiovascular disease	Wallace, T. *et al* (2016)	Systematic review and meta-analysis	Cardiovascular disease markers	European, Mexican, Chinese, and Iranian populations	PubMed, Web of Science, BIOSIS	Inception – July 2014	12	RCT (n = 12)	NA	2005–2014	Scottish Intercollegiate Guidelines Network (SIGN) grading system	Purified ACNs from natural berry extracts (whortleberry, bilberry, black currant), and natural berry extracts (chokeberry, red grape, whortleberry, elderberry)	19.2–640.0	Significantly decreases LDLc in hyperlipidemic populations; however, not in the healthy or individuals with other cardiovascular-related diseases Significantly increases in HDLc in hyperlipidemic subjects, healthy individuals, individuals with metabolic syndrome, and prehypertensive subjects That ACNs cause a significant reduction of total cholesterol that ranges between −5.86 and −25.53% in patients with metabolic syndrome , and in patients with dyslipidemia
Effects of ACN on serum lipids in dyslipidemia patients: a systematic review and meta-analysis	Liu, C. *et al* (2016)	Systematic review and meta-analysis	Serum lipids	Dyslipidemic subjects	PubMed, Web of Science, MEDLINE, Cochrane Library, China National Knowledge Infrastructure, and Wanfang Database	Inception – July 2015	6	RCTs (n = 6)	586/586	2008–2014	Cochrane risk-of-bias tool	Purified ACNs	90.0–320.0	Significantly reduces plasmatic levels of: TC (MD = −24.06, 95% CI: −45.58, −2.64 mg/dL; $I^2 = 93\%$). TGs (MD = −26.14, 95% CI: −40.20, −3.08 mg/dL; $I^2 = 66\%$) LDLc (MD= −22.10, 95% CI: −34.36, −9.85; mg/dL; $I^2 = 61\%$) Significantly increases the plasmatic levels of: HDLc (MD = 5.58, 95% CI: 1.02, 10.14 mg/dL; $I^2 = 90\%$)
Effects of ACNs on cardiometabolic health: a	Yang, L. *et al* (2017)			Healthy population/	MEDLINE, Embase, Cochrane database, OVID	Inception – February 2017	32	RCTs (n = 32)	1491 (759/732)	2005–2016	Cochrane risk-of-bias tool	Purified ACNs and berry extracts (whortleberry,	2.2–742.0	Significantly decreases the plasmatic levels of:

(continued)

Table 2 **Continued**

Title	Reference	Type of review	Outcome/s	Population	Databases	Data range	N=	Intervention type (n)	Participants involved in meta-analysis (total [participants/cases])	The publication date range of the included studies	Quality appraisal tool used	ACN source	Total ACN dose (mg/d)/Total dietary ACN intake estimation from FFQ (mg/d)	Reported results of ACNs on health outcomes
systematic review and meta-analysis of randomized controlled trials		Systematic review and meta-analysis	Glycemic regulation and lipid profile	cardiometabolic patients	EBM Reviews, and clinicaltrials.gov							cranberry, raspberry, blueberry, blackberry, dried purple carrot, maqui berry, strawberry, black soybean, pomegranate juice, cranberry juice, elderberry juice, tart cherry juice)		TC in 0.33 mmol/L ([12.76 mg/dL] SMD: −0.33; 95% CI: −0.62, −0.03; $I^2 = 86.9\%$) LDLc in 0.35 mmol/L ([13.53 mg/dL] SMD: −0.35; 95% CI: −0.66, −0.05; $I^2 = 85.2\%$) Significantly increases the plasmatic values of: HDLc values in 0.24 mmol/L ([9.28 mg/dL] SMD: 0.24; 95% CI: 0.00, 0.49; $I^2 = 81.1\%$) No significant effects in the TG, ApoA1 and ApoB plasmatic values
Effect of ACN supplementation on cardiometabolic biomarkers: a systematic review and meta-analysis of randomized controlled trials	Daneshzad, E. *et al* (2019)	Systematic review and meta-analysis	Cardiovascular disease risk biomarkers	Poland, UK, Italy, Korea, China, Japan, Norway, Austria, Mexico, US, Australia, and Iran	MEDLINE and Embase	Inception – August 2017	19	RCT (n = 19)	572	2000–2016	Jadad scoring system	Purified ACNs	31.5–1050.0	Significantly decreases the plasmatic levels of: HDLc: 7.40 mg/dL (95% CI = 6.04, 8.75; $P < 0.0001$; $I^2 = 84.6\%$) Significantly decreases the plasmatic levels of: LDLc: -10.67 mg/dL (95% CI: −14.97, −6.37)
Cancer Flavonoids, flavonoid subclasses and breast cancer risk: a meta-analysis of epidemiologic studies	Hui, C. *et al* (2013)	Meta-analysis	Breast cancer risk	NR	Cochrane Library, MEDLINE, Embase, and PubMed	Inception – July 2012	12	Prospective cohort (n = 6); nested case–control (n = 1): population-based case–control (n = 2); hospital-based case–control (n = 3)	9513/181 906	1997–2010	Funnel plot analysis	Dietary berry intake from FFQ (ie, blueberry, raspberry, strawberry, etc.)	∼4.0–21.0	No significant association for breast cancer risk (RR = 0.97, 95% CI: 0.87, 1.08)
Intake of ACNs and gastric cancer risk: a comprehensive meta-analysis on cohort and case–control studies	Yang, D. *et al* (2019)	Systematic review and meta-analysis	Gastric cancer risk	NR	PubMed, Embase, Web of Science, and Cochrane Library	Inception – June 2018	6	Cohort (n = 2); case–control (n = 4)	949.226	2004–2017	GRADE system	Dietary berry intake from FFQ (ie, blueberry, raspberry, strawberry, etc.)	>1.0	No significant association for gastric cancer risk (RR = 0.92, 95% CI: 0.81, 1.04) No significant evidence of association by subgroup analysis for: Gender (men: RR = 1.02, 95% CI: 0.73, 1.40; women: RR = 0.80, 95% CI: 0.52, 1.23) Tumor location (cardia: RR = 0.90, 95% CI: 0.62, 1.31; non-cardia: RR = 0.86, 95% CI: 0.69, 1.07)

Abbreviations: ACN, anthocyanin; CINAHL, cumulative index to nursing and allied health; CHD, coronary heart disease; CI, confidence interval; CRP, C-reactive protein; CVD, cardiovascular disease; FFQ, food frequency questionnaire; FMD, flow-mediated dilation; HDLc, high-density lipoprotein cholesterol; LDLc, low-density lipoprotein cholesterol; MD, mean difference; N/A, not available; RCT, randomized controlled trial; RR, relative risk; SMD, standard mean deviation; T2DM, type 2 diabetes mellitus; TC, total cholesterol; TGs, triglycerides; WMD, weighted mean difference.

obtained after the analysis of all 32 included studies. The RCTs included in the analysis presented high heterogeneity regarding the ACN doses used for the interventions, which ranged between 1.60 and 1323 mg/day.[25] However, after the meta-analysis, the authors reported that doses of ACNs of >300 mg/day can significantly reduce the pooled risk of high serum concentrations of CRP (−0.33 mg/L, 95% CI: −0.55 to −0.11, $P = 0.003$), IL-6 (−0.41 ρg/mL, 95% CI: −0.70 to −0.13, $P = 0.004$), TNF-α (−0.64 ρg/mL, 95% CI: −1.18 to −0.09, $P = 0.023$), ICAM-1 (−52.4 ng/mL, 95% CI: −85.7 to −19.1, $P = 0.002$), and VCAM-1 (−49.6 ng/mL, 95% CI: −72.7 to −26.5, $P < 0.001$).[25] Finally, ACNs doses of >300 mg/day also increased plasmatic adiponectin levels (0.75 μg/mL, 95% CI: 0.23 to 1.26, $P = 0.004$) as a biomarker of adipocyte saturation in the assessed populations.[25]

Endothelial function assessment by arterial flowmediated dilation. The effects of ACNs on endothelial function were assessed in 1 high-quality RCT-SRM of 29 RCTs [acute (n = 8), chronic (n = 21)].[24] Endothelial function and arterial flow-mediated dilation (FMD) outcomes were assessed from the SRM of 4/29 included RCTs, while pulse wave velocity results were obtained from the SRM of 2/29 included RCTs.[24] The 29 included RCTs were published between 2006 and 2016 and conducted in adult male, adult female, and postmenopausal female populations aged over 18 years from the United Kingdom, North America, China, Korea, Italy, Australia, Greece, and Israel.[24] As a result, from the SRM of 4/29 included RCTs, the authors concluded that acute ACN intake with doses of between 1 and 724 mg/day from various sources caused a significant 3.92% improvement in arterial FMD (SMD: 3.92%, 95% CI: 1.47–6.38, $P = 0.002$, $I^2 = 91.8\%$).[24] In the same way, after the SRM of the results of 4/29 included RCTs, chronic ACN consumption from diverse sources significantly improved arterial FMD (SMD: 0.84%, 95% CI: 0.55–1.12, $P < 0.001$; $I^2 = 62.5\%$).[24]

Finally, after the evaluation of the results of 2/29 included studies in the SRM, acute ACN supplementation significantly enhanced the pulse wave velocity by −1.27 m/s (SMD: −1.27, 95% CI: −1.96, −0.58, $P < 0.001$; $I^2 = 17.8\%$) and increased the vascular reactivity (SMD: 2.41, 95% CI: −0.91, −3.91, $P = 0.002$; $I^2 = 92.6\%$).[24] These benefits were noted for healthy individuals and nonhealthy populations that included obese, overweight, and hypertensive subjects.[24] No significant differences were noted for the arterial augmentation index, a measure of systemic arterial stiffness.[24] Hence, ACNs (mostly extracts or purified ACNs) significantly improve endothelial function in healthy subjects but also in people suffering from obesity, overweight, or hypertension.

Blood pressure and hypertension. In the OS-SRM of 20 OSs (cross sectional [n = 15], prospective cohort [n = 7]), published between 2002 and 2018, the association between dietary ACN intake and the occurrence of hypertension was assessed from 200 256 participants (45 732 cases of hypertension).[27] Dietary ACN intake was found to be significantly associated with an 8% reduction in the risk of hypertension, when comparing the highest against the lowest dietary exposure to ACNs (RR: 0.92, 95% CI: 0.88, 0.97; $I^2 = 74\%$),[27] although the specific values for the "highest" and "lowest" dietary exposures were not defined by the original authors of the SRM.[27]

However, the effects of ACNs on blood pressure and other CVD biomarkers were assessed in the RCT-SRM based on 12 RCTs published between 2005 and 2014,[8] and no significant effects of the ACN interventions on the participants' blood pressure values were noted for doses between 19.2 and 640.0 mg/day.[8] Interestingly, no adverse effects of ACNs were reported across the studies at levels of up to 640 mg/day.[8] Nonetheless, in one of the RCTs included in the RCT-SRM, an 85 mg/day dose of a flavonoid-rich chokeberry extract (*Aronia melanocarpa*; 25% ACNs) or a placebo was added to the statin therapy of postmyocardial infarction patients for 6 weeks, resulting in significant decreases in both the systolic and diastolic blood pressure values.[8,32]

Accordingly, in an RCT-SRM including 6 clinical studies (RCTs [n = 5], crossover [n = 1]), including 472 postmenopausal light smokers or healthy participants (in China, England, Norway, and Italy), no effects of ACN doses of between 162 and 640 mg/day on either systolic blood pressure (weighted mean difference: 1.15 mmHg, 95% CI: −3.17, 5.47; $I^2 = 56\%$) or diastolic blood pressure were identified (weighted mean difference: 1.06 mmHg, 95% CI: −0.71, 2.83; $I^2 = 0.00\%$).[28]

Additionally, in another RCT-SRM, the effects of the administration of any type of flavonoid-rich fruit or equivalent supplement, including ACNs, were compared against placebo or other interventions in 119 adult subjects with hypertension from 3 RCTs published between 2007 and 2016.[29] The subgroup analysis showed no significant effects of ACN intake on systolic or diastolic blood pressure values (mean difference [MD]: 0.96, 95% CI: −3.22, 1.30; $P = 0.41$, $I^2 = 0.00\%$) when compared against a placebo.[29] In accordance, no significant effects were observed on systolic or diastolic blood pressure ($P > 0.05$).[21]

Therefore, despite the reduced risk of hypertension found in OSs after ACN dietary intake, no effects of

ACN supplementation (mostly as extracts or purified ACNs) on hypertension were determined from the included RCTs.

Plasmatic lipid profile. The effects of ACNs on the lipid profiles of humans were assessed in 3 RCT-SRMs.[8,21,30] In the systematic review of 12 RCT articles, 10 studies describing the effects of ACNs on diverse CVD biomarkers were assessed in European (n = 5), Chinese (n = 2), Iranian (n = 2), and Mexican (n = 1) populations.[8] As a result, the consumption of ACN doses of between 19.2 and 640.0 mg/day significantly decreased the values of plasmatic LDLc by approximately 14.08%.[8] Notably, all 4 RCTs reporting positive results were conducted on hyperlipidemic (n = 3) and dyslipidemic populations (n = 1); healthy populations did not report significant changes in LDLc.[8] Moreover, ACN supplementation with doses of 19–140 mg/day also increased the high-density lipoprotein cholesterol (HDLc) plasmatic levels by approximately 11.79% in hyperlipidemic, dyslipidemic, healthy, metabolic syndrome, and prehypertensive individuals,[8] and significantly reduced the total cholesterol (TC) values by between 5.86% and 25.53% ($P < 0.01$) in patients with metabolic syndrome or hyperlipidemia.[8]

In addition, the cholesterol-lowering properties of ACNs were assessed in the RCT-SRM of 586 dyslipidemic subjects from 6 RCTs.[30] As a result, when compared against the placebo group, the consumption of ACN doses of 90–320 mg/day for 4–24 weeks significantly reduced the plasmatic levels of TC by 24 mg/dL (MD: −24.06, 95% CI: −45.58, −2.64 mg/dL; $I^2 = 93\%$), of triglycerides (TGs) by 26.14 mg/dL (MD = −26.14, 95% CI: −40.20, −3.08 mg/dL; $I^2 = 66\%$), and of LDLc by 22.10 mg/dL (MD: −22.10, 95% CI: −34.36, −9.85; mg/dL; $I^2 = 61\%$), while increasing the HDLc by 5.58 mg/dL (MD: 5.58, 95% CI: 1.02, 10.14 mg/dL; $I^2 = 90\%$).[30]

These ACN effects were particularly true for Iranian (MD: −50.58, 95% CI: −86.52, −14.64 mg/dL, $I^2 = 89\%$) and Chinese (MD: −6.59, 95% CI: −12.44, −0.73 mg/dL, $I^2 = 1\%$) populations.[30]

Moreover, in a high-quality RCT-SRM of 27 trials, the effects of ACNs on glycemic control and plasmatic lipids were assessed in 1491 volunteers (732 cardiometabolic patients).[21] It was found that the intake of 200–400 mg/day of ACNs from diverse sources was associated with a decrease in TC of 0.33 mmol/L ([12.76 mg/dL] SMD: −0.33; 95% CI: −0.62, −0.03; $I^2 = 86.9\%$) and a decrease in LDLc of −0.35 mmol/L ([13.53 mg/dL] SMD: −0.35; 95% CI: −0.66, −0.05; $I^2 = 85.2\%$) and marginally increased HDLc values of +0.24 mmol/L ([9.28 mg/dL] SMD: 0.24; 95% CI: 0.00, 0.49; $I^2 = 81.1\%$).[21] Moreover, the TG, ApoA1, and ApoB plasmatic values were not significantly modified.[21]

The information available on the effects of ACN intake (mostly as extracts or purified ACNs) on plasmatic lipids showed reduction in TGs, TC, and LDLc, while increasing HDLc plasmatic values.

Cancer. The associations between dietary ACN intake and cancer were assessed in 1 moderate-quality[31] and 1 high-quality OS-SRM.[22] In the first OS-SRM, the associations between ACN dietary intake and breast cancer risk were assessed from 9513 breast cancer patients and 181 906 controls involved in 12 OSs (prospective cohort [n = 6], nested case–control [n = 1], population-based case–control (n = 2), hospital-based case–control [n = 3]), published between 1997 and 2010.[31] As a result, there were no significant associations between ACN dietary intake and breast cancer risk (RR = 0.97, 95% CI: 0.87, 1.08).[31]

Additionally, the associations between ACN dietary intake and gastric cancer risk were assessed in 949 226 patients and controls from 6 OSs (cohort [n = 2], case–control [n = 4]) published between 2004 and 2017.[22] As a result, no significant associations between the dose–response relationship were found in this OS-SRM (RR = 0.92, 95% CI: 0.81, 1.04).[22] Furthermore, no significant associations were found for the linear or nonlinear dose response of ACNs in the subgroup analysis by gender (men: RR = 1.02, 95% CI: 0.73, 1.40; women: RR = 0.80, 95% CI: 0.52, 1.23) or for tumor location (cardia: RR = 0.90, 95% CI: 0.62, 1.31; noncardia: RR = 0.86, 95% CI: 0.69, 1.07).[22]

Thus, ACN dietary intake showed no significant associations with breast cancer or with gastric cancer risks.

DISCUSSION

This umbrella review has been compiled following the principles published by the Joanna Briggs Institute.[15] The main goal of the review was to summarize, contrast, and compare the currently available information on the effects of ACN consumption on human health from RCT-SRMs, and on the association between ACN consumption and various health outcomes from OS-SRMs. The review was performed following the principles published by Aromataris et al.[15] Consequently, the objectives, search strategy, presentation of results, and summary of evidence were extracted from the literature, providing a comprehensive analysis of the available evidence regarding the effects of ACN intake and supplementation on human health. In an attempt to provide a descriptive analysis of the available information, the present umbrella review included as many health

outcomes related to noncommunicable chronic diseases as possible.

Based on the information obtained from 5 OS-SRMs that included 57 OSs (32 prospective, 15 cross-sectional, and 10 case–control studies, containing 2 134 336 participants), ACN intake from various sources, such as berries, was significantly associated with an 8% reduction in the risk of hypertension, as reported by the authors.[27]

Based on the information obtained from 8 RCT-SRMs that included 139 RCTs and >4984 participants (surprisingly, 2 SRMs[8,24] did not report the original RCT participant numbers), ACN extracts improved the plasmatic lipid profile and endothelial function without having significant effects on blood pressure.

Thus, the information obtained from the included OS-SRMs and RCT-SRMs provides new perspectives for the management of cardiometabolic diseases in humans, with the exception of blood pressure, for which a reduction in hypertension risk was observed,[27] but intervention studies did not support blood pressure reduction.[8,21,26,28,29]

ACNs, glucose metabolism, and T2DM

T2DM is characterized by insulin resistance and high blood sugar in association with multiple long-term complications, including coronary heart disease, stroke, kidney failure and a reduction in blood flow to the extremities, causing significant morbidity and mortality.[33] In recent years, T2DM has considerably increased in prevalence among the general population, particularly for younger individuals, which contributes to longer exposure to the disease and increased risk of complications and numerous adverse effects.[34] Accordingly, the prevention and treatment of T2DM has attracted significant interest from the scientific and medical communities. In accordance, an analysis of the effects of ACN consumption on glycemic control,[21,26] as well as of the association between ACN intake and the risk of T2DM, was conducted.[20]

From epidemiological studies including over 200 894 participants, dietary ACN intake of approximately 22 mg/day coming from various sources reduced an individual's risk of T2DM by 15%.[20] The benefits of ACNs on the reduction of T2DM risk are dose dependent, since additional 5% reductions are reported per 7.5 mg/day increase in dietary ACN intake, mostly from berry consumption,[20] demonstrating the benefits of chronic ACN intake.

Moreover, the information retrieved from the RCT-SRMs regarding the effects of ACNs (mostly as ACN extracts or purified ACNs) on diverse glucose metabolism biomarkers demonstrates that ACN supplementation with doses of between 200 and 400 mg/day significantly reduces fasting glucose levels by 5.58 mg/dL and Hb1Ac values by 0.65 units.[21] In addition, ACN consumption of between 31.45 and 1050 mg/day significantly decreased the HOMA-IR by between 0.21[26] and 0.65 units in overweight-obese subjects.[21]

These results suggest that some of the effects of ACN consumption on glycemic control as observed in RCTs might explain the reduction in the risk of T2DM observed in healthy individuals consuming the highest dietary amounts of ACNs in general.[20]

Furthermore, one possible mechanism of action of the effects of ACN intake on glucose metabolism can be supported by some in vitro studies showing that ACNs promote the synthesis of glycogen and reduce gluconeogenesis in HepG2 cells.[35] The reduction in gluconeogenesis is caused by a decrease in the activity of 2 important enzymes, phosphoenolpyruvate carboxykinase and glucose-6-phosphatase, in response to the activation of PPARγ caused by incubation of HepG2 cells[35,36] and adipocytes[37,38] in a 1000 μg/mL concentration of ACN. Thus, the in vitro cell results demonstrate that 1000 μg/mL ACN extracts from mulberry increase cellular glucose uptake and reduce insulin resistance in adipocytes[37,38] and hepatic cells.[35,36]

Moreover, in a meta-analysis of 18 studies in which the associations between dietary flavonoid intake and T2DM risk were evaluated, a nonlinear association between ACN intake and T2DM risk was found, suggesting that the intake of recommended ACN doses should minimize the risk of T2DM.[39]

Finally, in vitro, ACNs downregulate the expression of the GLUT2 transporter in human intestinal Caco-2 cells,[38,40] possibly explaining why patients show diminished intestinal glucose absorption, reflected as better postprandial glycemia in healthy populations.[21,26]

Consequently, ACN intake significantly reduces the risk of T2DM in humans and aids in its management.

ACNs and arterial FMD functional assessment and vascular inflammation

To date, the ACN effects on endothelial function have been assessed only in 1 high-quality RCT-SRM, in which it was determined that acute ACN supplementation with ACN-rich foods, ACN-rich extracts or purified ACNs significantly increases arterial FMD values by 3.92%, reduces pulse wave velocity values by 1.27 m/s, and increases vascular reactivity in adults >18 years old.[24]

Additionally, chronic supplementation for between 1 and 12 weeks with 12–625 mg/day ACNs from ACN-rich foods, ACN-rich extracts, or purified ACNs to

healthy and nonhealthy populations, such as smokers, hypertensive patients, and patients with metabolic syndrome, significantly increases arterial FMD by 0.84%,[24] demonstrating beneficial effects on vascular function from both acute and chronic supplementation.

Acute ACN consumption, using doses of between 7 and 724 mg/day from various berry extracts, significantly improves vascular function, and increased arterial FMD possibly arises from the effects of various phenolic compounds, for instance, during the first 2 h, caused by the presence of vanillic and benzoic acids in plasma, while the ACN effects observed after 6 h appear to be caused by hippuric and homovanillic acid concentrations in the plasma of healthy males.[41,42]

Moreover, the acute benefits on vascular function, FMD, associated with ACN supplementation are provoked by the regulation of proteins involved in nitrous oxide (NO) metabolism, consequently enhancing the endothelial function observed in healthy humans.[41,43] The regulation of proteins involved in NO metabolism by ACNs reduces the expression of various adhesion molecules in endothelial cells in humans[25,41] and prevents peroxynitrite-mediated endothelial dysfunction, as demonstrated by incubating human umbilical vein endothelial cells in a 0.085 μM cyanidin-3-glucoside (an ACN metabolite) concentration in vitro.[43,44] The beneficial effects on FMD are not accompanied, however, by an ACN-mediated blood pressure reduction.

Finally, chronic ACN supplementation using doses of >300 mg/day from various berry extracts significantly decreases various markers of vascular and systemic inflammation, such as CRP, IL-6, TNF-α, ICAM-1, and VCAM-1, while increasing adiponectin levels in the plasma of healthy, hypertensive, and cardiometabolic patients,[25] thus demonstrating a beneficial influence of ACNs on vascular and systemic inflammation.

ACNs and blood pressure

There is contradictory information from RCTs regarding the effects of ACN intake on blood pressure values in human populations, and from OSs on the associations between ACN intake and blood pressure. Our analysis of OS-SRMs indicated that a chronic dietary ACN intake of approximately 500 mg/day is associated with an 8% reduction in the risk of hypertension in the general population.[27]

However, the effects of ACN supplementation using doses of between 162 and 640 mg/day for 4–12 weeks showed no effects on blood pressure values in postmenopausal women, light smokers, or healthy humans.[28] Thus, the association between ACN intake and a reduced risk of hypertension could be caused by other bioactive components present in ACN-rich fruits, such as soluble fiber.[45] Moreover, in OSs, the presence of inadvertent and unanalyzed confounding variables, such as recall bias or some unintended recollection errors,[46,47] could explain the reduction in the risk of hypertension observed in healthy individuals consuming larger amounts of ACN-rich fruits, providing >500 mg/day ACNs.[27]

Interestingly, ACNs could be of some aid in the treatment of acute myocardial infarction patients undergoing a secondary cardiovascular prevention regimen, since ACN supplementation with doses of between 162 and 640 mg/day for 6 weeks significantly reduced the severity of inflammation assessed through highly sensitive C reactive protein serum concentrations and oxidized LDLc concentrations, and lowered the systolic and diastolic blood pressure values by mean average values of 11 and 7.2 mmHg, respectively, when compared against a control group treated only with statins.[32]

ACNs and the plasmatic lipid profile

Evidence regarding the effects of ACN supplementation on plasma lipids in human RCTs consistently reports various clinically relevant beneficial effects.

It has been demonstrated that supplementation with 300 mg/day doses of ACNs can significantly decrease plasmatic TC concentrations to between 12.76 mg/dL and 24.06 mg/dL,[21,26,30] and LDLc concentrations to between 10.67 mg/dL and 22.10 mg/dL in hypercholesterolemic subjects.[21,26,30]

On the other hand, ACN doses of between 90 and 320 mg/day for between 4 and 24 weeks significantly reduced the plasmatic concentration of TGs by 26.14 mg/dL in dyslipidemic subjects in only one of the included studies.[30]

Finally, the 300 mg/day ACN dose demonstrated a significant increase in HDLc plasmatic concentrations of between 5.58 mg/dL[30] and 9.28 mg/dL[21] in patients suffering from hyperlipidemia, metabolic syndrome, and prehypertension.

The improvement in plasma lipids secondary to ACN supplementation might be related to the improvement in reverse cholesterol transport and HDL particle functionality that has been described in humans, acting beyond a simple increase in c concentrations.[48] In dyslipidemic subjects, ACN doses of between 200 and 400 mg/day for at least 4 weeks can enhance the cholesterol efflux capacity of HDL particles in healthy humans by inhibiting cholesteryl ester transfer protein (CETP).[30]

In addition, ACNs exert an antioxidant effect on HDLs by improving paraoxonase-1 activity in HDL particles, leading to increased HDL functionality in

hypercholesterolemic subjects,[49] and they have also shown interesting potential for the reduction of aortic cholesterol levels, as demonstrated in hyperlipidemic mice.[50]

The proposed ACN mechanism of action was found in vitro, demonstrating that various ACN metabolites, predominantly cyanidin-3-glucoside and peonidin-3-glucoside, can increase the luminal precipitation of cholesterol in human Caco-2 cells, which could be explained by the suppression of cholesterol uptake via competitive inhibition.[51]

In parallel, the luminal precipitation of cholesterol from a diet containing 1% ACNs for 6 weeks can explain the high ACN fecal excretion and lower plasmatic cholesterol values observed after ACN administration in hamsters.[52]

As a result, it has been demonstrated that ACN supplementation induces several beneficial changes in plasma lipids and can improve HDL functionality in humans.[8,21,26,30]

ACNs and cancer

Analysis of the association between an ACN dietary intake of at least 1 mg/day and cancer risk in humans assessed according to 2 high-quality OS-SRMs of cohort studies[22,31] showed no association with decreased risk of breast cancer[31] or gastric cancer.[22]

Potential associations between ACNs and the risk of other types of cancer, such as prostate, colon, or pancreatic cancer, have not been addressed thus far in high-quality SRMs, which indicates an ongoing opportunity for future research in the field.

Limitations

The fact that umbrella reviews are a type of systematic review that summarizes what is known regarding a particular subject and focuses only on the evidence available from the included SRMs results in various limitations related to the original studies included in the original SRMs, such as the amount of evidence available, the quality of the original studies, and the richness of their analyses.[53]

One of the most important limitations of the present umbrella review is that restricting the information from other reviews that differ from SRMs, such as narrative reviews, reduces the final amount of data available for analysis from other publications.[15,53] To address this and to amplify the number of SRMs, a broader search term and less stringent inclusion and exclusion criteria would need to be applied to find all possible relevant articles.

Additionally, special considerations must be taken when analysing the positive health effects of ACNs (observed in RCTs) or associations between ACNs and health benefits (observed in OSs) resulting from whole-food consumption, as the attribution of any result to one single bioactive compound is hindered because of the presence of other bioactive molecules that might account for a portion of the results, or because of the interaction between different bioactive compounds within a food, such as may occur with soluble fiber.[54–56]

Moreover, a summary of the outcomes of SRMs might result in "an optimistic picture" of evidence, mostly caused by positive publication bias[57]; therefore, restraint should be applied when interpreting the results of some of the outcomes assessed here.

Similarly, parallel considerations should be taken regarding the possible interactions between ACNs and cancer, since the effects of ACNs in cancer patients have not been assessed in RCT-SRMs thus far; thus, the results are not presented as a part of the present review.

In addition, OS-SRMs evaluate and describe the associations between the estimated ACN dietary intake and various health outcomes for very different populations with important differences in each population baseline diet: consumption of distinct amounts and types of foods with ACN contents hinders the interpretation of the results.

Finally, some consideration should be taken when interpreting the results from the present umbrella review, as the high heterogeneity (I^2) of the results reduces the strength of the conclusions.

CONCLUSIONS

From 5 OS-SRMs, including 57 studies and more than 2.1 million participants, ACN dietary intake might be significantly associated with a clinically relevant reduction in the risks of T2DM and hypertension, while no beneficial associations between ACN dietary intake and the risk of breast or gastric cancer were reported.

From 8 RCT-SRMs, including 139 RCTs and >4984 volunteers, it was suggested that purified ACN supplementation significantly reduce the HOMA-IR, fasting glucose, Hb1Ac, TC, TGs, and LDLc values, while also increasing the HDLc concentrations in the plasma of healthy volunteers and dyslipidemic patients.

Additionally, ACNs might improve arterial FMD, pulse wave velocity, and vascular reactivity, indicating some beneficial effects of ACNs over various markers of cardiometabolic disease.

The current evidence does not support any effects of ACN supplementation on systolic or diastolic blood pressure values.

The effects of ACN doses of between 200 mg/day and 400 mg/day for at least 4 weeks seem to provide significant health benefits, particularly for cardiometabolic health.

As a result, based on OS-SRMs, chronic ACN dietary intake might be associated with the prevention of T2DM and hypertension. According to RCT-SRMs, it has been suggested that supplementation using ACN-rich extracts or purified ACNs could be considered in the management of glucose metabolism, hypercholesterolemia, and the improvement of endothelial function in humans.

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Declaration of interest. The authors have no relevant interests to declare.

Supporting Information

The following Supporting Information is available through the online version of this article at the publisher's website.

Table S1 **Critical appraisal tool for risk of bias assessment in systematic reviews including randomized or nonrandomized studies in healthcare interventions (AMSTAR 2)**

Table S2 **Critical appraisal tool for the risk of bias in systematic reviews of observational studies (elaborated by the Joanna Briggs Institute)**

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