JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Review

Cite This: J. Agric. Food Chem. 2018, 66, 11531−11543

pubs.acs.org/JAFC

Anthocyanin Tissue Bioavailability in Animals: Possible Implications for Human Health. A Systematic Review

Berner Andrée Sandoval-Ramírez,[†] Úrsula Catalán,*[,†,§] Sara Fernández-Castillejo,[†] Laura Rubió,[∥] Alba Macià,[∥] and Rosa Solà[†,‡]

[†]Faculty of Medicine and Health Sciences, Medicine and Surgery Department, Functional Nutrition, Oxidation, and CVD Research Group (NFOC-Salut), Universitat Rovira i Virgili, 43201 Reus, Spain

[‡]Hospital Universitari Sant Joan de Reus (HUSJR), 43204 Reus, Spain

[§]Institut d'Investigació Sanitària Pere Virgili (IISPV), 43204 Reus, Spain

[∥]Food Technology Department, Agrotecnio Research Center, University of Lleida, Av/78 Alcalde Rovira Roure 191, 25198 Lleida, Spain

ABSTRACT: Anthocyanins (ACNs) are promising health-enhancing phenolic compounds. We focus on ACN animal tissue bioavailability to provide an evidentiary link between tissue ACNs and their associated health properties. We performed a systematic review of electronic libraries; 279 results were retrieved, and 13 publications met inclusion criteria. Extracted information included animal model employed, administration route, doses, analysis method, and ACN concentration values in tissues. Total ACN concentrations were detected in mice kidney (2.17×10^5 pmol/g), liver (1.73×10^5 pmol/g), heart (3.6×10^3 pmol/g), and lung (1.16×10^5 pmol/g); and in pig brain (6.08×10^3 pmol/g). ACNs showed a predominance of parent ACNs in long-term experiments versus an ACN metabolite predominance in short-term experiments. ACNs detected in animal tissues, such as cyanidin-3-glucoside, suggest it may have an important role in human health. This information could be useful to determine proper ACN-intake biomarkers in biological samples in futures studies.

KEYWORDS: *anthocyanins, tissue bioavailability, health, animal studies, mechanism-of-action, phenolic compounds*

INTRODUCTION

In recent years, preventive medicine has acquired a significantly more important role in healthcare systems primarily due to the increase in aged population and the prevalence of obesity, diabetes, metabolic syndrome, and hypertension[1,2] in which a healthy diet and lifestyle play a significant role.[3] Dietary changes and healthy eating patterns such as the Mediterranean diet are characterized by a high intake of fruit, vegetables, fish, nuts, and olive oil, that can reduce the risk for chronic noncommunicable diseases.[4,5] However, most bioactive molecules present in fruits and vegetables and the corresponding biochemical pathways that grant them their associated health benefits are still unclear.

Among the most well-known bioactive molecule groups found in fruits and vegetables are the phenolic compounds such as anthocyanins (ACNs). ACNs are water-soluble plant pigments responsible of giving red and blue coloration in fruits, flowers, seeds, and plants.[6] They are frequently found in the skin of many fruits and in the flesh of some berries,[7] with concentrations ranging from 0.1% up to 1.0% of the fruit's or vegetable's dry weight.[8] ACNs consist of a wide range of molecule classes and subclasses,[9] each one with its own properties and most of them capable of modulating or regulating wide and diverse biochemical pathways.[10,11]

It is well-known that the ACN's absorption site[12] and the chemical structure of anthocyanins[13] have an impact on the phenolic profiles that can be detected in human body fluids. These differences can be attributed to changes in pH between the gastric and colonic lumens,[14,15] but also because of an important ACN's metabolization by colonic microbiota which can occur within the first 2 h after the ingested ACNs reach the human colon.[16] Thus, colonic metabolization often results in new metabolites that were not present in the original ACN food source.[17−19] These colonic ACN metabolites have shown interesting potential for human health and could even have greater biological activity than their parent molecules, since colonic ACN metabolites are more abundant and are quite often better absorbed than their ACN parents.[20,21] Consequently, ACNs colonic metabolization has a direct impact in the amount and half-life of different metabolites of ACNs.[22] The mentioned differences in ACN absorption and metabolization render different plasmatic phenolic profiles that in turn may be of paramount importance when determining tissue profiles and the benefits of long-term versus short-term ACN ingestion; however, this remains an area of uncertainty. Thus, absorption and metabolization probably contributes to the ACN tissue bioavailability, and this might be a key relevant aspect for determining biomarkers to assess the intake of ACN containing foods. Moreover, bioavailability of ACNs in tissues and cells show great potential for the treatment and prevention of different pathological entities.[23−27]

In the current Review, we hypothesize on how the ACNs, either parent ACNs, such as cyanidin-3-glucoside (C3G),

Received: July 27, 2018
Revised: October 19, 2018
Accepted: October 22, 2018
Published: October 22, 2018

DOI: 10.1021/acs.jafc.8b04014
J. Agric. Food Chem. 2018, 66, 11531−11543

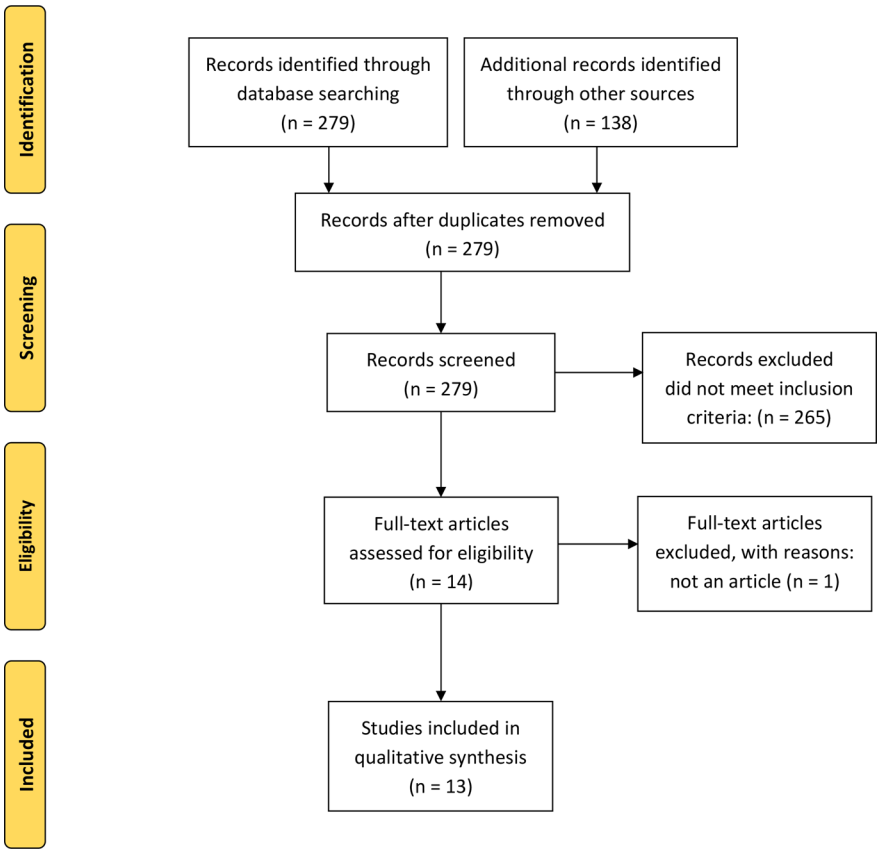

Figure 1. PRISMA statement flowchart for anthocyanin tissue availability adapted to animal studies.

delphindin-3-glucoside, malvidin-3-glucoside, peonidin-3-glucoside (P3G), and petunidin-3-glucoside, or their metabolites could be detected in target tissues might exert an effect in the cells in which they are present. In consequence, providing a link between ACNs present in tissues after their consumption and the described intracellular mechanisms of action are an explanation of ACN reported beneficial effects on human health. We adapted the concept published by de Ferrars et al.[13] and defined "parent" ACNs as an ACN structure from which other molecules, namely, metabolites, are obtained by substituting or adding radicals through the methylation, conjugation, sulfation, and glucuronidation. Thus, our primary goal is to perform a systematic review of the current knowledge reported on the tissue bioavailability of ACNs in different animal tissues after the administration of diverse ACN sources and consequently identifying possible bioactive molecules that could be clinically relevant. Moreover, through an up-to-date descriptive review of in vitro experiments, we aspire to provide an explanation for the reported in vivo ACN health effects.

MATERIALS AND METHODS

Search Strategy and Selection Criteria. For the present Review, our group adapted the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement (http://www.prisma-statement.org/), designed for clinical trials,[28] to the systematic review of animal studies due to the lack of a better and more standardized screening method. An electronic-based search in the scientific libraries PubMed (http://www.ncbi.nlm.nih.gov/pubmed) and Scopus (http://www.scopus.com) was performed. A broad search term of "anthocyanin AND bioavailability" was used for our search, since more specific options did not render all studies that could meet the inclusion criteria used for the present Review. Results were screened based on their titles, abstracts, and full-text availability according to our inclusion criteria: (1) animal interventions using ACNs extracts or ACN supplementation and (2) ACNs tissue bioavailability evaluation. All non-English publications, and studies that did not report specific metabolite concentration values in tissues were excluded from the discussion.

Data Extraction, Standardization, and Analysis. Two independent authors (B.A.S.-R. and Ú.C.) extracted published data from text and tables; for data published as graphics, approximates were estimated through scaling; all differences were resolved by a third reviewer (R.S.). The following information was extracted from all reviewed studies: (1) study characteristics including the animal model used, ACN administration routes, doses, and sample analysis method; and (2) total ACN, parent ACN, or ACN metabolite concentration assessed in animal tissues.

All concentration units were converted and homogenized into the international system of units (molar) using metabolite molecular weight published in the Phenol-Explorer (http://phenol-explorer.eu/).[29] After homogenization, data was analyzed and text, Tables, and Figures were elaborated, presented, and discussed.

RESULTS

Literature Search, Studies Selection, and Characteristics. A total of 279 results published from inception up to February 2018 were found in our initial screening. All entries titles and abstracts were assessed for relevance according to inclusion criteria; 265 studies did not meet inclusion criteria and 1 study was excluded because it was not a paper. Thirteen publications[10,11,30−40] were identified and further examined as full texts. The flow diagram of the selection process used is shown in Figure 1.

Of all publications evaluated, 7 short-term experiments[11,33−37,40] assessed ACN tissue bioavailability after a single dose of ACN

DOI: 10.1021/acs.jafc.8b04014
J. Agric. Food Chem. 2018, 66, 11531−11543

administered either orally or intravenously (IV). The remaining 6 experiments[10,30−32,38,39] assessed ACN tissue bioavailability after a long-term oral-controlled ACN supplementation. Out of the 13 studies, 5 were performed on Wistar rats,[30,33,35−37] 3 on mice,[11,32,34] 2 on Sprague−Dawley rats,[10,40] 2 on pigs,[31,38] and 1 on Zucker rats.[39]

High-performance liquid chromatography and mass spectrometry were the most used analysis techniques to determine ACN concentration in tissues. Kidney, liver, and brain were the most frequently analyzed organs with highest total ACN concentrations: 2.17×10^5 pmol/g in mice kidneys,[11] 1.73×10^5 pmol/g in mice liver,[32] and 6.08×10^3 pmol/g in young swine brain.[38] Complete information on general characteristics of studies that assessed ACN tissue bioavailability in animals as well as the total ACN concentrations detected in analyzed tissues are presented in Table 1.

Short versus Long-Term Tissue ACNs Bioavailability. One of the differences that was noted through our findings was that the ACN bioavailability profiles in animal tissues after a short versus long-term administration of ACNs showed different patterns. We observed a clear predominance of parent ACNs in long-term experiments, as shown in Table 3, versus ACNs metabolites predominance in short-term experiments reflected in Table 2. These differences will be further addressed and explained in more detail in the following sections.

Tissue Bioavailability in Short-Term Experiments. In short-term experiments, high methodology variability was observed. These experiments were performed after a single dose of an ACN concentrate either orally or IV. Oral doses presented high variability between 8 mg/kg grape extract[37] and 500 mg/kg C3G extract,[11] while IV doses ranged between 1 mg/kg C3G extract[11] and 5 mg/kg bilberry extract.[33] Animals were sacrificed between 0 min and 24 h after administration, although sacrifice was most commonly performed 15 min after ACN either oral or IV administration.[11,33,35,36]

4′-*O*-methylcyanidin-3-*O*-β-glucopyranoside highest concentration (19.18 pmol/g) in liver was found 15 min after a 5 mg/kg IV ACN dose after bilberry extract administration,[33] while in kidneys petunidin-3-glucoside was the highest ACN (2317.00 pmol/g) found at 15 min after a 670 nmol ACN IV dose.[36] C3G was the parent ACN found at highest concentrations in brain at 0.25 min (40.46 pmol/g) after the IV administration of bilberry extract.[35] Moreover, C3G was also found in gastrointestinal tract (8×10^5 pmol/g), lung (5.19×10^4 pmol/g), and prostate (4×10^4 pmol/g) with peak concentrations at different times after an oral single dose of C3G extract.[11] Complete information regarding peak concentrations of parent ACNs and ACN metabolites at different doses and times from short-term animal interventions are reported in Table 2.

Tissue Bioavailability in Long-Term Experiments. High heterogeneity in oral ACN administrated doses was also noted for long-term interventions: doses ranged from 27.5 mg/kg/day[38] up to 617.6 mg/kg/day.[32] Animal sacrifices were performed at different periods comprehended between 10 days and 8 weeks. In long-term experiments, animal brains were the most common organs analyzed.[10,30−32,35,38,41] In the analyzed brains, the highest concentration for an ACN was found for malvidin-3-glucoside (4.43 pmol/g) after an 82.5 mg/kg/day oral supplementation with bilberry extract for 3 weeks in young swine pigs.[38]

Cyanidin-3-rutinoside-5-β-D-glucoside was found in the heart (1.50×10^{-4} pmol/g), brain (1.85×10^{-4} pmol/g), liver (3.34×10^{-5} pmol/g), kidney (9.24×10^{-4} pmol/g), and bladder (2.16×10^{-4} pmol/g) after 2 weeks of 200 mg/kg/day oral supplementation with bilberry in Wistar rats.[30] Complete information regarding doses, maximum ACN peak or metabolite concentration, and animal organ tissues analyzed for long-term animal interventions are reported in Table 3.

ACNs Bioavailability in Animal Tissues. *Cardiac Tissue Bioavailability.* Out of the 13 studies assessed, 3 studies evaluated the presence of ACNs in animal hearts using different ACN sources.[11,30,32] For mice hearts obtained after a 2 week ACN 617.6 mg/kg/day oral supplementation with C3G extract, tissue ACN concentrations were not detected,[32] whereas ACN concentrations of 1.11 pmol/g were reported in heart tissue for oral ACN doses as low as 200 mg/kg/day in a 3 week intervention using bilberry extract in Wistar rats.[30] These differences in heart ACN detection were probably due to differences in sample processing proceedings, ACN source, or animal model used since analysis methods used for ACN detection did not differ between both studies. Interestingly, higher supplementation oral doses of 2000 mg/kg/day using bilberries in Wistar rats reported a 54% (0.51 pmol/g) reduction for heart tissue ACN bioavailability compared with lower doses (200 mg/kg/day) of bilberry extract,[30] suggesting the possible increase or upregulation in ACN's degradation and elimination mechanisms available inside cardiac cells.

In mice hearts, ACNs were also found in short-term experiments performed when a single C3G extract oral dose of 500 mg/kg was administered to mice, the study reported peak of C3G (1.4×10^4 pmol/g) 10 min post administration.[11] Cyanidin metabolites were the most commonly in animal hearts analyzed, with the highest concentration reported for long-term studies was 4.95×10^{-4} pmol/g after administrating a 200 mg/kg/day oral dose of bilberry for 3 weeks in Wistar rats,[30] and 1.40×10^4 pmol/g on heart for short-term studies after a single 500 mg/kg oral dose of C3G extract in mice.[11]

There is no consensus on ACN daily doses and administration times that are needed to detect ACNs in target tissues. However, as stated before, the minimum ACN time and oral dose supplementation to achieve heart tissue detection has been demonstrated in rats after 3 weeks of bilberry extract supplementation with 200 mg/kg/day.[30] This oral dose of 200 mg/kg/day in rats represents a human oral dose of 32.4 mg/kg/day, representing around 2 g of bilberry extract for a standard 70 kg human.[42]

Brain Tissue Bioavailability. ACN brain bioavailability was evaluated in 7 out of the 13 studies that met inclusion criteria for the present review.[10,30−32,35,38,43] ACNs were not detected in brain tissue after a 48 mg/kg/day oral ACN extract supplementation for an 8 week period with wild blueberry in Sprague−Dawley rats,[10] after 509 mg/kg/day oral dose of blueberry powder after 10 days in Zucker rats, or after a 617.6 mg/kg/day oral grape seed extract supplementation for 2 weeks in mice.[39]

However, a maximum ACN concentration of 6.08×10^3 pmol/g was found in analyzed brains after a 3 week experiment performed on young swine pigs in which an 82.5 mg/kg/day oral dose of tart cherry was administered, whereas an ACN distribution pattern was determined for every brain region.[38] In same study, also a direct correlation between dose consumed and tissue bioavailability was observed.[38]

In the brain, after an 82.5 mg/kg/day ACN oral dose of tart cherry, petunidin-3-glucoside was the ACN found in highest concentration (6.66 pmol/g), followed by malvidin-3-glucoside (4.43 pmol/g) and P3G (4.40 pmol/g).[38] Moreover, in

DOI: 10.1021/acs.jafc.8b04014
J. Agric. Food Chem. 2018, 66, 11531−11543

Table 1. General Characteristics of Studies Performing Anthocyanin Bioavailability in Animals and Total Anthocyanin Concentrations Detected in Analyzed Tissues[a]

			administration routes and dosage						total anthocyanin mean concentration (pmol/g) in different tissues											
			oral		IV															
author and ref	animal model	anthocyanin source	single dose	long-term	single dose	human equivalent	experiment duration	analysis method	heart	brain	liver	kidney	bladder	R. Fat	prostate	lung	testes	spleen	thymus	muscle	eyes
Del Bò et al.[10]	SD rat	wild blueberry powder		48 mg/kg/day		7.8 mg/kg/day	4 and 8 wk	HPLC		ND	ND										
Ichiyanagi et al.[33]	Wistar rat	bilberry extract	400 mg/kg		5 mg/kg		15 min	HPLC			206.14	673.26									
Aqil et al.[34]	athymic nude mice	blueberry powder	10 mg/mouse	5% diet sup			120 min/10 days	UPLC/LC-MS								≤0.25 ng[b]					
Forna7saro et al.[35]	Wistar rat	C3G extract			668 nmol		0, 25, 5, 10, 15, 20 min	UPLC-MS/MS		44.98[c]	599.05[c]	3.68×10^3[c]									
Vanzo et al.[36]	Wistar rat	C3G extract			670 nmol		0.25, 1, 5, 15 min	HPLC-DAD-MS			1011.00	138.00									
Chen et al.[39]	Zucker rat	grape seed extract		509 mg/kg/day		82.5 mg/kg/day	10 days	LC-MS OR LC-MS/MS		ND											
Marczylo et al.[11]	mice	C3G extract	500 mg/kg			40.5 mg/kg	5, 10, 20, 30, 60, 90, 120 min	HPLC	3.6×10^3		3.53×10^4	2.17×10^5			4.96×10^4	2.83×10^4					
					1 mg/kg		5, 10, 15, 20, 30, 60, 90, 120 min	HPLC	720.00		80.00	970.00			ND	430.00					
Chen et al.[38]	Pig	bilberry extract		27.5 mg/kg/day		20 mg/kg/day	3 wk	LC-MS/MS		1.76×10^3[d]											
				82.5 mg/kg/day		60 mg/kg/day	3 wk	LC-MS/MS		6.08×10^3[d]											
Borges et al.[40]	SD rat	raspberry juice	2.77 mL juice				0, 1, 2, 3, 4, 6, 12, 24 h	HPLC-PDA-MS/MS			ND	ND	ND								
Kirakosyan et al.[30]	Wistar rat	tart cherry powder		200 mg/kg/day		32.4 mg/kg/day	3 wk	LC-MS/MS	1.11	0.27	0.43	2.07	1.33	ND							
				2000 mg/kg/day		324.3 mg/kg/day	3 wk	LC-MS/MS	0.51	0.43	1.52	1.65	3.66	ND							
Vanzo et al.[37]	wistar rat	grape extract	8 mg/kg			1.3 mg/kg	10 min	HPLC			2.65×10^3	3.03×10^3									
							30 min	HPLC			3.02×10^3	2.11×10^3									
Milbury et al.[31]	pig	blueberry powder		2% blueberry powder			8 wk	HPLC-MS/MS		0.1683[d]											
Hiroyuki et al.[32]	mice	bilberry extract		617.6 mg/kg		50.1 mg/kg	2 wk	HPLC-DAD & LC-MS/MS	ND	ND	1.73×10^5	1.14×10^5		ND		1.16×10^5	1.49×10^5	ND	ND	ND	ND

[a]IV, intravenous; HPLC, high-performance liquid chromatography; UPLC, ultrahigh performance liquid chromatography; MS, mass spectrometry; LC, liquid chromatography; ND, not detected; G.I., gastrointestinal; R. Fat, retroperitoneal fat; SD, Sprague−Dawley; wk, week. [b]Exact values not reported. [c]Maximum detected value. [d]Data extracted from figures.

J. Agric. Food Chem. 2018, 66, 11531−11543 DOI: 10.1021/acs.jafc.8b04014

Table 2. Peak Parent and Anthocyanin Metabolite Concentration at Different Doses and Times From Short-Term Animal Interventions[a]

					parent or metabolite anthocyanin maximum concentration (pmol/g) at different time points (min) in analyzed tissues																
					heart	brain			liver						kidney					prostate	lung
parent or metabolite ACN	ref	dose	ACN source	administration route	10	0.25	2	15	0.25	1	10	15	20	30	0.25	2	10	15	30	5	10
4′-*O*-methyl cyanidin 3-*O*-*β*-D-glucopyranoside	33	5 mg/kg	bilberry	IV								19.18						46.12			
4′-*O*-methyl delphinidin 3-*O*-*β*-D-galactopyranoside	33	5 mg/kg	bilberry	IV								15.73						55.09			
4′-*O*-methyl petunidin 3-*O*-*β*-D-galactopyranoside	33	5 mg/kg	bilberry	IV								11.11						21.60			
cyanidin 3-*O*-*β*-D-galactopyranoside	33	5 mg/kg	bilberry	IV								5.41						14.19			
cyanidin-3-glycoside	35	668 nmol	C3G extract	IV		40.46			56.61						1.69						
	11	500 mg/kg	C3G extract	oral	1.40×10^4									3.23×10^4			3.86×10^5			4×10^4	5.19×10^4
cyanidin-3-*O*-*β*-D-glycoside-glucuronide	33	5 mg/kg	bilberry	IV								2.13						18.36			
delphindin-3-glucoside	36	670 nmol	C3G extract	IV						641.00											
	37	8 mg/kg	grape extract	oral										50.00					110.00		
malvidin 3-*O*-*β*-D-galactopyranoside	33	5 mg/kg	bilberry	IV								13.50						21.84			
malvidin 3-*O*-*β*-D-glucopyranoside	33	5 mg/kg	bilberry	IV								2.59						2.40			
malvidin-3-6-*O*-acetylglucoside	37	8 mg/kg	grape extract	oral							140.00			160.00			220.00		930.00		
malvidin-3-6-*O*-*p*-coumaryl-glucoside	37	8 mg/kg	grape extract	oral							290.00			580.00			40.00		120.00		
malvidin-3-glucoside	36	670 nmol	C3G extract	IV						370.00								546.00			
	37	8 mg/kg	grape extract	oral							1260.00			1830.00			1170.00		1450.00		
peonidin 3-glucoside	35	668 nmol	C3G extract	IV					539.38						1.99						
	37	8 mg/kg	grape extract	oral			2.07				290.00			310.00			230.00		270.00		
peonidin 3-*O*-*β*-D-galactopyranoside	33	5 mg/kg	bilberry	IV								7.24						54.07			
petunidin 3-*O*-*β*-D-galactopyranoside	33	5 mg/kg	bilberry	IV								3.46						9.53			
petunidin-3-glucoside	35	668 nmol	C3G extract	IV				2.45					3.06			2.82					
	36	670 nmol	C3G extract	IV														2317.00			

J. Agric. Food Chem. 2018, 66, 11531−11543
DOI: 10.1021/acs.jafc.8b04014

Table 2. continued

parent or metabolite ACN	ref	dose	ACN source	administration route	parent or metabolite anthocyanin maximum concentration (pmol/g) at different time points (min) in analyzed tissues																					
					heart		brain			liver					kidney					prostate		lung				
					10	0.25	2	15	0.25	1	10	15	20	30	0.25	2	10	15	30	5	10					
	37	8 mg/kg	grape extract	oral							660.00			880.00			1366.00		700.00							

[a]ND, not detected; IV, intravenous; G.I., gastrointestinal; C3G, cyanindin-3-glucoside. Homogenized values obtained from data collection. Parent anthocyanins are presented in bold.

brain, a P3G peak concentration of 2.07 pmol/g was found after 2 min for a single 8 mg/kg oral raspberry dose.[37]

Liver Tissue Bioavailability. Total ACN concentrations were reported in liver, ranging from not detected in Sprague–Dawley rats after an 8 week period of oral intervention of 48 mg/kg/day with wild blueberry extract,[10] to a detected concentration of 1.73×10^5 pmol/g in mice livers after a 2 week oral intervention with 617.6 mg/kg/day of bilberry extract.[32] In short-term studies, 4′-*O*-methyl cyanidin 3-*O*-*β*-D-glucopyranoside was the ACN metabolite found in highest concentration (19.18 pmol/g), 15 min after a 5 mg/kg IV dose of bilberry extract in Wistar rats.[33] In long-term experiments, cyanidin-3-rutinoside-5-*β*-D-glucoside was the ACN metabolite found in highest concentrations (1.16×10^{-3} pmol/g), after a 2000 mg/kg/day oral dose for 3 weeks of tart cherry extract in Wistar rats.[30]

Kidney Tissue Bioavailability. ACN concentrations detected in kidney ranged from 1.65 pmol/g after an oral supplementation of 2000 mg/kg/day with bilberry for 3 weeks,[30] up to 2.17×10^5 pmol/g for a C3G extract unique oral dose of 500 mg/kg presenting a maximal C3G concentration at 10 min after oral administration in rats.[11] In kidney a maximal C3G concentration was found at 10 min after a unique oral C3G extract administration,[11] while after a single oral dose of 500 mg/kg of raspberry other ACN metabolites such as malvidin-3-glucoside, P3G, and petunidin-3-glucoside maximum peaks were detected at 10, 10, and 15 min, respectively.[37]

Lung Tissue Bioavailability. In lung tissue, C3G was found at a peak concentration of 3.86×10^5 pmol/g after a single 5 mg/kg C3G extract IV administrated dose in mice;[11] and at a concentration of 1.15×10^4 pmol/g after an oral supplementation with 617.6 mg/kg of a C3G extract for 2 weeks in mice.[32] Beyond C3G, other parent ACNs such as delphinidin-3-glucoside (3.40×10^4 pmol/g) and P3G (8.80×10^4 pmol/g) were also found in lungs after oral doses of 617.6 mg/kg of supplementation with a C3G extract for 2 weeks in mice.[32]

ACN Bioavailability in Other Animal Tissues. ACNs were also detected in prostatic tissue of mice (total concentrations of 4.96×10^4 pmol/g), after a single oral 500 mg/kg dose of C3G extract,[11] and on testes of mice (1.16×10^5 pmol/g) after a 2 week intervention using a dose of 617.6 mg/kg/day of bilberry oral supplementation.[32] However, to date not enough bioavailability assays have been published regarding ACN presence in these tissues to properly determine the importance of specific phenolic compounds in relation of the health of these organs.

DISCUSSION

The present work aims to summarize the knowledge reported on the ACN's tissue bioavailability after the administration of different ACN sources in animals. Thus, identifying possible bioactive molecules in animal tissues could suggest clinical relevance for humans. Surprisingly, there is a remarkable lack of studies that describe both ACN tissue bioavailability and their pharmacodynamics explaining ACN effects. In order to solve this issue, we describe the relationship between the ACNs detected concentrations in animal tissues and their possible health effects, by providing a link between the findings of ACNs in in vivo animal assays and in vitro experiments, leading to determine specific mechanisms of action in cell or/and tissues. This allowed us to provide a potential explanation of ACN's health effects in humans.

DOI: 10.1021/acs.jafc.8b04014
J. Agric. Food Chem. 2018, 66, 11531−11543

Table 3. Maximum Peak Parent or Metabolite Anthocyanin Concentration Detected in Animal Tissues Obtained After Long-Term Animal Intervention Studies[a]

				parent or metabolite anthocyanin maximum concentration (pmol/g) at different time points (weeks) found in analyzed tissues									
				heart	brain		liver		kidney		lung	testes	bladder
parent or metabolite ACN	ref	ACN source	dose	3	3	8	2	3	2	3	2	2	3
cyanidin-3-rutinoside-5-β-D-glucoside	30	tart cherry	200 mg/kg/day	1.50×10^{-4}	1.85×10^{-4}			3.34×10^{-5}		9.24×10^{-4}			2.16×10^{-4}
	30	tart cherry	2000 mg/kg/day	2.28×10^{-4}	4.00×10^{-4}			1.16×10^{-3}		6.10×10^{-4}			4.32×10^{-4}
cyanidin-3-arabinoside	31	blueberry powder	2% sup			1.48×10^{-3}							
	38	bilberry extract	27.5 mg/kg/day		1.50								
	38	bilberry extract	82.5 mg/kg/day		3.18								
	31	blueberry powder	2% sup			5.92×10^{-4}							
cyanidin-3-glucoside	38	bilberry extract	27.5 mg/kg/day		0.85								
	38	bilberry extract	82.5 mg/kg/day		3.17								
	31	blueberry powder	2% sup			8.14×10^{-3}							
	32	bilberry	617.6 mg/kg/day				2.30×10^{4}		2.50×10^{4}		1.15×10^{4}	ND	
cyanidin-3-glucosyl rutinoside	30	tart cherry	200 mg/kg/day	6.26×10^{-5}	ND			ND		ND			5.69×10^{-4}
	30	tart cherry	2000 mg/kg/day	1.03×10^{-4}	ND			ND		ND			3.09×10^{-3}
cyanidin-3-rutinoside	30	tart cherry	200 mg/kg/day	3.98×10^{-4}	ND			2.46×10^{-4}		1.90×10^{-4}			4.64×10^{-4}
	30	tart cherry	2000 mg/kg/day	1.67×10^{-4}	ND			ND		4.63×10^{-4}			ND
delphindin-3-galactoside	38	bilberry extract	27.5 mg/kg/day		0.21								
	38	bilberry extract	82.5 mg/kg/day		3.32								
	31	blueberry powder	2% sup			5.18×10^{-3}							
delphindin-3-glucoside	38	bilberry extract	27.5 mg/kg/day		0.21								
	38	bilberry extract	82.5 mg/kg/day		2.90								
	32	bilberry	617.6 mg/kg/day				3.40×10^{4}		1.14×10^{5}		3.40×10^{4}	ND	
malvidin-3-galactoside	38	bilberry extract	27.5 mg/kg/day		0.93								
	38	bilberry extract	82.5 mg/kg/day		3.97								
	31	blueberry powder	2% sup			1.85×10^{-2}							
malvidin-3-glucoside	38	bilberry extract	27.5 mg/kg/day		1.43								

J. Agric. Food Chem. 2018, 66, 11531−11543 DOI: 10.1021/acs.jafc.8b04014

Table 3. continued

				parent or metabolite anthocyanin maximum concentration (pmol/g) at different time points (weeks) found in analyzed tissues									
				heart	brain		liver		kidney		lung	testes	bladder
parent or metabolite ACN	ref	ACN source	dose	3	3	8	2	3	2	3	2	2	3
	38	bilberry extract	82.5 mg/kg/day		4.43								
	31	blueberry powder	2% sup			5.33×10^{-2}							
malvidin-3-glucoside/ peonidin-3-glucoside	32	bilberry	617.6 mg/kg/day				1.08×10^{5}		8.80×10^{4}		7.05×10^{4}	1.49×10^{5}	
peonidin 3-glucoside	38	bilberry extract	27.5 mg/kg/day		0.51								
	38	bilberry extract	82.5 mg/kg/day		4.40								
	31	blueberry powder	2% sup			1.55×10^{-2}							
peonidin-3-arabinoside	31	blueberry powder	2% sup			2.22×10^{-3}							
peonidin-3-galactoside	38	bilberry extract	27.5 mg/kg/day		NR								
	38	bilberry extract	82.5 mg/kg/day		3.80								
	31	blueberry powder	2% sup			3.70×10^{-3}							
peonidin-3-rutinoside	30	tart cherry	200 mg/kg/day	4.95×10^{-4}	8.37×10^{-5}			3.35×10^{-4}		9.52×10^{-4}			7.71×10^{-5}
	30	tart cherry	2000 mg/kg/day	1.54×10^{-5}	3.33×10^{-5}			1.17×10^{-4}		5.74×10^{-4}			1.43×10^{-4}
petunidin-3-galactoside	38	bilberry extract	27.5 mg/kg/day		ND								
	38	bilberry extract	82.5 mg/kg/day		3.84								
	31	blueberry powder	2% sup			2.52×10^{-2}							
petunidin-3-glucoside	38	bilberry extract	27.5 mg/kg/day		0.23								
	38	bilberry extract	82.5 mg/kg/day		6.66								
	32	bilberry	617.6 mg/kg/day				8.00×10^{3}		ND		ND	ND	

[a]ND, not detected; sup, supplementation; NR, Not reported. Homogenized values obtained from data collection. Parent anthocyanin are presented in bold.

J. Agric. Food Chem. 2018, 66, 11531−11543
DOI: 10.1021/acs.jafc.8b04014

Tissue ACN Profile Differences in Short versus Long-Term in Animal Oral Interventions. From current data, we suggest that the difference in tissue ACN bioavailability profiles obtained after short and long-term oral assays performed in animal interventions could be explained by the saturation of the absorption mechanisms, mainly at gastric level via bilitranslocase, and by the further colonic metabolization of non-absorbed ACNs after an oral consumption.[10] Gastric absorption is then followed by hepatic metabolization in which ACN metabolites are created by glucosidation, methylation, and glucuronidation, and then liberated into the plasma, causing a rise of ACN metabolite forms that are further delivered to the different tissues.[17]

Furthermore, from our results, in oral short-term experiments, a predominance of parent ACNs in tissues is detected, whereas the long-term oral administration of ACNs can cause the saturation of the ACN gastric absorption mechanisms, mainly bilitranslocase located in the mucosecretory and parietal cells of the stomach.[44] This saturation leads to the hydrolysis of parent ACNs into their metabolites. As a result, total ACN metabolite concentration increases in the stomach, following later stages of the digestion process when ACNs are susceptible to be metabolized by colonic microbiota.[12,17] Therefore, in long-term ACN administration, colonic microbiota reconvert ACNs into their metabolized versions, that are then absorbed through the colonic epithelia, transferred to the bloodstream, and from there distributed to the rest of tissues in animals.[17] This colonic process is also probably the same for humans. Whereas in short-term exposure, the main absorption is performed through the gastric epithelia.[22,44]

A good example of how ACN gut microbiota metabolization and colonic absorption are relevant to their bioavailability is the generation of the two major molecules product of C3G's degradation: protocatechuic acid (PCA) and pholoroglucinaldehyde (PGA). After C3G's ingestion and gastric absorption, C3G's remnant concentrations arrive at the colon, where gut microbiota start its degradation yielding PCA out of C3G's B-ring and PGA out of its A-ring.[11,22] Afterward, these molecules are absorbed, giving rise to their respective plasmatic concentration peaks and therefore rending maximum concentration times (T-max; 3.3 ± 0.7 h for PGA and 2.8 ± 1.1 h for PCA), much later than their parent C3G (1.8 ± 0.2 h).[13] This information suggests that the observed time/dose response over tissue ACN profiles might be of relevance for tissue-specific health effects derived from ACN administration. As consequence, the result is that short-term oral exposure to ACNs leads to a higher presence of parent ACNs, while the long-term administration renders a more diverse pattern in which ACN metabolites are more commonly found.

ACN's Mechanisms of Action and Possible Health Repercussions. Since ACN mechanisms of action in humans cannot be determined due to the intrinsic methodologic limitations and ethical concerns to detect their presence in human tissues, this subject has not yet been properly studied. Because of that, complementary to animal studies, in vitro studies performed on cellular models become relevant when determining important bioactive ACN molecules and their effects on biochemical pathways involved in the treatment or prevention of a series of pathologies.[45−48] ACNs have shown great potential in cellular models of experimentation as bioactive molecules capable of not just reducing oxidative stress,[49−51] but also possibly being capable of modifying pathways from different types of cancer (hepatocarcinoma,[52] colorectal cancer,[53,54] and breast cancer[55,56]) or modulating obesity and its associated low-grade inflammation state.[57]

ACNs and Cardiac Health. Cardioprotective effects of some ACNs such as C3G have been demonstrated in mice, both in cellular and in animal model experiments,[58] since it has been shown to be able to reduce doxorrubicin's, an anticancer drug, cardiotoxicity in myocytes; where after high doses of pure C3G extract, cellular death was reduced after a 3 week oral supplementation.[58] Another study evaluated on an isoproterenol-induced myocardial infarction mice model, after 28 days of ACN oral administration. In this study, mice showed reduced plasmatic protein levels of creatine kinase muscle/brain (CK-MB), a cardiac necrosis biomarker, increased levels of intracellular enzymatic antioxidants, and decreased levels of apoptotic markers probably achieving C3G's effects through the activation of β_1 adrenergic receptors in cardiac cells,[23] which in turn could reduce cytochrome *c* intracellular concentrations, therefore leading to fewer cardiac cellular damage and apoptosis.[59]

Moreover, after 4 weeks of 10 mg/kg/day C3G oral supplementation in rats with myocardial infarction, left ventricle dilatation and body mass loss were prevented while not showing any improvements in cardiac structure and function.[58] These cardioprotective effects of C3G were not observed after 8 weeks of ACN administration of the same doses.[60] The discrepancy the results obtained after 4 and 8 weeks of daily C3G consumption need to be addressed.

However, ACN metabolization in the heart might be generated to counteract possible adverse effects of ACN activity, since high concentrations of phenolic compounds can cause rather negative intracellular effects, the opposite of what has been demonstrated for lower ACN concentrations as reported before.[61]

Up to the date of the present Review, few studies have evaluated the effect of oral ACNs in humans and showed a lower systolic blood pressure, lower plasmatic triglyceride (TG) levels, and healthier TG/HDL cholesterol ratio.[6,62−64] No experimental studies assessing protective effects of ACN supplementation after myocardial infarction or heart failure in humans have been performed.

ACNs and Brain Health. In brain, it has been demonstrated that ACNs are able to cross the blood-brain barrier;[65] however, to the best of our knowledge, the exact mechanism by which it happens is still unclear.

In animal models, ACN natural dietary supplementation not only reduced oxidative stress but also neurodegeneration and memory impairment for a mice model of Alzheimer's disease, which could be explained by the regulation of the phosphorylated-phosphatidylinositol 3-kinase-Akt-glycogen synthase kinase 3 beta (p-Pl3k/Akt/GSK3β) pathway.[27] The p-Pl3k/Akt/GSK3β reduced reactive oxygen species elevations, further preventing apoptosis, neurodegeneration,[27] and glial cell death induced by H_2O_2, as a consequence delaying the age-related degenerative changes in brain cells.[66] Some ACN's possible mechanisms of action have been described in in vitro experiments were the improvement of free radical scavenging, reactive carbonyl trapping, antiglycation, anti-amyloid β (Aβ) fibrillation, and microglial neuroprotective effects in murine cell cultures,[50] and in the human neuroblastoma cell line SK-N-SH.[24]

The effects of ACNs on brain tissue were observed at oral doses as low as 15 mg/kg/day in mice,[67] which represents a human equivalent of 1.3 mg/kg/day (91 mg/day for a 70 kg average person).[42] The ACN brain effects could be enhanced

DOI: 10.1021/acs.jafc.8b04014
J. Agric. Food Chem. 2018, 66, 11531−11543

by the use of nanovehicles by loading polyethylene glycol-gold nanoparticles with ACNs to increase brain bioavailability.[67] As result, ACN exerts an enhanced cellular protection against Aβ induced oxidative stress involved in Alzheimer's diseases.[68] The nanoparticle delivery system could be a good strategy to increase intracellular concentrations of ACNs in other tissues. Moreover, ACN antioxidant protective effects could provide beneficial effects not only in Alzheimer's disease, but also in other neurodegenerative diseases such as Huntington's demonstrated in mice where supplementation with ACNs for 3 weeks showed an improvement in motor functions,[69,70] and Parkinson's disease where ACNs acted mainly by their free radical scavenging properties.[71] Thus, the demonstrated ACN effects support their potential for the treatment and prevention of neurodegenerative diseases.

ACNs hold various pharmacokinetic and pharmacodynamics profiles due to their structural differences, as demonstrated with models that used C-labeled ACN molecules that evidenced their specific properties. In humans, after a 500 mg bolus dose of isotopically labeled C3G, maximum plasmatic concentrations ranged between 10 and 2000 nM and T-max values comprehended between 2 and 20 h.[13] In accordance with these findings, we suggest that sustained consumption and ACN source could be of importance when analyzing their profiles present in the tissues.

ACNs and Hepatic Health. There is a considerable amount of published evidence about the presence of ACNs on hepatic tissue, surely because its paramount role in ACN metabolization,[11,13,22] and its central role in the degradation and excretion of many molecules. Evidence indicates that ACNs could help reduce liver inflammation demonstrated by the lower activity of alanine aminotransferase and aspartate aminotransferase, two key hepatic inflammation biomarkers, reported in a murine model of hepatic damage induced by IV injected lipopolysaccharide and *Propionibacterium acnes,* in which a positive reduction of inflammation was observed after the intake of 50−150 mg/kg/day bilberry extract for 7 days.[72] However, there is a remarkable lack of studies that assess the impact of specific ACN presence in the liver as to prevent hepatic diseases.

ACNs and Renal Health. The evidence that suggests that the presence of malvidin-3-glucoside, P3G, and petunidin-3-glucoside in renal tissue prevented and delayed the progression not only of renal disease against cisplatin-induced acute kidney injury that is produced after its use as an anticancer treatment,[73] but also of ischemia-reperfusion injury as demonstrated in mice.[74] In consequence, these results suggest a potential aid in preventing and delaying the progression of acute kidney injury in humans. On the other hand, ACNs have shown nonspecifically inhibition properties against the connective tissue growth factor's expression, also known as CCN2. This growth factor has been identified as an important molecule in the development of kidney failure in diabetic nephropathy, through the retardation of tumor growth factor-β signaling pathway.[75] ACN supplementation with doses as low as 10 mg/kg every 2 weeks for 4 doses in humans retarded glomerular angiogenesis and inhibited endothelial tube formation promoted by high glucose-exposed mesangial conditioned media.[75]

We suggest that ACN source is of importance when analyzing different polyphenol profiles in different animal tissues; therefore, not all ACN sources could provide the same health benefits for humans. Despite the promising effects of ACN on kidney protection, up to date no human studies regarding the applications of ACN therapy in kidney disease or glomerular injuries have been described.

Health Implications of ACNs in Lung and Other Tissues. ACNs have shown anti-lung-cancer properties as supported by the results from ACNs' successful inhibition of lung cancer cell migration and invasion by suppressing matrix metalloproteinase (MMP)-2 and MMP-9, as well as different proteins related to in cancer proliferation, adhesion, and angiogenesis involved in lung cancer development.[76] These anticancer benefits might be specifically provided by P3G, an ACN which has demonstrated to inhibit the invasion, motility, and secretion of MMPs such as MMP-2, MMP-9, and urokinase-type plasminogen activator in lung cancer cells, by inhibiting extracellular signal-regulated kinase (ERK)-1/2, a mitogen-activated protein kinase (MAPK) family member involved in the regulation of MMP molecules as demonstrated in a lung cancer cell in vitro model.[77] Furthermore, so-called suboptimal in vitro concentrations of a combination of ACNs have demonstrated to act synergistically inhibiting the growth of aggressive non-small-cell lung cancer cells, possibly by their inhibitory effects on molecules like β-catenin, cyclin B1, and MMP-9 as well as the inhibition of TNFα-induced nuclear factor-kappa B (NF-κB) activation.[78]

Delphinidin is another ACN that has shown anticancer properties. It is capable of inducing cell apoptosis in lung cancer cells by inhibiting the epidermal growth factor receptor (EGFR)/vascular endothelial growth factor receptor 2 (VEGFR2) pathway,[79] and through the suppression of hypoxia-inducible factor 1-alpha (HIF-1α).[80] However, up to date, studies performed on humans have not shown lung cancer preventive benefits, as is the case of the Kuopio Ischemic Heart Disease Risk Factor Study, an ongoing prospective study performed in 2682 middle-aged men from Finland, in which a nonsignificant lung cancer risk reduction of 20% for men was found when comparing the highest and lowest ACN consumption quartiles.[81]

One other relevant area of interest that has not been addressed in this Review is the importance of ACNs in the gastrointestinal tract for the prevention of colorectal cancer,[53,54] mainly due to the shortage of studies that provide evidence of a complete ACN profile in gastrointestinal tissues. Nonetheless, reports of total ACN concentration values of 8.1 μg/g[82] or 4.48 × 105 pmol/g[11] of intestinal mucosa were described in mice. Moreover, evidence exists regarding the ability of ACNs to inhibit intestinal tumor development in ApcMin mice and growth of human colon cancer cell lines,[83,84] and to induce cytotoxicity and decrease viability of Caco-2 cells, facts that could hinder the growth of tumoral cells in vivo.[85] These studies demonstrate the presence of ACNs in colorectal tissues and ACN capability for colon cancer prevention. Though, the lack of more studies in animals and human populations make difficult the correlation of ACN consumption and colorectal cancer prevention.

Limitations of the Review. One of the greatest limitations on performing our review was the heterogeneity between the study methodologies, regarding the animal models employed, doses used, experiment duration, ACN sources and profiles, which could hinder the direct comparisons between studies. Another limitation observed was that ACN source composition was rarely described in published articles, making difficult to determine the molecular origin of the ACN profiles demonstrated, which is of paramount importance to differentiate the possible best ACN sources for specific tissues or health effects or pathologies. Published studies were found to be either focused on the metabolism, bioavailability, or

DOI: 10.1021/acs.jafc.8b04014
J. Agric. Food Chem. 2018, 66, 11531−11543

health effects, but there are no studies that integrate this information altogether. As a consequence, under these conditions, it is not easy to determine which parent or ACN metabolite is actually responsible for observed health effects in in vivo studies.

Final Remarks. The presence of parent or ACN metabolites in animal tissues could explain the myriad of health benefits attributed to oral or IV administration of ACNs. C3G and its metabolites are one of the most frequently found metabolites in tissues. ACN source, dose, and consumption time are of paramount importance when analyzing ACN profiles in target tissues.

From the analyzed information obtained through this Review, we suggest that C3G, present in target tissues, could have an interesting potential for the reduction of myocardial infarction negative tissue effects and neurodegenerative diseases such as Alzheimer's and Parkinson's, and could also help delay or even reverse acute renal failure. Therefore, the published evidence indicates that the ACNs detected in animal tissues, such as C3G, may have an important role and could be one of the most promising bioactive molecules for human health. Moreover, this information could be useful to determine proper ACN-intake biomarkers in biological samples in futures studies.

AUTHOR INFORMATION

Corresponding Author

*Tel: (+34) 977 75 93 75. E-mail: ursula.catalan@urv.cat.

ORCID

Úrsula Catalán: 0000-0001-8884-9823

Author Contributions

Study conception and design: B.A.S-R., Ú.C., and R.S. Acquisition of data: B.A.S-R. and Ú.C. Analysis and interpretation of data: B.A.S-R., Ú.C., R.S., and S.F.-C. Drafting of the manuscript: B.A.S-R. Critical revision: Ú.C., R.S., S.F.-C., L.R., and A.M.

Funding

The AppleCOR Project (Subproject AGL2016-76943-C2-2-R and Suproject AGL2016-76943-C2-1-R) has been possible with the support of Ministerio de Economía, Indústria y Competitividad, the Agencia Estatal de Investigación (AEI) and the European Regional Development Fund (ERDF). B.A.S.-R. enjoys a 2017MFP-COFUND-30 predoctoral fellowship contract. This project has received funding from the European Union's Horizon 2020 research and innovation program under the Marie Skłodowska-Curie Grant Agreement No. 713679 and from the Universitat Rovira i Virgili (URV). Ú.C. has a Pla estratègic de recerca i innovació en salut (PERIS) postdoctoral grant (SLT002/16/00239; Catalunya, Spain) from Generalitat de Catalunya; NFOC-Salut group is a consolidated research group of Generalitat de Catalunya, Spain (2017 SGR 522).

Notes

The authors declare no competing financial interest.

ABBREVIATIONS USED

ACN, anthocyanin; PRISMA, Preferred Reporting Items for Systematic Reviews and Meta-Analyses; IV, intravenous; P3G, peonidin-3-glucoside; C3G, cyanidin-3-glucoside; CK-MB, creatine kinase; Aβ, amyloid β; Pl3k/Akt/GSK3β, phosphorylated-phosphatidylinositol 3-kinase-Akt-glycogen synthase kinase 3 beta; CCN2, connective tissue growth factor; TNF-α, tumor necrosis factor α; MMP, matrix metalloproteinase; ERK, extracellular signal-regulated kinase; MAPK, mitogen-activated protein kinase; NF-κB, nuclear factor kappa B; EGFR, epidermal growth factor receptor; VEFGR2, vascular endothelial growth factor receptor 2

REFERENCES

(1) World Health Organization. *Preventing Chronic Diseases: A Vital Investment*; Geneva, 2005.

(2) World Health Organization. *World Health Statistics 2017: Monitoring Health for The Sustainable Development Goals*; Geneva, 2017.

(3) O'Flaherty, M.; Guzman, M. Keeping Public Health Clean: Food Policy Barriers and Opportunities in the Era of the Industrial Epidemics. *AIMS public Heal* **2016**, *3* (2), 228−234.

(4) Estruch, R.; Ros, E.; Salas-Salvadó, J.; Covas, M.-I.; Corella, D.; Arós, F.; Gómez-Gracia, E.; Ruiz-Gutiérrez, V.; Fiol, M.; Lapetra, J.; et al. Primary Prevention of Cardiovascular Disease with a Mediterranean Diet. *N. Engl. J. Med.* **2013**, *368* (14), 1279−1290.

(5) Georgoulis, M.; Kontogianni, M. D.; Yiannakouris, N. Mediterranean Diet and Diabetes: Prevention and Treatment. *Nutrients* **2014**, *6* (4), 1406−1423.

(6) Khoo, H. E.; Azlan, A.; Tang, S. T.; Lim, S. M. Anthocyanidins and Anthocyanins: Colored Pigments as Food, Pharmaceutical Ingredients, and the Potential Health Benefits. *Food Nutr. Res.* **2017**, *61* (1), 1361779.

(7) Delgado-Vargas, F.; Jiménez, A. R.; Paredes-López, O. Natural Pigments: Carotenoids, Anthocyanins, and Betalains - Characteristics, Biosynthesis, Processing, and Stability. *Crit. Rev. Food Sci. Nutr.* **2000**, *40* (3), 173−289.

(8) Manach, C.; Scalbert, A.; Morand, C.; Rémésy, C.; Jiménez, L. Polyphenols: Food Sources and Bioavailability. *Am. J. Clin. Nutr.* **2004**, *79*, 727−747.

(9) Hooper, L.; Kay, C.; Abdelhamid, A.; Kroon, P. A.; Cohn, J. S.; Rimm, E. B.; Cassidy, A. Effects of Chocolate, Cocoa, and Flavan-3-Ols on Cardiovascular Health: A Systematic Review and Meta-Analysis of Randomized Trials. *Am. J. Clin. Nutr.* **2012**, *95* (3), 740−751.

(10) Del Bò, C.; Ciappellano, S.; Klimis-Zacas, D.; Martini, D.; Gardana, C.; Riso, P.; Porrini, M. Anthocyanin Absorption, Metabolism, and Distribution from a Wild Blueberry-Enriched Diet (Vaccinium Angustifolium) Is Affected by Diet Duration in the Sprague-Dawley Rat. *J. Agric. Food Chem.* **2010**, *58* (4), 2491−2497.

(11) Marczylo, T. H.; Cooke, D.; Brown, K.; Steward, W. P.; Gescher, A. J. Pharmacokinetics and Metabolism of the Putative Cancer Chemopreventive Agent Cyanidin-3-Glucoside in Mice. *Cancer Chemother. Pharmacol.* **2009**, *64* (6), 1261−1268.

(12) Fang, J. Bioavailability of Anthocyanins. *Drug Metab. Rev.* **2014**, 508−520.

(13) de Ferrars, R. M.; Czank, C.; Zhang, Q.; Botting, N. P.; Kroon, P. A.; Cassidy, A.; Kay, C. D. The Pharmacokinetics of Anthocyanins and Their Metabolites in Humans. *Br. J. Pharmacol.* **2014**, *171* (13), 3268−3282.

(14) Wu, H.-Y.; Yang, K.-M.; Chiang, P.-Y. Roselle Anthocyanins : Antioxidant Properties And. *Molecules* **2018**, *23* (6), 1357.

(15) Cabrita, L.; Petrov, V.; Pina, F. On the Thermal Degradation of Anthocyanidins: Cyanidin. *RSC Adv.* **2014**, *4* (36), 18939−18944.

(16) Keppler, K.; Humpf, H. U. Metabolism of Anthocyanins and Their Phenolic Degradation Products by the Intestinal Microflora. *Bioorg. Med. Chem.* **2005**, *13* (17), 5195−5205.

(17) Hribar, U.; Ulrih, N. The Metabolism of Anthocyanins. *Curr. Drug Metab.* **2014**, *15* (1), 3−13.

(18) Kamiloglu, S.; Capanoglu, E.; Grootaert, C.; van Camp, J. Anthocyanin Absorption and Metabolism by Human Intestinal Caco-2 Cells—A Review. *Int. J. Mol. Sci.* **2015**, 21555−21574.

(19) Mueller, D.; Jung, K.; Winter, M.; Rogoll, D.; Melcher, R.; Richling, E. Human Intervention Study to Investigate the Intestinal

DOI: 10.1021/acs.jafc.8b04014
J. Agric. Food Chem. 2018, 66, 11531−11543

Accessibility and Bioavailability of Anthocyanins from Bilberries. *Food Chem.* **2017**, *231*, 275−286.
(20) Ormazabal, P.; Scazzocchio, B.; Varì, R.; Santangelo, C.; D'Archivio, M.; Silecchia, G.; Iacovelli, A.; Giovannini, C.; Masella, R. Effect of Protocatechuic Acid on Insulin Responsiveness and in Fl Ammation in Visceral Adipose Tissue from Obese Individuals : Possible Role for PTP1B. *Int. J. Obes.* **2018**, DOI: 10.1038/s41366-018-0075-4.
(21) Williamson, G.; Clifford, M. N. Colonic Metabolites of Berry Polyphenols: The Missing Link to Biological Activity? *Br. J. Nutr.* **2010**, S48−S66.
(22) Czank, C.; Cassidy, A.; Zhang, Q.; Morrison, D. J.; Preston, T.; Kroon, P. A.; Botting, N. P.; Kay, C. D. Human Metabolism and Elimination of the Anthocyanin, Cyanidin-3-Glucoside: A13C-Tracer Study. *Am. J. Clin. Nutr.* **2013**, *97* (5), 995−1003.
(23) Jana, S.; Patel, D.; Patel, S.; Upadhyay, K.; Thadani, J.; Mandal, R.; Das, S.; Devkar, R. Anthocyanin Rich Extract of Brassica Oleracea L. Alleviates Experimentally Induced Myocardial Infarction. *PLoS One* **2017**, *12* (8), e0182137.
(24) Thummayot, S.; Tocharus, C.; Suksamrarn, A.; Tocharus, J. Neuroprotective Effects of Cyanidin against Aβ-Induced Oxidative and ER Stress in SK-N-SH Cells. *Neurochem. Int.* **2016**, *101*, 15−21.
(25) Loef, M.; Walach, H. Fruit, Vegetables and Prevention of Cognitive Decline or Dementia: A Systematic Review of Cohort Studies. *J. Nutr., Health Aging* **2012**, 626−630.
(26) Airoldi, C.; La Ferla, B.; D'Orazio, G.; Ciaramelli, C.; Palmioli, A. Flavonoids in the Treatment of Alzheimer's and Other Neurodegenerative Diseases. *Curr. Med. Chem.* **2018**, *25*, 3228.
(27) Ali, T.; Kim, T.; Rehman, S. U.; Khan, M. S.; Amin, F. U.; Khan, M.; Ikram, M.; Kim, M. O. Natural Dietary Supplementation of Anthocyanins via PI3K/Akt/Nrf2/HO-1 Pathways Mitigate Oxidative Stress, Neurodegeneration, and Memory Impairment in a Mouse Model of Alzheimer's Disease. *Mol. Neurobiol.* **2018**, *55*, 6076−6093.
(28) Moher, D.; Liberati, A.; Tetzlaff, J.; Altman, D. G.; Altman, D.; Antes, G.; Atkins, D.; Barbour, V.; Barrowman, N.; Berlin, J. A. Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. *Ann. Intern. Med.* **2009**, 264−269.
(29) Neveu, V.; Perez-Jimenez, J.; Vos, F.; Crespy, V.; du Chaffaut, L.; Mennen, L.; Knox, C.; Eisner, R.; Cruz, J.; Wishart, D.; et al. Phenol-Explorer: An Online Comprehensive Database on Polyphenol Contents in Foods. *Database* **2010**, *2010* (0), bap024−bap024.
(30) Kirakosyan, A.; Seymour, E. M.; Wolforth, J.; McNish, R.; Kaufman, P. B.; Bolling, S. F. Tissue Bioavailability of Anthocyanins from Whole Tart Cherry in Healthy Rats. *Food Chem.* **2015**, *171*, 26−31.
(31) Milbury, P. E.; Kalt, W. Xenobiotic Metabolism and Berry Flavonoid Transport across the Blood?Brain Barrier. *J. Agric. Food Chem.* **2010**, *58* (7), 3950−3956.
(32) Sakakibara, H.; Ogawa, T.; Koyanagi, A.; Kobayashi, S.; Goda, T.; Kumazawa, S.; Kobayashi, H.; Shimoi, K. Distribution and Excretion of Bilberry Anthocyanines in Mice. *J. Agric. Food Chem.* **2009**, *57* (17), 7681−7686.
(33) Ichiyanagi, T.; Shida, Y.; Rahman, M. M.; Hatano, Y.; Konishi, T. Bioavailability and Tissue Distribution of Anthocyanins in Bilberry (Vaccinium Myrtillus L.) Extract in Rats. *J. Agric. Food Chem.* **2006**, *54* (18), 6578−6587.
(34) Aqil, F.; Vadhanam, M. V.; Jeyabalan, J.; Cai, J.; Singh, I. P.; Gupta, R. C. Detection of Anthocyanins/Anthocyanidins in Animal Tissues. *J. Agric. Food Chem.* **2014**, *62* (18), 3912−3918.
(35) Fornasaro, S.; Ziberna, L.; Gasperotti, M.; Tramer, F.; Vrhovšek, U.; Mattivi, F.; Passamonti, S. Determination of Cyanidin 3-Glucoside in Rat Brain, Liver and Kidneys by UPLC/MS-MS and Its Application to a Short-Term Pharmacokinetic Study. *Sci. Rep.* **2016**, *6*, 1−11.
(36) Vanzo, A.; Vrhovsek, U.; Tramer, F.; Mattivi, F.; Passamonti, S. Exceptionally Fast Uptake and Metabolism of Cyanidin 3-Glucoside by Rat Kidneys and Liver. *J. Nat. Prod.* **2011**, *74* (5), 1049−1054.
(37) Vanzo, A.; Terdoslavich, M.; Brandoni, A.; Torres, A. M.; Vrhovsek, U.; Passamonti, S. Uptake of Grape Anthocyanins into the Rat Kidney and the Involvement of Bilitranslocase. *Mol. Nutr. Food Res.* **2008**, *52* (10), 1106−1116.
(38) Chen, T. Y.; Kritchevsky, J.; Hargett, K.; Feller, K.; Klobusnik, R.; Song, B. J.; Cooper, B.; Jouni, Z.; Ferruzzi, M. G.; Janle, E. M. Plasma Bioavailability and Regional Brain Distribution of Polyphenols from Apple/Grape Seed and Bilberry Extracts in a Young Swine Model. *Mol. Nutr. Food Res.* **2015**, *59* (12), 2432−2447.
(39) Chen, T. Y.; Ferruzzi, M. G.; Wu, Q. L.; Simon, J. E.; Talcott, S. T.; Wang, J.; Ho, L.; Todd, G.; Cooper, B.; Pasinetti, G. M.; et al. Influence of Diabetes on Plasma Pharmacokinetics and Brain Bioavailability of Grape Polyphenols and Their Phase II Metabolites in the Zucker Diabetic Fatty Rat. *Mol. Nutr. Food Res.* **2017**, *61* (10), 1700111.
(40) Borges, G.; Roowi, S.; Rouanet, J. M.; Duthie, G. G.; Lean, M. E. J.; Crozier, A. The Bioavailability of Raspberry Anthocyanins and Ellagitannins in Rats. *Mol. Nutr. Food Res.* **2007**, *51* (6), 714−725.
(41) Li, D.; Wang, P.; Luo, Y.; Zhao, M.; Chen, F. Health Benefits of Anthocyanins and Molecular Mechanisms: Update from Recent Decade. *Crit. Rev. Food Sci. Nutr.* **2017**, *57* (8), 1729−1741.
(42) Nair, A.; Jacob, S. A Simple Practice Guide for Dose Conversion between Animals and Human. *J. Basic Clin. Pharm.* **2016**, *7* (2), 27.
(43) Yang, L.; Ling, W.; Du, Z.; Chen, Y.; Li, D.; Deng, S.; Liu, Z.; Yang, L. Effects of Anthocyanins on Cardiometabolic Health: A Systematic Review and Meta-Analysis of Randomized Controlled Trials. *Adv. Nutr.* **2017**, *8* (5), 684−693.
(44) Nicolin, V.; Grill, V.; Micali, F.; Narducci, P.; Passamonti, S. Immunolocalisation of Bilitranslocase in Mucosecretory and Parietal Cells of the Rat Gastric Mucosa. *J. Mol. Histol.* **2005**, *36* (1−2), 45−50.
(45) Belwal, T.; Nabavi, S. F.; Nabavi, S. M.; Habtemariam, S. Dietary Anthocyanins and Insulin Resistance: When Food Becomes a Medicine. *Nutrients* **2017**, 1111.
(46) Kelley, D.; Adkins, Y.; Laugero, K. A Review of the Health Benefits of Cherries. *Nutrients* **2018**, *10*, 368.
(47) Tsuda, T. Dietary Anthocyanin-Rich Plants: Biochemical Basis and Recent Progress in Health Benefits Studies. *Mol. Nutr. Food Res.* **2012**, *56*, 159−170.
(48) Fang, J. Classification of Fruits Based on Anthocyanin Types and Relevance to Their Health Effects. *Nutrition* **2015**, 1301−1306.
(49) Huang, W.-Y.; Wu, H.; Li, D.-J.; Song, J.-F.; Xiao, Y.-D.; Liu, C.-Q.; Zhou, J.-Z.; Sui, Z.-Q. Protective Effects of Blueberry Anthocyanins against H 2 O 2 -Induced Oxidative Injuries in Human Retinal Pigment Epithelial Cells. *J. Agric. Food Chem.* **2018**, *66* (7), 1638.
(50) Ma, H.; Johnson, S. L.; Liu, W.; DaSilva, N. A.; Meschwitz, S.; Dain, J. A.; Seeram, N. P. Evaluation of Polyphenol Anthocyanin-Enriched Extracts of Blackberry, Black Raspberry, Blueberry, Cranberry, Red Raspberry, and Strawberry for Free Radical Scavenging, Reactive Carbonyl Species Trapping, Anti-Glycation, Anti-β-Amyloid Aggregation, and Mic. *Int. J. Mol. Sci.* **2018**, *19* (2), 461.
(51) Xu, Z.; Rothstein, S. J. ROS-Induced Anthocyanin Production Provides Feedback Protection by Scavenging ROS and Maintaining Photosynthetic Capacity in Arabidopsis. *Plant Signaling Behav.* **2018**, *13*, e1451708.
(52) de Sales, N.; Silva da Costa, L.; Carneiro, T.; Minuzzo, D.; Oliveira, F.; Cabral, L.; Torres, A.; El-Bacha, T. Anthocyanin-Rich Grape Pomace Extract (Vitis Vinifera L.) from Wine Industry Affects Mitochondrial Bioenergetics and Glucose Metabolism in Human Hepatocarcinoma HepG2 Cells. *Molecules* **2018**, *23* (3), 611.
(53) Fernández, J.; García, L.; Monte, J.; Villar, C. J.; Lombó, F. Functional Anthocyanin-Rich Sausages Diminish Colorectal Cancer in an Animal Model and Reduce Pro-Inflammatory Bacteria in the Intestinal Microbiota. *Genes* **2018**, *9* (3), 133.
(54) Chen, L.; Jiang, B.; Zhong, C.; Guo, J.; Zhang, L.; Mu, T.; Zhang, Q.; Bi, X. Chemoprevention of Colorectal Cancer by Black Raspberry Anthocyanins Involved the Modulation of Gut Microbiota and SFRP2 Demethylation. *Carcinogenesis* **2018**, *39* (3), 471−481.

DOI: 10.1021/acs.jafc.8b04014
J. Agric. Food Chem. 2018, 66, 11531−11543

(55) Zhou, J.; Zhu, Y. F.; Chen, X. Y.; Han, B.; Li, F.; Chen, J. Y.; Peng, X. L.; Luo, L. P.; Chen, W.; Yu, X. P. Black Rice-Derived Anthocyanins Inhibit HER-2-Positive Breast Cancer Epithelial-Mesenchymal Transition-Mediated Metastasis in Vitro by Suppressing FAK Signaling. *Int. J. Mol. Med.* **2017**, *40* (6), 1649−1656.
(56) Nanashima, N.; Horie, K.; Chiba, M.; Nakano, M.; Maeda, H.; Nakamura, T. Anthocyanin-Rich Blackcurrant Extract Inhibits Proliferation of the MCF10A Healthy Human Breast Epithelial Cell Line through Induction of G0/G1 Arrest and Apoptosis. *Mol. Med. Rep.* **2017**, *16* (5), 6134−6141.
(57) Lee, Y. M.; Yoon, Y.; Yoon, H.; Park, H. M.; Song, S.; Yeum, K. J. Dietary Anthocyanins against Obesity and Inflammation. *Nutrients* **2017**, 1089.
(58) Petroni, K.; Trinei, M.; Fornari, M.; Calvenzani, V.; Marinelli, A.; Micheli, L. A.; Pilu, R.; Matros, A.; Mock, H. P.; Tonelli, C.; et al. Dietary Cyanidin 3-Glucoside from Purple Corn Ameliorates Doxorubicin-Induced Cardiotoxicity in Mice. *Nutr., Metab. Cardiovasc. Dis.* **2017**, *27* (5), 462−469.
(59) Skemiene, K.; Rakauskaite, G.; Trumbeckaite, S.; Liobikas, J.; Brown, G. C.; Borutaite, V. Anthocyanins Block Ischemia-Induced Apoptosis in the Perfused Heart and Support Mitochondrial Respiration Potentially by Reducing Cytosolic Cytochrome C. *Int. J. Biochem. Cell Biol.* **2013**, *45* (1), 23−29.
(60) Raj, P.; McCallum, J. L.; Kirby, C.; Grewal, G.; Yu, L.; Wigle, J. T.; Netticadan, T. Effects of Cyanidin 3−0-Glucoside on Cardiac Structure and Function in an Animal Model of Myocardial Infarction. *Food Funct.* **2017**, *8* (11), 4089−4099.
(61) Acín, S.; Navarro, M. A.; Arbonés-Mainar, J. M.; Guillén, N.; Sarría, A. J.; Carnicer, R.; Surra, J. C.; Orman, I.; Segovia, J. C.; De La Torre, R.; et al. Hydroxytyrosol Administration Enhances Atherosclerotic Lesion Development in Apo E Deficient Mice. *J. Biochem.* **2006**, *140* (3), 383−391.
(62) Reis, J. F.; Monteiro, V. V. S.; de Souza Gomes, R.; do Carmo, M. M.; da Costa, G. V.; Ribera, P. C.; Monteiro, M. C.; et al. Action Mechanism and Cardiovascular Effect of Anthocyanins: A Systematic Review of Animal and Human Studies. *J. Transl. Med.* **2016**, *14*, 315.
(63) Cassidy, A.; Bertoia, M.; Chiuve, S.; Flint, A.; Forman, J.; Rimm, E. B. Habitual Intake of Anthocyanins and Flavanones and Risk of Cardiovascular Disease in Men. *Am. J. Clin. Nutr.* **2016**, *104* (3), 587−594.
(64) OH, J. S.; KIM, H.; VIJAYAKUMAR, A.; KWON, O.; KIM, Y.; CHANG, N. Association of Dietary Flavonoid Intake with Prevalence of Type 2 Diabetes Mellitus and Cardiovascular Disease Risk Factors in Korean Women Aged 30 Years. *J. Nutr. Sci. Vitaminol.* **2017**, *63* (1), 51−58.
(65) Rashid, K.; Wachira, F. N.; Nyabuga, J. N.; Wanyonyi, B.; Murilla, G.; Isaac, A. O. Kenyan Purple Tea Anthocyanins Ability to Cross the Blood Brain Barrier and Reinforce Brain Antioxidant Capacity in Mice. *Nutr. Neurosci.* **2014**, *17* (4), 178−185.
(66) Ereminas, G.; Majiene, D.; Sidlauskas, K.; Jakstas, V.; Ivanauskas, L.; Vaitiekaitis, G.; Liobikas, J. Neuroprotective Properties of Anthocyanidin Glycosides against H 2 O 2 -Induced Glial Cell Death Are Modulated by Their Different Stability and Antioxidant Activity in Vitro. *Biomed. Pharmacother.* **2017**, *94*, 188−196.
(67) Wei, J.; Zhang, G.; Zhang, X.; Xu, D.; Gao, J.; Fan, J.; Zhou, Z. Anthocyanins from Black Chokeberry (*Aroniamelanocarpa* Elliot) Delayed Aging-Related Degenerative Changes of Brain. *J. Agric. Food Chem.* **2017**, *65* (29), 5973−5984.
(68) Ali, T.; Kim, M. J.; Rehman, S. U.; Ahmad, A.; Kim, M. O. Anthocyanin-Loaded PEG-Gold Nanoparticles Enhanced the Neuroprotection of Anthocyanins in an Aβ1−42 Mouse Model of Alzheimer's Disease. *Mol. Neurobiol.* **2017**, *54* (8), 6490−6506.
(69) Møllersen, L.; Moldestad, O.; Rowe, A. D.; Bjølgerud, A.; Holm, I.; Tveterås, L.; Klungland, A.; Retterstøl, L. Effects of Anthocyanins on CAG Repeat Instability and Behaviour in Huntington's Disease R6/1 Mice. *PLoS Curr.* **2016**, *8*, ecurrents.hd.58d04209ab6d5de0844db7ef5628ff67.
(70) Kreilaus, F.; Spiro, A. S.; Hannan, A. J.; Garner, B.; Jenner, A. M. Therapeutic Effects of Anthocyanins and Environmental Enrichment in R6/1 Huntington's Disease Mice. *J. Huntington's Dis.* **2016**, *5* (3), 285−296.
(71) Subash, S.; Essa, M. M.; Al-Adawi, S.; Memon, M. A.; Manivasagam, T.; Akbar, M. Neuroprotective Effects of Berry Fruits on Neurodegenerative Diseases. *Neural Regener. Res.* **2014**, *9* (16), 1557−1566.
(72) Luo, H.; Lv, X.-D.; Wang, G.-E.; Li, Y.-F.; Kurihara, H.; He, R.-R. Anti-Inflammatory Effects of Anthocyanins-Rich Extract from Bilberry (*Vaccinium Myrtillus* L.) on Croton Oil-Induced Ear Edema and *Propionibacterium Acnes* plus LPS-Induced Liver Damage in Mice. *Int. J. Food Sci. Nutr.* **2014**, *65* (5), 594−601.
(73) Qi, Z.-L.; Wang, Z.; Li, W.; Hou, J.-G.; Liu, Y.; Li, X.-D.; Li, H.-P.; Wang, Y.-P. Nephroprotective Effects of Anthocyanin from the Fruits of *Panax Ginseng* (GFA) on Cisplatin-Induced Acute Kidney Injury in Mice. *Phytother. Res.* **2017**, *31* (9), 1400−1409.
(74) Isaak, C. K.; Wang, P.; Prashar, S.; O, K.; Brown, D. C.; Debnath, S. C.; Siow, Y. L. Supplementing Diet with Manitoba Lingonberry Juice Reduces Kidney Ischemia-Reperfusion Injury. *J. Sci. Food Agric.* **2017**, *97* (9), 3065−3076.
(75) Wang, S.; Li, B.; Li, C.; Cui, W.; Miao, L. Potential Renoprotective Agents through Inhibiting CTGF/CCN2 in Diabetic Nephropathy. *J. Diabetes Res.* **2015**, 962383.
(76) Lu, J. N.; Panchanathan, R.; Lee, W. S.; Kim, H. J.; Kim, D. H.; Choi, Y. H.; Kim, G. S.; Shin, S. C.; Hong, S. C. Anthocyanins from the Fruit of Vitis Coignetiae Pulliat Inhibit TNF-Augmented Cancer Proliferation, Migration, and Invasion in A549 Cells. *Asian Pac. J. Cancer Prev.* **2017**, *18* (11), 2919−2923.
(77) Ho, M.-L.; Chen, P.-N.; Chu, S.-C.; Kuo, D.-Y.; Kuo, W.-H.; Chen, J.-Y.; Hsieh, Y.-S. Peonidin 3-Glucoside Inhibits Lung Cancer Metastasis by Downregulation of Proteinases Activities and MAPK Pathway. *Nutr. Cancer* **2010**, *62* (4), 505−516.
(78) Kausar, H.; Jeyabalan, J.; Aqil, F.; Chabba, D.; Sidana, J.; Singh, I. P.; Gupta, R. C. Berry Anthocyanidins Synergistically Suppress Growth and Invasive Potential of Human Non-Small-Cell Lung Cancer Cells. *Cancer Lett.* **2012**, *325* (1), 54−62.
(79) Pal, H. C.; Sharma, S.; Strickland, L. R.; Agarwal, J.; Athar, M.; Elmets, C. A.; Afaq, F. Delphinidin Reduces Cell Proliferation and Induces Apoptosis of Non-Small-Cell Lung Cancer Cells by Targeting EGFR/VEGFR2 Signaling Pathways. *PLoS One* **2013**, *8* (10), e77270.
(80) Kim, M.-H.; Jeong, Y.-J.; Cho, H.-J.; Hoe, H.-S.; Park, K.-K.; Park, Y.-Y.; Choi, Y. H.; Kim, C.-H.; Chang, H.-W.; Park, Y.-J.; et al. Delphinidin Inhibits Angiogenesis through the Suppression of HIF-1α and VEGF Expression in A549 Lung Cancer Cells. *Oncol. Rep.* **2017**, *37* (2), 777−784.
(81) Mursu, J.; Nurmi, T.; Tuomainen, T.-P.; Salonen, J. T.; Pukkala, E.; Voutilainen, S. Intake of Flavonoids and Risk of Cancer in Finnish Men: The Kuopio Ischaemic Heart Disease Risk Factor Study. *Int. J. Cancer* **2008**, *123* (3), 660−663.
(82) Cooke, D.; Schwarz, M.; Boocock, D.; Winterhalter, P.; Steward, W. P.; Gescher, A. J.; Marczylo, T. H. Effect of Cyanidin-3-Glucoside and an Anthocyanin Mixture from Bilberry on Adenoma Development in the Apc ^{Min} Mouse Model of Intestinal Carcinogenesis-Relationship with Tissue Anthocyanin Levels. *Int. J. Cancer* **2006**, *119* (9), 2213−2220.
(83) Bobe, G.; Wang, B.; Seeram, N. P.; Nair, M. G.; Bourquin, L. D. Dietary Anthocyanin-Rich Tart Cherry Extract Inhibits Intestinal Tumorigenesis in APC ^{Min} Mice Fed Suboptimal Levels of Sulindac. *J. Agric. Food Chem.* **2006**, *54* (25), 9322−9328.
(84) Kang, S.-Y.; Seeram, N. P.; Nair, M. G.; Bourquin, L. D. Tart Cherry Anthocyanins Inhibit Tumor Development in Apc(Min) Mice and Reduce Proliferation of Human Colon Cancer Cells. *Cancer Lett.* **2003**, *194* (1), 13−19.
(85) Kubow, S.; Iskandar, M.; Melgar-Bermudez, E.; Sleno, L.; Sabally, K.; Azadi, B.; How, E.; Prakash, S.; Burgos, G.; Felde, T. zum. Effects of Simulated Human Gastrointestinal Digestion of Two Purple-Fleshed Potato Cultivars on Anthocyanin Composition and Cytotoxicity in Colonic Cancer and Non-Tumorigenic Cells. *Nutrients* **2017**, *9* (9), 953.

DOI: 10.1021/acs.jafc.8b04014
J. Agric. Food Chem. 2018, 66, 11531−11543