

Bioavailability and Antioxidant Activity of Black Chokeberry (*Aronia melanocarpa*) Polyphenols: *in vitro* and *in vivo* Evidences and Possible Mechanisms of Action: A Review

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Abstract: Black chokeberry (*Aronia melanocarpa*) is a distinctive berry with a high content of polyphenol compounds and possesses one of the highest *in vitro* antioxidant activities among fruits. The bioavailability of aronia polyphenols seems to be low, but there is ample evidence for chokeberry health benefits including antidiabetic, cardioprotective, hepatoprotective, antimutagenic, and anticarcinogenic effects. This review presents the available information for the bioavailability and antioxidant activity of chokeberry polyphenols and explains the possible mechanisms of action *in vivo* in the prevention and treatment of oxidative stress-related diseases. The review shows the available data for chokeberry antioxidant activity *in vitro*, in isolated cells and cell lines, and *in vivo*, in both human subjects and animals. It is evident that *in vivo* antioxidant action of chokeberry extends far beyond radical scavenging and includes suppression of reactive oxygen and nitrogen species formation, inhibition of prooxidant enzymes, restoration of antioxidant enzymes, and probably cellular signaling to regulate the level of antioxidant compounds and enzymes. The original contribution of this work is that it compiles the available information up to date and outlines the gaps and future directions in the assessment of chokeberry antioxidant action *in vivo*.

Introduction

Life on earth is inconceivable without oxygen (O_2), but in higher concentration this vital element is toxic to aerobes. Most of the damaging effects of O_2 are due to oxygen radicals, which include superoxide ($O_2^{\cdot-}$), hydroperoxyl (HOO^{\cdot}), hydroxyl (HO^{\cdot}), peroxy (ROO^{\cdot}), and alkoxy (RO^{\cdot}) radicals (Gilbert 1981). These, together with the nonradicals hydrogen peroxide (H_2O_2), ozone (O_3), and singlet oxygen (1O_2) constitute the so-called reactive oxygen species (ROS). ROS together with the reactive nitrogen species (RNS) nitric oxide (NO), peroxynitrite ($ONOO^-$), peroxynitrate, and so on, are constantly produced in our bodies through numerous physiological reactions and processes. Experimental evidence has directly or indirectly suggested that there are 6 major reactive species causing oxidative damage in the human body. These species are superoxide anion, H_2O_2 , peroxy radicals, hydroxyl radical, singlet oxygen, and peroxynitrite. Superoxide is formed *in vivo* by NADPH oxidase in phagocytic cells

by other enzymes like xanthine oxidase and xanthine dehydrogenase which reduce O_2 to $O_2^{\cdot-}$ and by the auto-oxidation of many biomolecules like glyceraldehydes, FMN, FADH₂, adrenalin, noradrenalin, and dopamine (Kirsch 2003). The most important source of $O_2^{\cdot-}$ *in vivo* is the mitochondrial electron transport chain and hemoglobin in human erythrocytes, which also could be a source of superoxide radicals (Rifkind 2004). Hydroxyl radicals are the most potent oxidants among ROS. Physiologically they are being produced mainly through Fenton-like reactions catalyzed by transition metal ions, UV-induced homolytic cleavage of H_2O_2 (von Sonntag 1987), γ -ray-assisted homolytic fission of water, and hypochlorous acid reacting with $O_2^{\cdot-}$ (Folkes and others 1995). Peroxy and alkoxy radicals are good oxidizing agents, which can easily abstract hydrogen atom from different biomolecules. *In vivo* they are formed through the reaction of carbon-centered radicals with O_2 or by the decomposition of organic peroxides (Forni and Willson 1986). H_2O_2 is continuously produced in many tissues *in vivo* and mitochondria are the biggest contributors to its generation both by monoamine oxidases and by dismutation of $O_2^{\cdot-}$ (Halliwell and Gutteridge 2007). Singlet oxygen is often generated by photosensitization reactions and its detrimental effect is expressed mainly in skin and eye damage. Peroxynitrite is generated by the reaction of NO with superoxide radical and the biggest contributors for NO generation are the nitric oxide synthase enzymes. To counteract the assault of all ROS and RNS, living cells have elaborated a complex biological defense system composed of enzymatic and nonenzymatic antioxidants that convert

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ROS/RNS to harmless species. The term antioxidant is defined as any substance that in low concentrations, compared to those of an oxidizable substrate, significantly delays or prevents oxidation of the substrate (Halliwell and Gutteridge 2007). By mechanism of action antioxidants are divided into preventive and chain-breaking antioxidants. Preventive antioxidants act as the first line of defense by suppressing the formation of ROS and RNS. The scavenging antioxidants remove active species rapidly before they can attack biologically essential molecules. For example, superoxide radical is converted to oxygen and H_2O_2 by superoxide dismutase (SOD) and H_2O_2 can then be converted to water and oxygen by catalase. In contrast, no enzymatic action is known to scavenge $ROO\cdot$, $HO\cdot$, 1O_2 , and $ONOO^-$ (Huang and others 2005). Therefore, the burden of defense relies on a variety of nonenzymatic antioxidants such as vitamins C and E and many phytochemicals such as polyphenols that have the ability to scavenge oxidants and free radicals. These scavenging antioxidants act as the second line of defense *in vivo*. Various enzymes function in a third line of defense by repairing damage, clearing wastes, and reconstituting lost function. In addition, the adaptation mechanism functions as a fourth line of defense in which appropriate antioxidants are generated at the right time and transferred to the best position in the right concentration. Furthermore, there is increasing evidence showing that some antioxidants act as a cellular signaling messenger to regulate the level of antioxidant compounds and enzymes (Niki 2010). Usually there is a balance between the antioxidants and the prooxidants *in vivo*, but several factors like stress, radiation, nutrition, polluted atmosphere, smoking, and so on, disrupt the oxidative balance leading to so-called oxidative stress, which imposes the necessity to contribute exogenous antioxidants with the diet. Oxidative stress is a physiological state, which is believed to be a prerequisite for the development of many diseases including cardiovascular disease (CVD), stroke, and neurodegenerative disorders such as Alzheimer's disease and Parkinson's disease (Davies 2000; Fenkel and Holbrook 2000; Sayre and others 2008). The injury caused by oxidative stress can affect all organ systems. For example, LDL oxidation is the initial step in atherosclerosis, leading to CVD and oxidized DNA is the basis in mutagenesis and is involved in carcinogenesis (Berliner and others 1995).

A growing amount of evidence indicates that the consumption of plant foods is correlated with a lower risk from development of arteriosclerosis and oxidative stress-related diseases (Ellingsen and others 2008). In contrast, diets poor in plant-based foods and rich in animal products and ingredients are related to an increased risk for CVD and certain types of cancer (Rissanen and others 2003). Most of the antioxidants taken with the diet are of plant origin and the richest sources are herbs, cereals, fruits and vegetables in which polyphenol substances, carotenoids, vitamin C, and vitamin E are the biggest contributors to their antioxidant activity. In the last decades, polyphenolic compounds have gained much attention and have been subject to thorough research because of their antioxidant properties and beneficial effect beyond vitamin action. Polyphenols are the most abundant antioxidants taken with the diet (Ross and Kasum 2002), with over 8000 known compounds, which makes them one of the largest chemical groups in the plant kingdom. Natural polyphenols are structurally diverse and vary from single molecules such as phenolic acids to highly polymerized structures like tannins (Harborne and Simmonds 1964).

Aronia (*Aronia melanocarpa*), with the common name chokeberry, originates from the eastern parts of North America. Around 1900 it was transferred to Europe and in the 1960s the plant was established as a cultivar in the former Soviet Union. Nowadays

aronia berries are widely distributed mainly in the east-south and central parts of Europe and cultivated as an industrial crop (Hardin 1973; Seidemann 1993; Strigl and others 1995). Aronia berries are distinctive with a high content of polyphenols and possess one of the highest antioxidant activities among plant species. In the last few years there has been increasing research interest in chokeberry, generating a significant number of scientific publications. Recently, several articles reviewed the chemical composition and medicinal potential of the berries (Kulling and Rawel 2008), their potential health benefits (Valcheva-Kuzmanova and Belcheva 2006) and their clinical effectiveness (Chrubasik and others 2010). So far there is no review available assessing the bioavailability of chokeberry polyphenols and the connection between their *in vivo* antioxidant activity and potential health benefits. The aim of this effort was to review the available information on the bioavailability and antioxidant activity of chokeberry and to explain the antioxidant mechanisms implicated in the prevention and treatment of oxidative stress-related diseases by aronia. Reviewed are the available data for chokeberry antioxidant activity *in vitro*, in isolated cells and cell lines, and *in vivo*, in both human subjects and animals. The original contribution of this work is that it compiles the available information up to date and outlines the gaps and future direction in the assessment of chokeberry antioxidant action *in vivo*.

Antioxidant Activity of Aronia and Its Major Polyphenols *in vitro*

Kulling and Rawel (2008) reviewed the polyphenol constituents of aronia fruit very well. In Table 1 we present updated information including the polyphenol composition of chokeberry juice. It is known that processing of berries into juice can significantly affect polyphenol composition; and quite often bioavailability studies and clinical trials are performed with juices, since they are more convenient to store and ingest in comparison to berries. Chokeberries are a rich source of anthocyanins (ACN), proanthocyanidins (PACNs), and hydroxycinnamic acids. Flavonols (quercetin glycosides) and flavan-3-ols (epicatechin) are also present as minor components in the berries. The chemical structures of the major chokeberry polyphenols are presented in Figure 1. The total amount of anthocyanins in fresh berries varies in the range 357 to 1790 mg/100 g fresh weight (FW). Compared to other berries the aronia anthocyanin profile is very simple consisting almost exclusively of cyanidin glycosides, namely cyanidin-3-arabinoside, cyanidin-3-galactoside, cyanidin-3-glucoside, and cyanidin-3-xyloside. Cyanidin-3-galactoside and cyanidin-3-arabinoside are the predominant representatives with a cumulative content >90% in the berries (Oszmianski and Wojdylo 2005). There is also 1 paper reporting minor amounts of pelargonidin-3-arabinoside and traces of pelargonidin-3-galactoside (Wu and others 2004). Chokeberry proanthocyanidins consist exclusively of (-)-epicatechin units bonded by a C4 to C8 linkage. Their content in fresh berries varies in the range 664 to 2120 mg/100 g and the following composition of proanthocyanidins has been reported: monomers (0.78%), dimers (1.88%), trimers (1.55%), 4 to 6-mers (6.07%), 7 to 10-mers (7.96%), and >10-mers (81.72%) (Wu and others 2004). Hellstrom and others (2009) reported that different varieties of chokeberry contain between 80 and 95% extractable PACNs. The hydroxycinnamic acids are represented by significant amounts of chlorogenic (61 to 193 mg/100 g FW) and neochlorogenic acids (85 to 123 mg/100 g FW). Quercetin and several quercetin glycosides

Table 1–Polyphenol constituents in aronia berries and juices.

Sample	Berries (mg/100 g)	Reference	Juices (mg/L)	Reference
Total polyphenols	719	Jakobek and others 2007b	7093	Valcheva-Kuzmanova and others 2007b
	778–1285	Rop and others 2010	8100	Hellstrom and others 2010
	1064	Jakobek and others 2007a	9154	Jakobek and others 2007c
	2556	Zheng and Wang 2003		
	6902	Benvenuti and others 2004		
	3440 (DW)	Kolesnikov and Gins 2001		
	3760 (DW)	Hudec and others 2006		
	4210 (DW)	Kahkonen and others 1999		
	7849 (DW)	Oszmianski and Wojdylo 2005		
Total proanthocyanidins	664	Wu and others 2004	2800	Hellstrom and others 2010
	1740–2170	Hellstrom and others 2009	2934	Skoczynska and others 2007
	5182 (DW)	Oszmianski and Wojdylo 2005		
Total anthocyanins	357	Jakobek and others 2007b	1068	Valcheva-Kuzmanova and others 2007b
	428	Zheng and Wang 2003	1250–2500	Fuchs and others 1996
	434	Jakobek and others 2007a	3042	Jakobek and others 2007c
	461	Benvenuti and others 2004		
	470–1790	Fuchs and others 1996		
	1480	Wu and others 2006		
Antocyanins				
Cyanidin-3-arabinoside	99	Jakobek and others 2007a	51	Skoczynska and others 2010
	94–155	Rop and others 2010	647	Jakobek and others 2007c
	142	Zheng and Wang 2003	1000	Hellstrom and others 2010
	146	Slimestead and others 2005		
	399	Wu and others 2004		
	582 (DW)	Oszmianski and Wojdylo 2005		
Cyanidin-3-galactoside	101–120	Rop and others 2010	125	Skoczynska and others 2010
	126	Zheng and Wang 2003	1817	Jakobek and others 2007c
	279	Jakobek and others 2007a	2100	
	315	Slimestead and others 2005		
	990	Wu and others 2004		
	1282 (DW)	Oszmianski and Wojdylo 2005		
Cyanidin-3-glucoside	1.7	Zheng and Wang 2003	7.1	Skoczynska and others 2010
	10	Slimestead and others 2005	74	Jakobek and others 2007c
		Jakobek and others 2007a		
	12	Wu and others 2004		
	38	Oszmianski and Wojdylo 2005		
	42 (DW)			
Cyanidin-3-xyloside	10	Slimestead and others 2005	5.9	Skoczynska and others 2010
	15	Jakobek and others 2007a		
	47	Zheng and Wang 2003		
	52	Wu and others 2004		
	53 (DW)	Oszmianski and Wojdylo 2005		
	2.3	Wu and others 2004		
Pelargonidin-3-arabinoside				
Flavonols				
Quercetin derivatives	>17	Slimestead and others 2005		
Quercetin	7.1	Jakobek and others 2007a	118	Valcheva-Kuzmanova and others 2007b
		Häkkinen and others 1999		
	8.9	Jakobek and others 2007b		
	9.2			
Quercetin-3-galactoside	30.2	Zheng and Wang 2003	28.3	Skoczynska and others 2010
	37(DW)	Oszmianski and Wojdylo 2005		
Quercetin-3-glucoside	27.3	Zheng and Wang 2003	22.5	Skoczynska and others 2010
	21 (DW)	Oszmianski and Wojdylo 2005		
	15 (DW)	Oszmianski and Wojdylo 2005	16.8	Skoczynska and others 2010
Quercetin-3-rutinoside			11.5	Skoczynska and others 2010
Quercetin 3-vicianoside			11.7	Skoczynska and others 2010
Quercetin 3-robinobioside				
Kaemferol	0.53	Jakobek and others 2007a		
	0.69	Jakobek and others 2007b		
Flavan-3-ols				
Epicatechin	47–84	Rop and others 2010	14.8	Skoczynska and others 2010
	15.4 (DW)	Oszmianski and Wojdylo 2005		
Hydroxycinnamic acids				
Chlorogenic acid	61	Slimestead and others 2005	455	Skoczynska and others 2010
	113 -196	Rop and others 2010	800	Hellstrom and others 2010
	302 (DW)	Oszmianski and Wojdylo 2005		
Neochlorogenic acid	84 -117	Rop and others 2010	492	Skoczynska and others 2010
	123	Slimestead and others 2005	1200	Hellstrom and others 2010
	291(DW)	Oszmianski and Wojdylo 2005		

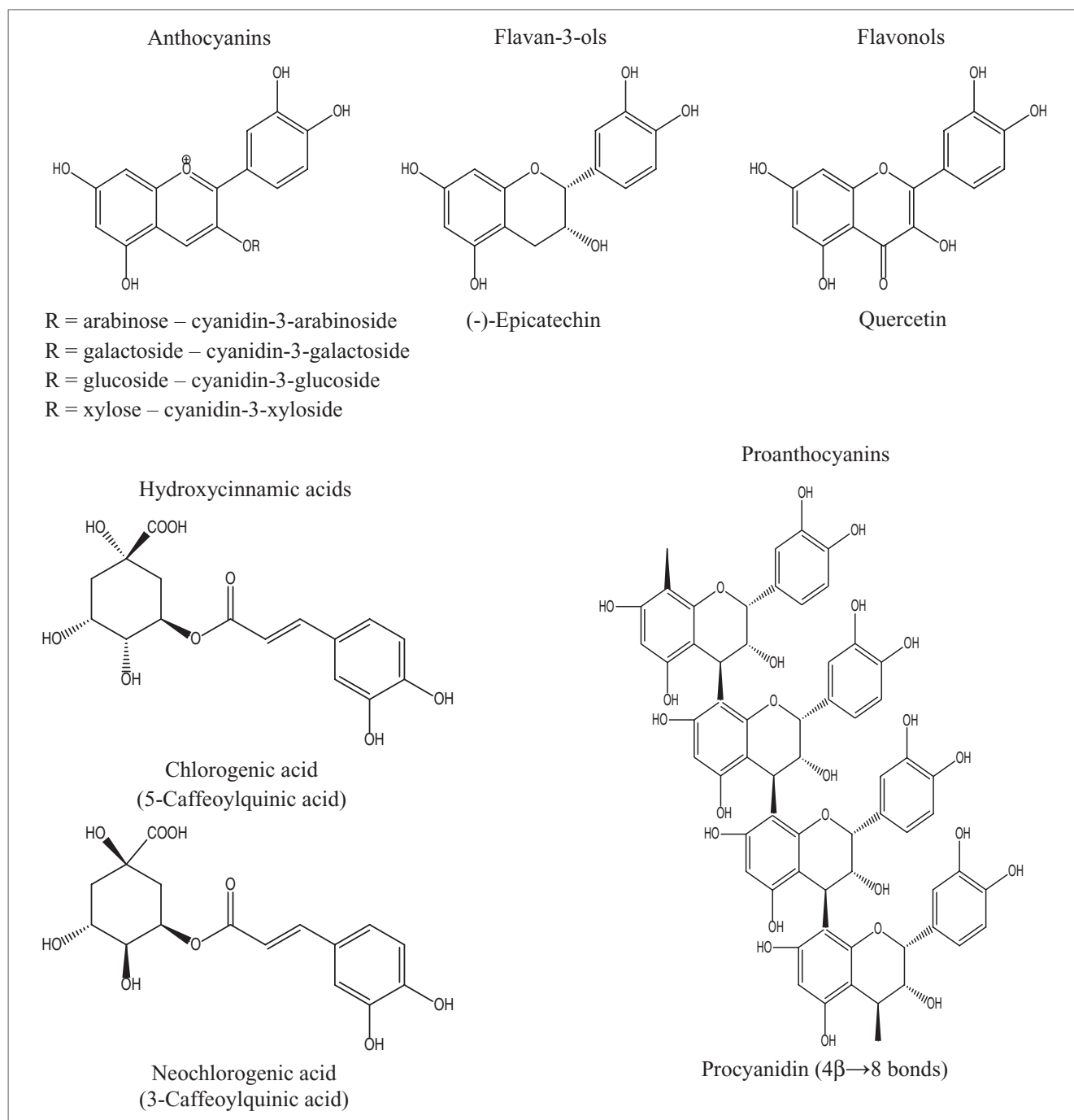


Figure 1—Chemical structures of chokeberry main polyphenols.

(quercetin-3-galactoside, quercetin-3-glucoside, and quercetin-3-rutinoside) were also detected in aronia berries but in relatively low concentrations of about 71 mg/100 g FW. Rop and others (2010) reported that epicatechin is also present in the berries in concentrations of 47 to 84 mg/100 g FW. The content of total phenols of aronia berries has been determined to be in the range 690 to 2556 /100 g FW and 3440 to 7849 mg/100 g DW (Table 1). It is evident that chokeberries vary significantly in their contents of total and individual polyphenol components, which could be attributed to the analytical techniques used and various environmental and genetic factors such as cultivar, harvest time, habitat, fertilization, climate, maturation, and so on.

Table 2 summarizes the available data for antioxidant activity of aronia berries, extracts, and juices measured by different *in vitro* assays. It is obvious that aronia antioxidant action can be assessed with numerous assays including ORAC, TRAP, DPPH, ABTS, and HORAC which rely on the generation of different radicals, acting by different mechanisms. Depending upon the reactions involved, these assays can roughly be classified as 2 types: assays based on hydrogen atom transfer (HAT) reactions and assays based on electron transfer (ET). The majority of HAT-based assays apply a competitive reaction scheme in which antioxidant and substrate compete for thermally generated peroxy radicals through the decomposition of azo compounds. These assays include

Table 2—Antioxidant activity of aronia and aronia products determined by different *in vitro* assays.

Sample	ORAC _H ^a , μmol TE/g	ORAC _L ^b , μmol TE/g	TRAP μmol TE/g	DPPH,		ABTS		No scavenging, % inhibition	Inhibition of lipid peroxidation, % inhibition	HORAC, μmol GAE/g	Hydroxyl radical scavenging, % inhibition	Superoxide anion scavenging, % inhibition
				Units	Result	Units	Result					
Berries	158 ^c 160.2 ^d	2.4 ^c		μmol TE/100 g μmol TE/g AAE/kg EC ₅₀ (mg) μmol TE/mL mmol TE/L	279 (DW) ^e 181 ^f 8.9–16 ^g 1.8 ^h 72.4 ⁱ 63 ^j	μmol TE/100 g μmol TE/g	439 (DW) ^e 79 ^f	27–41 ^g	12–20 ^g		22–34 ^g	21–37 ^g
Juice												
Juice concentrate Extract	5165 ^l		4051 ^l	mg TE/mL IC ₅₀ (mg/L)	60 ^k 36 ^m	mg TE/mL	103.2 ^k			1265 ^l		

^a ORAC_H = Hydrophilic ORAC.
^b ORAC_L = Lipophilic ORAC.
^c References: Wu and others 2004.
^d Zheng and Wang 2003.
^e Oszmianski and Wojtylo 2005.
^f Jakobek and others 2007b.
^g Rop and others 2010.
^h Benvenuti and others 2004.
ⁱ Jakobek and others 2007c.
^j Valcheva Kuzmanova and others 2007d.
^k Bermudez-Soto & Tomas-Barberan 2004.
^l Denev and others 2010.
^m Gabrielska and others 1999.

inhibition of lipid peroxidation, ORAC, and TRAP. ET-based assays measure the capacity of an antioxidant in the reduction of an oxidant, which changes color when reduced. ET-based assays include ABTS and DPPH methods (Huang and others 2005). The HORAC method measures the metal-chelating activity of antioxidants under the conditions of Fenton-like reactions and hence indicates the protecting ability against formation of hydroxyl radical. The methods used embrace different aspects of the antioxidant action and give a broader view on the antioxidant potential of aronia. This is important since it is recommended to use more than 1 antioxidant assay for a detailed understanding of the principles of antioxidant properties of substances (Ciz and others 2010). Since antioxidants scavenge ROS/RNS by hydrogen atom transfer, HAT methods more than others are considered as physiologically relevant. ORAC and TRAP methods assess the radical scavenging activity of the sample against peroxy radicals, which physiologically are the most important ones. Aronia polyphenols are potent inhibitors of lipid peroxidation, which is very important since lipids are very susceptible to peroxidation *in vivo*. DPPH and ABTS assays are very rapid and easy to perform but have some serious limitations. For example, DPPH[•] radical is a resistant nitrogen radical in contrast to highly reactive physiologically relevant radicals. Often antioxidants, which react fast with peroxy radicals react very slowly with DPPH. There is also published evidence that DPPH[•] reacts reversibly with some polyphenols resulting in altered radical-scavenging values (Huang and others 2005).

Several studies found very good correlation between the total phenol content and antioxidant activity of aronia samples measured by ORAC, TRAP, and TEAC (Wu and others 2004; Jakobek and others 2007a; Denev and others 2010). In all cases the correlation was better than that of anthocyanins and antioxidant activity, showing that all polyphenols in chokeberry determine antioxidant properties rather than only the dominating anthocyanins.

Table 3 summarizes *in vitro* antioxidant activity of the most important phenolic constituents of chokeberry. Antioxidant activity of phenolic compounds is determined by several structural characteristics: the number and arrangement of the hydroxyl groups and the presence of 3-OH group and ortho-dihydroxy substitution in the B ring of flavonoids, as well as others. A 2, 3 double bond in conjugation with the 4-oxo function in the C ring is also a prerequisite for good antioxidant activity (Bors and others 1990; Salah and others 1995; Rice-Evans and others 1996). Among the polyphenols present in aronia quercetin possesses many of the structural characteristics necessary for potent antioxidant activity, and it is the most potent antioxidant among the aronia monomer phenolics followed by cyanidin glycosides and chlorogenic acid. Zheng and Wang (2003) estimated that anthocyanins, flavonols, and hydroxycinnamic acids contribute about 59.4% (95 μmol) of the total antioxidant activity of chokeberry without assuming the possible synergism/antagonism between the individual antioxidants. This means that 40% of the antioxidant activity of chokeberries could be attributed to PACNs making them the biggest contributors to aronia antioxidant activity.

Bioavailability of Polyphenols

The capacity of antioxidants *in vivo* is determined by many factors, which should be taken into consideration in its assessment. One such factor is bioavailability. The antioxidants should be absorbed, transported, distributed, and retained properly in the biological fluids, cells, and tissues. Since a bioavailability study

Table 3—Antioxidant activity of aronia main polyphenols determined by different assays.

Compound	ORAC, $\mu\text{molTE}/\text{mg}^{\text{a}}$	DPPH, % scavenged radicals ^b 17 μM	MeLo emulsion, % inhibition ^b		Inhibition of lipid peroxidation, % inhibition ^b	
			50 μM	250 μM	10 μM	25 μM
Cyanidin-3-galactoside	11.5	25	47	85	86	89
Cyanidin-3-arabinoside	12.3	26	50	76	98	99
Cyanidin-3-glucoside	14.8	32	52	83	92	92
Cyanidin-3-xyloside	11.9					
Quercetin		34	77	95	96	97
Quercetin-galactoside	13.4					
Quercetin-glucoside	15.8					
Chlorogenic acid	7.4	38	35	65	7	80

References: ^a Zheng and Wang 2003;
^b Kahkonen and Heinonen 2003.

allows determination of real exposure of the human organism to the tested compounds, such results are an important and essential topic. The capacity and efficacy of antioxidants *in vivo* may be assessed most accurately by the effect of antioxidant compounds on the level of oxidation in biological fluids and tissues, such as plasma, erythrocytes, urine, and cerebrospinal fluids, from humans and experimental animals. Saliva and tears may also be used. Research on polyphenol bioavailability allows us to correlate polyphenol intakes with one or several accurate measures of bioavailability (such as concentrations of key bioactive metabolites in plasma and tissues) and with potential health effects in epidemiologic studies (Manach and others 2004). Bioavailability appears to differ greatly among the various phenolic compounds and the most abundant ones in our diet are not necessarily those that have the best bioavailability profile. Metabolism of dietary antioxidants is another factor, strongly affecting their bioavailability. Usually, after absorption, polyphenols are subjected to 3 main types of conjugation: methylation, sulfation, and glucuronidation. Interestingly, unlike other flavonoids that are absorbed and excreted, most anthocyanins do not appear to undergo extensive metabolism of the parent anthocyanidin to glucuronide, sulfate, or methyl derivatives (Miyazawa and others 1999; McGhie and others 2003; Ichiyanagi and others 2005). Moreover, the structure of the resulting metabolites could be totally different from the parent compounds, and they may, or may not exert antioxidant properties. Plasma concentrations are good markers for bioavailability, but may vary according to the nature of the polyphenol and the food source. Usually they are on the order of nanomolar to low micromolar concentrations (Hollman and others 1997; Graefe and others 2001). Generally, among the polyphenols present in aronia catechin and quercetin are more efficiently absorbed than anthocyanins, which appear in plasma at very low concentrations (Miyazawa and others 1999; Cao and others 2001; Matsumoto and others 2001). An objective way to measure the antioxidant action of an antioxidant *in vivo* is the use of oxidative stress/oxidative defense biomarkers. Recently, Niki (2010) reviewed them outlining the most significant ones. Oxidation products of lipids (Maoodi and others 2008; Niki 2008; Yin and others 2009; Nakanishi and others 2009), oxidative modification and expression of proteins and sugars (Hawkins and others 2009; Rabbani and Thornalley 2009), and strand breaks of DNA and oxidation products of DNA bases (Dizdaroglu and others 2002) have been used as oxidative stress biomarkers. Some of the most used biomarkers summarized by Niki (2010) are: (1) lipid oxidation—ethane and pentane in exhaled gas; thiobarbituric acid-reactive substances (TBARS); conjugated dienes; hydroperoxide aldehydes; ketones; isoprostane; neuroprostane; isofuran; neurofuran; lyso PC, oxidized LDL, oxys-

terols; (2) protein oxidation—protein carbonyl hydroperoxides; disulfide -SS-; -SOH, -SOOH, -SOOOH; aldehyde-modified protein; hydroperoxide-modified protein; crosslinked protein; dityrosine, albumin dimer, creatol, myeloperoxide; (3) DNA oxidation—Commet assay, thiamine glycol; 5-hydroxyuracil; 8-hydroxyguanine; 8-nitro-, chloro-, bromo-guanine. The ratio of *cis,trans/trans,trans*-HODE (Nam and others 2007; Yoshida and others 2007), linoleoyl tyrosine 2-deoxyguanosyl ester (a conjugate product from unsaturated fatty acid, protein, and DNA; Khatib and others 2007) and acrolein (Tomitori and others 2005) has also been proposed. Antioxidants (ratio of oxidized/reduced forms of glutathione and coenzyme) could be used as well. As can be seen from this review, just a few of these biomarkers have been used in the investigation of aronia health benefits.

Bioavailability of Aronia Polyphenols Anthocyanins

Anthocyanins are broadly distributed and many plants, including berries, contain several structurally diverse anthocyanins. They appear to be poorly absorbed in the small intestine, so significant amounts probably pass into the large intestine where bacterial degradation occurs. There are reports that cyanidin-based anthocyanins undergo cleavage of the sugar moiety followed by ring fission of the released cyanidin, which produces 3,4-dihydroxybenzoic acid (protocatechuic acid; Aura and others 2005; Vitaglione and others 2007; Galvano and others 2008). Detecting and quantifying the trace levels of complex anthocyanin profiles in plasma and urine after absorption, excretion, and potential phase I and phase II metabolism appears to be very difficult. Fortunately, because of its simple anthocyanin profile aronia is preferred for bioavailability studies of cyanidin and its glycosides. That is why aronia anthocyanins have been investigated most thoroughly in comparison to its other phenolic constituents. Kay and others (2004) investigated the metabolic conversion of aronia-derived cyanidin glycosides in human subjects. Volunteers consumed approximately 20 g chokeberry extract containing 1.3 g cyanidin 3-glycosides. Cyanidin-3-galactoside accounted for 66.0% of the detected anthocyanins in the urine and serum samples. The metabolites were identified as glucuronide conjugates, as well as methylated and oxidized derivatives of cyanidin-3-galactoside and cyanidin glucuronide. The consumption of 4 cyanidin glycosides (cyanidin 3-galactoside, cyanidin 3-arabinoside, cyanidin 3-xyloside, and cyanidin 3-glucoside) resulted in the appearance of at least 10 individual anthocyanin metabolites in human urine and serum. Urine samples (22 to 24 h) showed cyanidin 3-galactoside and metabolized derivatives of cyanidin 3-galactoside to persist in

the urine at levels of 0.011 to 0.013 nmol/L. Additionally, the concentrations of identifiable anthocyanins and anthocyanin metabolites in the serum were observed at a level of 350.8 nmol/L within 2 h postconsumption, with a cumulative total serum concentration reaching 591.7 nmol/L. The data obtained indicated the presence of both cyanidin-3-galactosides and cyanidin glucuronides in the urine. Mono- and dimethylated cyanidin-3-galactoside derivatives and glucuronide derivatives were also detected. Interestingly 1 anthocyanin metabolite occurred in the urine but not in the serum, which may indicate that this metabolic product is either formed exclusively in the kidney, accumulates in the kidney, or the concentration of this metabolite in the serum may have been below the detection limit of the analytical technique used. This compound was highly metabolized cyanidin 3-galactoside. The parent aglycone cyanidin was not identified, neither in urine nor in the serum samples. Total urinary analysis revealed that cyanidin galactoside and its metabolites accounted for 84.0% of the identified anthocyanins. Of these, 55.3% was the parent compound cyanidin-3-galactoside. In the serum, cyanidin galactosides accounted for 89.4% (529.3 nmol/L) of the anthocyanins, with 66.0% being the parent compound. It must be noted that the study design included the ingestion of an extremely high dose of anthocyanins (1.3 g) and the metabolic route under these circumstances may differ from the route following the ingestion of a diet-relevant dose of anthocyanins. In a more recent study Wu and others (2005) investigated the urinary excretion of anthocyanins in chokeberry-fed pigs. A total of 18 different ACN-based compounds, including 4 major original ACNs and 14 metabolites were identified in the urine in contrast to the 11 ACN-based compounds in the urine described in the human study of Kay and others (2004). Of the total ACN-based compounds in the urine, cyanidin-3-galactoside accounted for 60.7% of the total, which is similar to findings of Kay and others (2004). In another pharmacokinetic study a more diet-relevant dose of chokeberry anthocyanins (721 mg cyanidin-3-glycosides) was given to human subjects orally (Kay and others 2005). The results indicated that cyanidin-3-glycosides are rapidly absorbed and metabolized extensively following a moderate-to-high oral dose in humans. In contrast to the other studies with aronia ACNs, the parent anthocyanins represented only 32.0% of the total anthocyanins detected with 68.0% identified as conjugated metabolites. Additionally only 32.5% (347.85 μg) of the anthocyanins excreted in the urine (total 24 h) were the parent compounds with 67.5% (723.69 μg) occurring as conjugated metabolites. The metabolites were identified as glucuronidated and methylated derivatives of the parent cyanidin-3-glycosides. Glucuronidation was the major metabolic pathway observed for anthocyanin metabolism, representing 59.8% and 57.8% of the total anthocyanins detected in the blood and urine, respectively, and methylation was the second most commonly observed metabolic transformation. In an elegant study Wiczowski and others (2010) investigated the bioavailability of anthocyanins from chokeberry juice providing 0.8 mg of anthocyanins/kg of body weight using healthy volunteers. In contrast to other studies, this was the first time when fresh chokeberry juice with a dietary-relevant dose of anthocyanins was used in a bioavailability study. Eight cyanidin derivatives were found in blood and urine after juice consumption. The maximum plasma anthocyanin concentration of 2.9 nmol/L was reached at 1.3 h. The anthocyanins' urine excretion rate was the highest within the first 2 h. In total 0.02% of the ingested anthocyanins was excreted by the renal route during 24 h, mainly as metabolites of cyanidin. After consumption, analysis of both plasma and urine of all volunteers showed that chokeberry anthocyanins were absorbed intact

as cyanidin glycosides as well as being metabolized to methylated and/or glucuronidated derivatives. Anthocyanins appeared in the blood within 30 min after the consumption of chokeberry juice, which authors related to absorption in the upper part of the digestive tract, suggesting at least in part absorption of anthocyanin from the stomach. Similarly to the other studies the glucuronidation was the major metabolic route of chokeberry anthocyanins, while methylation played a minor role in the metabolism of those. The elimination half-life of plasma total anthocyanins was calculated as 2.4 h.

It is evident that the available bioavailability data of aronia anthocyanins give controversial information. There are big differences in the number of the recovered metabolites in blood and urine and the amounts of the recovered parent anthocyanins in the human and animal studies. These differences could be attributed to the variation in the administered doses of anthocyanins, the specifics of animal and human metabolism and the analytical technique used. Metabolism of ingested flavonoids is not necessarily the same in animal models as it is in human subjects and frequently the dosages used in such studies are untranslatable to a regular human dietary regimen. In summary, it could be concluded that chokeberry anthocyanins are recovered in blood and urine in nanomolar concentrations. Glucuronidation and methylation seem to be the major pathways in aronia anthocyanin metabolism, whereas sulfation was not observed. It is likely that conjugation affects the biological activity of anthocyanins and these metabolic products are likely, in part, responsible for the reported health benefits associated with the consumption of anthocyanins.

Proanthocyanidins

Proanthocyanidins differ from most other plant polyphenols because of their polymeric nature and high molecular weight. This particular feature should limit their absorption through the gut barrier; oligomers larger than trimers are unlikely to be absorbed in the small intestine in their native forms. There are numerous feeding studies with animals and human subjects indicating that polymeric procyanidins are not absorbed (Espin and others 2007). Most pass unaltered to the large intestine where they are catabolized by the colonic microflora yielding a diversity of phenolic acids (Deprez and others 2000; Gonthier and others 2003a; Appeldoorn 2009a) including 3-(3-hydroxyphenyl)propionic acid and 4-O-methyl-gallic acid, which are absorbed into the circulatory system and excreted in urine. *In vitro* experiments using single layers of Caco-2 cells as a model of absorption in the small intestine showed that only the dimers and trimers of flavanols are able to cross the intestinal epithelium (Deprez and others 2001). Procyanidin B2 is very poorly absorbed in rats, whereas procyanidin B3 is not absorbed (Baba and others 2002; Donovan and others 2002). There is also a report of minor quantities of procyanidin dimers B1 and B2 being detected in human plasma (Sano and others 2003). Individual procyanidins in an extremely high dose (1 g/kg body weight) were fed to rats after which dimers, trimers, tetramers, and pentamers were detected in plasma (Shoji and others 2006). It remains to be determined whether procyanidins can be similarly detected in urea-extracted plasma after the ingestion of more nutritionally relevant doses.

So far, no bioavailability study has been conducted with aronia-isolated proanthocyanidins and there is only 1 piece of indirect evidence for the bioavailability of aronia proanthocyanidins. It is a study with purple aronia (*Aronia arbutifolia*) performed by Jakešević and others (2011) which revealed that in the caecal contents of chokeberry-fed groups, 4-hydroxyphenylacetic acid (4-HPA) and

3-hydroxyphenylacetic acid (3-HPA) were identified as metabolites. The detected phenolic acids were probably metabolites of microbial degradation of aronia proanthocyanidins. Interestingly 3,4-dihydroxyphenylacetic acid (diHPA), which is the main product of proanthocyanidin microbial degradation, was not detected in the chokeberry-fed mice. Instead, the authors detected 3-HPA and 4-HPA, which are suggested to be metabolites of diHPA dehydroxylation at meta and para positions (Appeldoorn and others 2009a). Despite their poor absorption proanthocyanidins may exert local activity in the gastrointestinal tract or activity mediated by phenolic acids produced through microbial degradation. Their local action may nevertheless be important because the intestine is particularly exposed to oxidizing agents and may be affected by inflammation and numerous diseases such as cancer (Halliwell and others 2000). Polyphenol concentrations in the colon can reach several hundred micromoles per liter (Scalbert and Williamson 2000) and, together with a few carotenoids, they constitute the only dietary antioxidants present in the colon, because vitamins C and E are absorbed in the upper segments of the intestine.

Hydroxycinnamic acids

Despite the scarcity of studies performed on the bioavailability of hydroxycinnamic acids, when ingested in the free form, these compounds are rapidly absorbed from the small intestine and are conjugated and, in particular, glucuronidated in the same way that flavonoids are (Cremin and others 2001). However, the only representatives in aronia, chlorogenic and neochlorogenic acids, are naturally esterified and this impairs their absorption. Human tissues and biological fluids do not possess esterases capable of hydrolyzing chlorogenic acid to release caffeic acid (Plumb and others 1999; Olthof and others 2001; Rechner and others 2001), which is a more potent antioxidant. This has also been observed in rats (Azuma and others 2000; Andreasen and others 2001). Only the colonic microflora would be capable of carrying out this hydrolysis and some of the bacterial strains involved have been identified (Couteau and others 2001), but the efficiency of absorption of phenolic acids is markedly reduced when they are present in the esterified form rather than in the free form (Gonthier and others 2003b).

Flavonols and flavan-3-ols

Flavonols and flavan-3-ols are present in very small amounts in chokeberries and probably because of that they have not been subjected to bioavailability studies. Since they possess the highest antioxidant activity, it is worth to discuss their fate *in vivo* and to judge indirectly, the bioavailability of aronia flavonols and flavan-3-ols. Investigations with other products, such as onions that contain substantial amounts of flavonols, indicate that quercetin-O-glucosides are hydrolyzed and a number of metabolites including quercetin-3'-O-sulphate, quercetin-3'-O-glucuronide, and isorhamnetin-3-O-glucuronide appear in plasma in submicromolar concentrations. The profile of urinary metabolites is markedly different to that in plasma, indicating that phase II metabolism is operative (Mullen and others 2006).

Studies with rats have led to suggestions that flavan-3-ol monomers may be removed from the bloodstream in the liver and returned to the small intestine in the bile (Kida and others 2000; Kohri and others 2001). To what extent enterohepatic transport of flavan-3-ol metabolites occurs in human subjects remains to be established. The bioavailability studies with flavan-3-ols are characteristic for the high urinary excretion (Manach and others

2005; Stalmach and others 2009) and also for the substantial recoveries of flavan-3-ols in ileal fluid by human subjects with an ileostomy (Stalmach and others 2010).

In regards to the bioavailability of aronia polyphenols can be concluded that, although very abundant in aronia, proanthocyanidins are poorly absorbed and their action is thus restricted to the lower intestine. The same appears to be true for anthocyanins. The intake of monomeric flavonols and flavanols with chokeberries is very low and it is not very likely that they have a significant influence on plasma polyphenol concentrations. Chlorogenic and neochlorogenic acids are found in significant concentrations in chokeberries, but esterification decreases their intestinal absorption.

Antioxidant activity of chokeberry polyphenols in isolated cells and cultured cells

Cultured cells have often been used as a substrate to evaluate the protective effects of antioxidants against various oxidants. The advantage of using cultured cells is that various oxidants and cell types, including model systems for some specific disease, can be used for the evaluation of antioxidant effect (Niki 2010). However, together with the advantages, the use of such *in vitro* systems is pursued by some drawbacks as well. For example, the concentration of the antioxidant added into the culture medium should be chosen very carefully (Niki 2010). Many of the antioxidant studies with cell cultures are performed with aronia extracts containing polyphenols at nonphysiologically achievable levels. At physiological concentrations, these polyphenols may not exhibit the reported effect. Therefore, these cell line systems must be used mainly to investigate the underlying mechanisms of oxidative stress and antioxidant action of the extracts. Following ingestion much of the dietary flavonoids and polyphenols appear in the circulatory system, not as the parent compounds but as glucuronide, methyl, and sulfate metabolites with totally different structures from the parent compounds. In the case of aronia it is favorable that its anthocyanins appear to a high extent unaltered in serum and urine which makes the performed studies with isolated cells and cell lines more physiologically plausible and related to the potential health benefits.

Antioxidant activity of aronia polyphenols in platelets

Ryszawa and others (2006) investigated the effects of chokeberry extracts on superoxide production and aggregation in platelets from subjects with significant cardiovascular risk factors (hypertension, hypercholesterolemia, smoking, and diabetes mellitus). Superoxide production was significantly increased in patients with cardiovascular risk profile when compared to controls. Aronia polyphenol-rich extracts caused a significant concentration-dependent decrease in superoxide production only in patients with cardiovascular risk factors, while no effect was observed in the control group. Aronia extracts abolished the difference in superoxide production between risk factor patients and controls and exerted significant concentration-dependent antiaggregatory effects in both studied groups, indicating that these effects may be independent of its ability to modulate superoxide production. The antiaggregatory effects of chokeberry extracts were similar irrespective of an aggregation-inducing agent (collagen or thrombin). Moreover, they appear to be independent of platelet NO release as NOS inhibition by L-NAME did not lead to their abrogation. In another study (Olas and others 2008a) chokeberry extract (total polyphenols 309.6 mg/g) in concentrations of 5 to 50 $\mu\text{g}/\text{mL}$ showed protective effects against peroxynitrite-induced oxidative/nitritative

damage to human platelet proteins and lipids and significantly inhibited platelet protein carbonylation and thiol oxidation as estimated with 5,5'-dithio-bis-(2-nitro-benzoic acid). The tested extract caused a distinct reduction of platelet lipid peroxidation induced by peroxynitrite. The exposure of blood platelets to peroxynitrite at a concentration of 0.1 mM resulted in a distinct depletion of free thiol groups in platelet proteins. The presence of the tested extract from aronia protected platelet protein thiols from oxidation induced by ONOO⁻ and this effect was dose-dependent. The aronia extract suppressed peroxynitrite toxicity, expressed as TBARS. In another set of experiments, the authors observed an increased level of biomarkers of oxidative/nitrative stress such as 3-nitrotyrosine in platelet proteins from breast cancer patients. This study provided evidence of antioxidant properties of the extract and its ability to scavenge peroxynitrite, which forms in the vascular system and may cause oxidative/nitrative stress and damage some biomolecules in platelets such as proteins and lipids (Olas and others 2004). In an *in vitro* model system Kedzierska and others (2010) showed that the commercial chokeberry extract (Aronox), due to antioxidant action, distinctly reduced the oxidative/nitrative stress in platelets isolated from patients with invasive breast cancer. The authors observed that all markers of oxidative stress were significantly higher in the blood platelets obtained from the patients in comparison with the control group. Aronia extract was also found to improve antiplatelet action of human umbilical vein endothelial cells towards ADP-activated platelets, and this effect was dose-dependent (Luzak and others 2010). The antiplatelet effect of aronia extract was shown to be higher than that of resveratrol indicating the extract may play an important role as a component of the human diet in the prevention of cardiovascular or inflammatory diseases where blood platelets are involved (Olas and others 2008b). The experiments have shown that aronia extracts (5 to 50 µg/mL) reduce platelet adhesion, aggregation, and generation of O₂⁻ in blood platelets. Another study investigated the influence of aronia extract on clot formation (using human plasma and purified fibrinogen) and brin lysis using the model of hyperhomocysteinemia (Malinowska and others 2012). Aronia extract reduced the adverse effects of homocysteine on hemostatic properties of fibrinogen or plasma, suggesting its possible protective properties in hyperhomocysteinemia-induced CVD. Moreover, aronia extract increased the plasma antioxidant activity, in the model of hyperhomocysteinemia, which may modulate the hemostatic properties of human plasma. Another set of *in vitro* experiments showed that aronia extract prolonged clotting time and decreased the maximal velocity of fibrin polymerization in human plasma. Moreover, thrombin incubation with the extract resulted in the inhibition of amidolytic activity of this enzyme (Bijak and others 2011).

Effect of aronia polyphenols on neutrophils

It is known that activated neutrophils generate extremely high amounts of ROS, but these are normally targeted at pathogens inside intracellular phagosomes. This beneficial antimicrobial function, if not controlled, contributes to the tissue-damaging effects of inflammatory reactions. Zielinska-Przyjemaska and others (2007) investigated the *in vitro* effect of aronia juice on oxidative metabolism and apoptosis of neutrophils from obese and nonobese individuals. Neutrophils were isolated and oxidant production was characterized by luminol-dependent chemiluminescence (CL) and flow cytometric dichlorofluorescein oxidation assay. Caspase-3 activity, a marker of apoptosis execution, in human neutrophils was used. Neutrophils from obese individuals had a significantly higher

H₂O₂ production and CL response compared to controls. The oxidative metabolism of neutrophils was decreased by aronia juice treatment in both groups. In a model of gastric digestion aronia juice significantly decreased the oxidative metabolism of neutrophils in healthy and obese subjects. The juice inhibited H₂O₂ production within the range of 10 to 50% v/v and the CL response. This activity can be explained either by the scavenger effect of the juice on ROS or by the functional changes that cells undergo in the presence of the juice. Phorbol 12-myristate 13-acetate (PMA) is an oncogenic substance that induces a translocation-activation process by protein kinase C (PKC). The inhibition of the respiratory burst by phagocytes could depend on interference of the juice with the PMA-dependent activation of PKC. The authors observed proapoptotic effects of nonstimulated neutrophils after 24 h of incubation with aronia juice and suggested that chokeberry polyphenols interfere with NADPH oxidase and/or ROS and promote apoptosis.

Antioxidant activity in endothelial cells

Vascular inflammation is a primary event in the pathogenesis of atherosclerosis, which can elicit acute coronary syndromes. An early stage in the inflammatory process is endothelial activation, which is directly responsible for the recruitment of circulating leucocytes. This process is self-maintaining and is mediated through the cell adhesion molecules (CAMs) expressed on the surface of endothelial cells, including ICAM-1 and VCAM-1 (Galkina and Ley 2007). Altered expression of CAMs has been implicated in a variety of chronic inflammatory conditions, including atherosclerosis. Pharmacological agents that display an inhibitory effect on endothelial cell activation should have antiinflammatory activity (Libby 2006). In a study performed by Zapolska-Downar and others (2012), human aortic endothelial cells (HAECs) were pretreated with various concentrations (primarily 50 µg/mL) of aronia extract prior to treatment with TNFα (10 ng/mL). The tested extract inhibited the proinflammatory response of endothelial cells by inhibiting the expression of ICAM-1 and VCAM-1, as determined by using flow cytometry and real-time RT-PCR, respectively, attenuated the phosphorylation of NF-κB p65, and decreased intracellular ROS production in TNFα-treated HAECs. Moreover, the inhibitory effect of the tested extract on the TNFα-induced VCAM-1 expression was similar to that of ibuprofen and PDTc, and on TNFα-stimulated ICAM expression was much greater than that observed for the 2 antiinflammatory drugs. The preincubation of the HAECs with aronia extract resulted in a reduction in TNFα-stimulated increased adhesiveness for peripheral blood mononuclear leucocytes (PBMLs), which may be an important mechanism by which aronia extract exerts its antiinflammatory and antiatherogenic effects. It was also found that TNFα induced higher levels of NF-κB phosphorylation that could be decreased in HAECs when pretreated with the aronia extract, suggesting that the antiinflammatory properties of this extract *in vitro* are mediated, at least in part, by the inhibition of NF-κB activation. The NF-κB redox-sensitive transcription factor plays a key role in TNFα-induced ICAM-1 and VCAM-1 expression (de Winther and others 2005) and its role in the pathogenesis of atherosclerosis has been indicated since its active form is present in atherosclerotic lesions (Brand and others 1996). It is known that ROS are implicated in the activation of NF-κB and interfere with signaling pathways, which leads to phosphorylation and subsequent degradation of phosphorylating kinases IκB. Based on the results, the authors assumed that the inhibitory effect of chokeberry extract on ICAM-1 and VCAM-1 expression and NF-κB

activation were due to antioxidant properties. In another set of experiments it was found that aronia extract produced dose- and endothelium-dependent vasorelaxation of coronary arterial rings and the effect was higher compared to bilberry and elderberry extracts (Bell and Gochenaur 2006). It is known that ROS impair endothelial NO functions through direct damage to the endothelium as well as by chemical quenching of NO. Factors that can enhance or protect the endothelial NO system, or scavenge and inactivate ROS, have the potential to prevent CVD. The authors suggested that the vasoprotective effect of aronia extract could be attributed to the high antioxidant activity of the extract, which would tend to reduce the effective concentration of superoxide or ROS reaching the arterial endothelium. More importantly, this ability to prevent loss of endothelium-dependent, NO-mediated relaxation was achieved at an extremely low concentration that could be achieved in human plasma after oral consumption of aronia products. In another set of experiments *Aronox* extract significantly decreased 7β -hydroxycholesterol-induced apoptosis and ROS generation and ameliorated the collapse of the mitochondrial transmembrane potential with subsequent inhibition of cytochrome C release in endothelial cells. Furthermore, down-regulation of Bcl-2 and up-regulation of caspase-3 activation were reversed by *Aronox* (Zapolska-Downar and others 2008).

Antioxidant activity in cancer cell lines

ROS and RNS, and damage caused by these species, are implicated in the pathogenesis of a variety of diseases, including cancers (Valko and others 2006; Fang and others 2009; Trachootham and others 2009). There is much evidence that during cancer the endogenous antioxidant protective system of the body is depleted. Changes in glutathione content have been reported in several malignancies. In cancer patients the content of reduced glutathione in erythrocytes is also decreased, which may promote oxidative stress in patients with invasive breast cancer and patients with benign breast cancer (Kumar and others 1995; Della Rovere and others 2000; Ścibior and others 2008). During cancer therapy (radiotherapy or chemotherapy) ROS may also be generated in patients. Results (Kim and others 2009) showed that doxorubicin can induce platelet cytotoxicity through ROS generation, decreased glutathione levels and protein thiol depletion. The toxic side effects of chemotherapy may be associated also with damage to different cellular components induced by ROS. Blood platelets isolated from patients with breast cancer may produce not only $O_2^{\cdot-}$, but also peroxynitrite (Kedzierska and others 2010), and moreover the level of $O_2^{\cdot-}$ after phase I of the chemotherapy was significantly higher than the level of $O_2^{\cdot-}$ in blood platelets obtained before or after the surgery, indicating the role of chemotherapy in the generation of ROS in blood platelets (Kedzierska and others 2012). Olas and others (2010) showed that the level of total glutathione, cysteine, and cysteinylglycine in plasma from patients with invasive breast cancer and patients with benign breast diseases was 50% lower than the level of these thiols in the control plasma obtained from healthy volunteers. Moreover, the levels of low-molecular-weight thiols in reduced forms (GSH, CSH, and CGSH) and in oxidized forms (GSSG, CSSC, and CGSSGC) in plasma from the patients were changed compared to the healthy group. On the contrary, the level of homocysteine in plasma from patients with invasive breast cancer was 15% higher than in plasma of controls. In the presence of aronia extract (50 μ g/mL) changes in the amount of thiols in plasma from patients with invasive breast cancer and patients with benign breast diseases were significantly reduced *in vitro* and the effect was stronger compared to that of pure resveratrol with the

same concentration. The authors proposed that aronia polyphenols may induce an increase in antioxidant capacity through enhancement of plasma SOD or other enzymes. In colon cancer models, Malik and others (2003) revealed that 24-h exposure of human HT-29 colon cancer cells to 50 μ g monomeric anthocyanin/mL of aronia extract resulted in 60% growth inhibition. The treated cells showed a blockage at G1/G0 and G2/M phases of the cell cycle. The cell cycle arrest coincided with an increased expression of the p21^{WAF1} and p27^{KIP1} genes and decreased expression of cyclin A and B genes. Prolonged exposure to the extract resulted in no further change in the cell numbers, indicating a cytostatic inhibition of cell growth. The authors reported that chokeberry extract inhibited cyclooxygenase-2 (COX-2) gene expression in HT-29 cells in a concentration-dependent manner, but did not translate into decreased protein levels or protein activity. Normal colon cells, NCM460, did not show a significant change in expression of either COX-1 or COX-2 genes. Cyclooxygenase enzymes catalyze the oxygenation of arachidonic acid, leading to formation of prostaglandins. Numerous epidemiological studies have shown that inhibition of COX genes is linked to colon cancer prevention, and upregulation in the expression of COX-2 has been observed in colorectal adenomas and carcinomas. Drugs, especially nonsteroidal antiinflammatory drugs, which inhibit the COX-2 enzyme, can delay or prevent colon cancer (Prescott and Fitzpatrick 2000). In a similar study by the same authors (Zhao and others 2004) chokeberry anthocyanin-rich extract was investigated for its potential chemopreventive activity against colon cancer. The growth of colon-cancer-derived HT-29 and nontumorigenic colonic NCM460 cells exposed to the extract (10 to 75 μ g of monomeric anthocyanin/mL) was monitored. HT-29 cell growth was inhibited by 50% after 48 h of exposure to 25 μ g/mL extract. Most importantly, the growth of NCM460 cells was not inhibited at lower concentrations of the extract, illustrating greater growth inhibition of colon cancer, as compared to nontumorigenic colon cells. The reasons for the different susceptibilities of colon cancer versus nontumorigenic cell lines are unknown but may involve differences in gene expression between NCM cells and tumor cell lines (Nimmrich and others 2000).

In another model study Bermudez-Soto and others (2007a) demonstrated that repeated exposure to chokeberry juice has a potent *in vitro* antiproliferative effect toward the human colorectal cancer cell line Caco-2 and that this antiproliferative effect may be mediated through arrest of the cells at the G/M checkpoint. Through analysis of gene expression they detected changes in the mRNA levels of several tumor markers typical for colon cancer and of proteins involved in proliferation and cell cycle that may be associated to the treatment. Among these was the tumor suppressor carcinoembryonic antigen-related cell adhesion molecule 1 (CEACAM1) whose expression is known to be reduced in the majority of early adenomas and carcinomas. The authors suggested that CEACAM1, which has a significant regulatory role on cell proliferation of particular interest at early stages of cancer development may be a potential target for chemoprevention by aronia polyphenols. In an early study (Gasiorowski and others 1997) it was shown that chokeberry markedly inhibited the mutagenic activity of benzo[a]pyrene and 2-amino fluorene in the Ames test and a significant decrease of sister chromatid exchanges frequency induced by benzo(a)pyrene was observed in human blood-derived lymphocytes cultured *in vitro*. In a study performed by Sueiro and others (2006) anthocyanins markedly inhibited the generation and release of superoxide radicals by human granulocytes, and some of the subfractions extracted from wild and cultivated chokeberry

showed >90% inhibitory activity to L1210 leukemia cells at a concentration of 25 $\mu\text{g}/\text{mL}$ and 50 $\mu\text{g}/\text{mL}$. Results of Lala and others (2006) showed a protective effect of chokeberry extract in colon carcinogenesis *in vitro* and indicated multiple mechanisms of action. The extract from *A. melanocarpa* has also shown anticancer effects, mediated by the increase of tumor suppressor genes as well as by reduction of oxidative stress and resulting DNA damage important for the proliferation of cancer cells.

Antioxidant activity of aronia polyphenols in pancreatic β -cells

Rugina and others (2011) evaluated the protective action of nanomolar concentrations of chokeberry extract against the oxidative stress induced by high doses of glucose (50 and 100 mM) in pancreatic β -cells. The results indicated the scavenging effect of chokeberry anthocyanins on the intracellular ROS species, and anthocyanins restored dose-dependently the strong decrease of glutathione (induced by high glucose), as compared to untreated cells. Enhanced oxidative stress due to high glucose contributes to pathological changes in diabetes-related liver complications. The glutathione (GSH) antioxidant system is critical for counteracting oxidative stress-induced intracellular injury. In a very recent study, Zhu and others (2012) evaluated the mechanism of the anthocyanin-mediated regulation of GSH synthesis and reduction in intracellular ROS levels. The authors observed that treatment of human HepG2 cells with the anthocyanin cyanidin-3-glucoside significantly reduced ROS levels induced by high glucose. Cyanidin-3-glucoside incubation increased glutamate-cysteine ligase expression, which occurred independent of the Nrf1/2 transcription factors, and mediated the reduction in ROS levels. The anthocyanin increased phosphorylation of cAMP response element binding protein (CREB) through protein kinase A (PKA) activation. The decrease in ROS levels by cyanidin-3-glucoside led to a significant inhibition of phospho-mitogen-activated protein kinase kinase-4 (MKK4)-Jun N-terminal kinase (JNK) signaling and thus a decrease in proapoptotic Fas expression. Consequently, the anthocyanin markedly ameliorated cell apoptosis and improved viability in HepG2 cells.

In vivo Evidence for Chokeberry Antioxidant Activity Antioxidant activity in the gastro-intestinal tract (GIT)

It should be noted that the role of antioxidants in the GIT may be very important. The diet contains various prooxidants, including metals such as iron, copper and heme, lipid hydroperoxides, aldehydes, and nitrite, and elevated levels of lipid peroxides have been observed in the postprandial state. The activation of phagocytes in the gut may also increase the ROS/RNS, and gastric juice may promote lipid peroxidation (Kanner and others 2001). These prooxidants may induce oxidative stress in the gastrointestinal tract to induce stomach ulcer and develop stomach, colon, and rectal cancers. The antioxidants contained in foods may suppress such oxidative stress and related diseases in the gastrointestinal tract before they are absorbed (Halliwell and others 2000). Many of the colonic metabolites still have free -OH groups and maintain the antioxidant activity, so they can also contribute to enhance plasma antioxidant capacity. Thus, in addition to the original phenolic compounds, their metabolites also have to be considered to understand the biological and antioxidant functions of foods (Fernandez-Pancho and others 2008).

Bermudez Soto and others (2007b) investigated the effects of an *in vitro* gastric and pancreatic digestion on the stability and composition of the major polyphenols in chokeberry juice in a model

system. Digestion was carried out with a mixture of pepsin-HCl for 2 h, followed by incubation with pancreatin and bile salts at 37 °C. Gastric digestion had no substantial effect on any of the major phenolic compounds in chokeberry. However, aronia polyphenols were significantly altered during the pancreatic digestion and this effect was more marked for anthocyanins (approximately 43% were lost during the 2-h treatment with pancreatin). Flavonols and flavan-3-ols decreased by 26% and 19%, respectively. Neochlorogenic acid decreased by 28%, whereas chlorogenic acid was increased by 24%. The losses were attributed mostly to the chemical conditions during pancreatic digestion instead of interactions with the digestive enzymes. The main advantage of this study was the concentration of polyphenols used. Such concentrations (0.5 to 50 $\mu\text{g}/\text{mL}$) especially in the lower threshold, seem to be achievable in plasma during supplementation with aronia extracts. However, there are important discrepancies between *in vitro* and *in vivo* results, such as those reported for the dimeric flavan-3-ols in model systems (Zhu and others 2002), which differed significantly by their fate in a *in vivo* human study (Rios and others 2002). Ingestion of aronia juice by rats increased stomach pH from 3.44 (control group) to 3.69–3.85 and ileal pH from 6.16 to 6.23–6.46. The juice decreased the concentration of cecal ammonia, especially at medium and high doses, increased microbial α -glucosidase, decreased β -glucosidase, and had no effect on β -glucuronidase activities in the cecal digesta (Wróblewska and others 2008). Additionally, increased content of butyrate in short-chain fatty acids in cecal digesta was observed. In a very recent study, Jakešević and others (2011) fed mice for 10 d with purple chokeberry powder which has the same anthocyanin profile (cyanidin-3-galactoside – 66%) as found in black chokeberry. Four anthocyanins were detected in the chokeberry powder and 3 of these anthocyanins were found in the cecum and colon of mice. Interestingly, the concentration of cyanidin-3-arabinoside was only slightly lower compared to the galactoside in the intestines, although its concentration was more than 30% higher in the chokeberry powder compared to cyanidin-3-arabinoside. This indicates that cyanidin-3-galactoside is to higher extent absorbed and/or degraded by gut microflora than the arabinoside. Cyanidin-3-xyloside was found in the cecum and colon of mice almost in the same amount as in the powder, which indicates that it was quite resistant to the degradation in the gut. The minor component in the powder cyanidin-3-glucoside was not detected in the intestines.

In the studies performed by Tanaka and Yuda (1996) and Valcheva-Kuzmanova and others (2005) aronia juice was shown to reduce significantly and dose-dependently the number and area of indomethacin-induced gastric ulcers in rats. The administration of indomethacin resulted in extensive lipid peroxidation, as evidenced by the accumulation of malondialdehyde (MDA) in the gastric mucosa, which is in agreement with other studies indicating that ROS are involved in the development of mucosal damage by nonsteroidal antiinflammatory drugs such as indomethacin (Naito and others 1998). The gastroprotective effect of aronia juice was accompanied by a significant decrease in lipid peroxidation evaluated as gastric and plasma MDA concentrations. Besides that aronia juice caused an increase in mucus production, which is another protective mechanism against indomethacin-induced gastric ulceration. The reduction of MDA in rat blood plasma by chokeberry supplementation was demonstrated also by Frejnagel and Zduńczyk (2008) suggesting that the extract had a preventive effect. The gastroprotective effect against ethanol-induced acute gastric hemorrhagic lesions in rats was demonstrated by

Matsumoto and others (2004), suggesting that the anthocyanin fraction was responsible for this effect. Anthocyanin-rich extract from aronia was fed for 14 wk to male rats treated with a colon carcinogen, azoxymethane (Lala and others 2006). The number and multiplicity of colonic aberrant crypt foci, colonic cell proliferation, urinary levels of oxidative DNA damage, and expression of cyclo-oxygenase genes were measured as biomarkers of colon cancer. The lower levels of these specific biomarkers in treated rats, with respect to controls, suggest a protective role of the extract in colon carcinogenesis and indicate multiple mechanisms of action. As already mentioned, some of the bacterial strains of the colonic microflora are capable to hydrolyze the abundant chlorogenic acid in chokeberries releasing caffeic acid, which is a more potent antioxidant. Moreover, 4-HPA and 3-HPA were identified in the cecal contents of aronia-fed mice. The antioxidant activity and biological properties of these phenolic metabolites have been poorly assessed, but it has been shown that phenylacetic acids possess anticarcinogenic activities (Samid and others 1997; Thibout and others 1999) and diHPA or 4-HPA were more effective than their precursors (rutin and quercetin) inhibiting *in vitro* platelet aggregation (Kim and others 1998). It is expected that 3,4-dihydroxyphenylacetic acid is a potent antioxidant since it has 2 ortho hydroxyl groups present in its structure resulting in elevated antioxidant activity. It has been shown that diHPA exhibit considerable antiproliferative activity in LNCaP prostate cancer and in HCT116 colon cancer cells (Gao and others 2006). And diHPA exerted antiinflammatory properties by reducing the secretion of proinflammatory cytokines, TNF- α , IL-1b, and IL-6, all involved in the early stages of atherosclerosis from lipopolysaccharide-stimulated human peripheral blood mononuclear cells.

Hepatoprotective effect of aronia polyphenols in relation to antioxidant activity

Aronia juice intake was shown to inhibit the endogenous generation of carcinogenic N-nitrosamines in rats treated with aminopyrine plus sodium nitrate. In consequence, histopathological changes observed in the liver of rats fed with nitrosamine precursors were prevented by co-treatment with aronia juice. The juice exerted a positive effect on blood and liver variables, which was demonstrated by decreased concentrations of glutamic-oxaloacetic transaminase, glutamic-pyruvic transaminase, and uric acid in serum, and lipid content in hepatocytes (Atanasova-Goranowa and others 1997). The hepatoprotective effect of aronia juice was confirmed also against carbon tetrachloride (CCl₄)-induced acute liver damage in rats (Valcheva-Kuzmanova and others 2004). The administration of CCl₄ increased plasma aspartate transaminase (AST) and alanine transaminase (ALT) activities, induced lipid peroxidation (as measured by MDA content in rat liver and plasma) and caused a depletion of liver reduced glutathione (GSH). Aronia juice dose dependently reduced the necrotic changes in rat liver and inhibited the increase of plasma AST and ALT activities induced by CCl₄, and prevented MDA formation and depletion of GSH content in rat liver. The authors proposed that the possible mechanisms of this effect might be similar to those proposed for the flavonoid silymarin (Lettéron and others 1990) which inhibited CCl₄-induced lipid peroxidation and hepatotoxicity in mice by a dual mechanism: by decreasing the metabolic activation of CCl₄ by cytochrome P450 into free radicals as well as by scavenging free radicals. A more recent study by Krajka-Kuźniak and others (2009) examined the effect of chokeberry juice alone or in combination with N-nitrosodiethylamine

(NDEA) on phase I and phase II enzymes and DNA damage in rat liver. The forced feeding with chokeberry juice alone decreased the activities of enzymatic markers of cytochrome P450, CYP1A1, and 1A2. NDEA treatment also decreased the activity of CYP2E1 but enhanced the activity of CYP2B. Pretreatment with chokeberry juice further reduced the activity of these enzymes. Modulation of P450 enzyme activities was accompanied by the changes in the relevant proteins levels indicating that juice augmented DNA damage. Further study by the same authors examined the hepatic and mammary gland carcinogen-metabolizing enzyme expression altered by the polycyclic aromatic hydrocarbon 7,12-dimethylbenz[a]anthracene (DMBA) in female rats (Szafer and others 2011). Pretreatment with chokeberry juice reduced the activity of CYP1A1 and increased that of CYP2B involved in metabolic activation/detoxication of DMBA in rat liver, as well as expression and activity of phase II enzymes but it had no effect on these parameters in the mammary gland and DMBA-induced DNA damage in rat blood cells. This indicates that metabolic alterations induced by chokeberry feeding are tissue-specific and depend on the class of carcinogen. Several studies have revealed that the polyphenols present in chokeberries modulate the phase I and phase II enzymes and are involved also in NDEA activation and detoxication (Krajka-Kuzniak and others 2004; Szafer and others 2008). Recently Shih and others (2007) demonstrated that anthocyanins exert their antioxidative effect through activation of phase II enzymes. The results obtained in rat liver Clone 9 cells showed that treatment of anthocyanins leads to positive effects on elevating the antioxidant capacity, including activated expression of glutathione-related enzymes (glutathione reductase, glutathione peroxidase, and glutathione S-transferase) and recruited GSH content. In addition, the activity of NAD(P)H:quinone oxidoreductase (NQO1) was also promoted under the treatment of anthocyanin. This functions as the defense system against programmed cell death induced by H₂O₂. All these data suggest that anthocyanins are potent chemopreventive phytochemicals and could stimulate the antioxidant system to resist oxidant-induced injury.

Hyperglycemia is considered one of the main factors in the development of hepatic clinical manifestations in patients with diabetes-related metabolic syndrome. Persistent and chronic hyperglycemia elicits an increase in oxidative stress, which in turn reduces the capacity of the antioxidant defense system and accelerates the progress of diabetic complications through diverse pathways (Brownlee 2001). Oxidative stress, due to the generation of excessive ROS, interrupts intracellular homeostasis in the redox status and sometimes induces cell damage and apoptosis or necrosis, thus potentially contributing to the devastating injury and dysfunction of liver tissue (Rains and Jain 2011). Therefore, oxidative stress and peroxidative liver damage seem to be a critical target in the prevention of diabetic liver disorders. In mammals the glutathione (GSH)-dependent antioxidant system plays a fundamental role in cellular defense against reactive oxidant species. The cytoprotective functions of GSH are due to its ability to directly react with reactive electrophiles (Thompson and others 2009). Failure to adequately replenish the depleted cellular GSH stores suddenly compromises cellular redox balance and cell viability. In the already mentioned study by Zhu and others (2012) it was demonstrated *in vivo* that the treatment of diabetic db/db mice with cyanidin-3-glucoside increased the GSH synthesis in the liver through PKA-CREB-dependent induction of Gclc expression. The effect was dose-dependent and largely increased the GSH/GSSG ratio, which is considered an indicator of oxidative defense/stress. The oxidative stress determined by lipid

peroxidation, neutrophil infiltration, and hepatic steatosis was attenuated in cyanidin-3-glucoside-treated db/db mice as well. All these results demonstrated that the anthocyanin cyanidin-3-glucoside has the effect of activating GSH synthesis through a novel antioxidant defense mechanism against excessive ROS production, contributing to the prevention of hyperglycemia-induced hepatic oxidative damage. The production of ROS may also stimulate proinflammatory cytokines, which leads to hepatocellular damage and cellular inflammation and, ultimately, the development of diabetic liver disorders. Consistently, cyanidin-3-glucoside is capable of inhibiting hyperglycemia-induced steatosis that occurs as a result of oxidative stress, supporting the fact that hepatic injury caused by hyperglycemia is antagonized by cyanidin-3-glucoside. Therefore, upregulation of the Gclc system by anthocyanin may be an important protective mechanism against excessive oxidative stress and the activation of stress-signaling pathways in the body. However, it remains to be determined whether the effects reported are specific to cyanidin-3-glucoside or can be extended to the other anthocyanins presented in chokeberry.

Hypotensive and lipid-lowering effect

The mechanism of the antioxidant effect of flavonoids related to cardioprotection is complex and goes far beyond radical scavenging, including both activity enhancement and protection of enzymes such as paroxynase, peroxide dismutase, glutathione reductase, as well as inhibition of inducible NO synthase, NADPH oxidase, and lipoxygenase (Law and others 1999).

Blood pressure lowering properties of aronia juice and extract were observed in several studies. Treatment of spontaneously hypertensive rats with the commercial aronia extract *Aronox* showed significantly lower systolic blood pressure compared with the control group (Park and Park 2011; Hellstrom and others 2010). The effect seemed to be short-term and was generally highest after 3 h from the intake. Juice and polyphenols indicated weak angiotensin-converting enzyme (ACE)-inhibitory activity measured *in vitro*. Authors of a later study hypothesized that chokeberry polyphenols enhance endothelial NO production with an ACE-independent mechanism, for example, by activation of endothelial nitric oxidase enzyme. Vasorelaxing properties of polyphenols appear to be endothelium-dependent and recent study by Appeldoorn and others (2009b) have strongly suggested that the prime mechanism involves enhanced activation and/or increased expression of endothelial NO synthase. The hypotensive effect was revealed also by Naruszewicz and others (2007) in a double-blind, placebo-controlled, parallel trial with 44 patients who had survived myocardial infarction and had received statin therapy for at least 6 mo. The same study indicated that chokeberry polyphenols reduced the severity of inflammation in patients after myocardial infarct and can be used clinically for the secondary prevention of ischemic heart disease. Compared to a placebo, aronia flavonoids significantly reduced serum 8-isoprostans and oxygenated forms of LDL (Ox-LDL). As a result of oxidative stress in the vascular wall OxLDL are formed and they are then taken up by macrophages using CD-36 and LOX-1 receptors, thus contributing to the formation of foam cells which are the basis for the development of early atherosclerotic lesions (Han and Nicholson 1998). Furthermore, lowering of the level of adhesion molecules VCAM, ICAM, and MCP-1 was also significant as compared to the placebo and was associated with an increase in adiponectin concentration. Additionally, a significant drop in the hsIL-6 and hsCRP level occurred in the group treated with chokeberry extract, which is probably related to a significant reduction in the level of oxidative

stress in patients treated with chokeberry polyphenols. Another consequence of the reduction in the oxidative stress levels caused by chokeberry flavonoids is a reduction in Ox-LDL in the blood. An immunohistochemical study performed by Kawa and others (2008a) revealed that quercetin-3-O-glucuronide, one of the main quercetin metabolites in the circulatory system, accumulates in macrophage-derived foam cells of human atherosclerotic lesions, but not in the normal aorta. In addition, mRNA expression of the class A scavenger receptor and CD36, which play key roles in the formation of foam cells, was suppressed by treatment with quercetin-3-O-glucuronide. Similarly, immunohistochemical studies have also shown that epicatechin-3-O-gallate is also specifically localized in macrophage-derived foam cells and similarly suppresses gene expression of CD36 (Kawa and others 2008b). These findings provide novel insights into the bioavailability of dietary flavonoids and their potential mechanism in the prevention of CVD. Determination of the actual bioavailability of polyphenol metabolites in tissues may be even more important than is knowledge of their plasma concentrations. Data are still very scarce, even from animal studies but polyphenols have been detected in a wide range of tissues in mice and rats, including the brain (Datla and others 2001; Abd El Mohsen and others 2002), endothelial cells (Youdim and others 2000), heart, kidney, spleen, pancreas, prostate, uterus, ovary, mammary gland, testes, bladder, bone, and skin (Chang and others 2000; Coldham and Sauer 2000; Kim and others 2000). It is still difficult to say whether some polyphenols accumulate in specific target organs; probably the endothelium is likely to be one of the primary sites of flavonoid action. Schramm and others (1999) showed that a rapid, energy-dependent transport system is active in aortic endothelial cells for the uptake of morin and it is possible that this system also transports other hydroxylated phenolic compounds.

The involvement of LDL cholesterol and triglycerides in the development and progression of atherosclerosis is one of the best-demonstrated cases in modern physiology. A positive correlation between LDL cholesterol or triglycerides and the risk of cardiovascular events has been observed in many large-scale population studies. The advantages of reducing these lipid level have been shown with many intervention trials, since hyperlipidemia, characterized by increased low-density lipoprotein (LDL) cholesterol and decreased high-density lipoprotein cholesterol, is one of the major risk factors for atherosclerosis and CVD. Lipid lowering by chokeberry products has been reported in several studies. Jurgonski and others (2008) investigated the changes in the gut, blood, and internal organs of a rat prooxidative model with prediabetes and hyperlipidemia after dietary supplementation with aronia extract. In order to mimic the metabolic syndrome in rats, the authors used a combination of a high-fructose diet, described as hypertriglyceridemic and prooxidative, supplemented with saturated fat, as well as an injection of a low dose of streptozotocin (STZ), known as a causative agent of oxidative stress and, partly, necrosis of the β islet cells. Dietary supplementation with chokeberry extract decreased the activity of maltase and sucrase as well as increased the activity of lactase in the mucosa of the small intestine. Its ingestion led also to the improvement of antioxidant status, especially the concentration of a lipid peroxidation indicator (TBARS) in organ tissues (liver, kidney, and lung), which was normalized. **Cholesterol-lowering and distinct hypoglycemic actions were also observed.** **The observed hypoglycemic action of the extract is in accordance with the hypoglycemic effect of chokeberry fruit juice seen in experiments on the STZ-induced diabetic rat model,** where authors discussed as possible mechanisms of action cell stimulation

of glucose uptake and glycogen synthesis, increase in insulin secretion, as well as protection of pancreatic β cells from STZ- and glucose-induced oxidative stress (Valcheva-Kuzmanova and others 2007a). Aronia juice significantly hindered an increase in plasma lipids (total cholesterol, LDL cholesterol, and triglycerides) in rats fed a cholesterol-containing diet (Valcheva-Kuzmanova and others 2007b,c). In a human intervention study with men diagnosed with mild hypercholesterolemia, regular chokeberry juice drinking resulted in reductions of total cholesterol, LDL cholesterol, and triglycerides (Skoczynska 2007). Moderate, but significant decreases in serum glucose, homocysteine, and fibrinogen concentrations were also observed. These beneficial metabolic changes were associated with a significant hypotensive effect, as the result of chokeberry juice drinking. Although the chokeberry juice administration did not change the total HDL cholesterol level, it increased the HDL subfraction of cholesterol, which is involved in reverse cholesterol transport, in postprandial lipid metabolism mediated by lipoprotein lipase, and takes part in coagulation as a cofactor of proteins C and S. The beneficial increase in HDL 3 cholesterol in the serum of men treated with chokeberry juice could be connected with a reduction of cholesterol content in the arterial wall as well as increased antithrombotic activity. Metabolic changes observed in men with lipid disturbances were associated with reduction in systolic and diastolic blood pressure as well. The lipid-lowering effect of aronia extract was found also in another very recent human intervention study performed by Sikora and others (2012). Although the exact mechanism is unclear, several potential mechanisms may account for the lipid-lowering effects of chokeberries. Such mechanisms may be the inhibition of cholesterol absorption as demonstrated by tea catechins, improved catabolism of lipoproteins as demonstrated for cyanidin, and increase in bile flow, biliary bile cholesterol, and biliary bile acids as demonstrated for grapefruit juice and naringin. Other mechanisms accounting for the cholesterol-lowering effects of flavonoids are inhibition of the enzyme 3-hydroxy-3-methylglutaryl-CoA reductase, leading to a reduction in cholesterol synthesis, and inhibition of the enzyme acyl-CoA: cholesterol acyltransferase, leading to a decrease in cholesterol esterification and a subsequent decrease in its absorption and inclusion into lipoproteins. These enzyme-inhibiting activities have been demonstrated for naringin, hesperetin, quercetin, and hesperidin. Lipid-lowering properties of aronia could be referred to as preventive towards hyperlipidemia-induced oxidative stress diseases.

Anticoagulant properties

Sikora and others (2012) observed significant inhibition of platelet aggregation after 1 mo of aronia extract administration by men. Supplementation with aronia extract did not influence the general number of platelets in the blood of the examined patients but led to lengthening of the time required to reach the aggregation maximum. In the case of coagulation induced by endogenous thrombin, a significant decrease in the overall potential for coagulation was induced after 1 or 2 mo of supplementation. Moreover, after 1 mo a beneficial reduction in the overall potential for clot formation and fibrinolysis was observed. It is evident that the anticoagulant activity of aronia extracts could not be attributed to its radical-scavenging effect but may be due to the inhibition of some enzymes, which is part of the third line of antioxidant defense.

Other evidence for aronia antioxidant activity *in vivo*

Pilaczynska-Szczesniak and others (2005) found that supplementation of aronia juice suppressed the oxidative stress resulting

from elevated physical activity. This was revealed in an intervention study in which of 150 mL of chokeberry juice, containing 23 mg/100 mL of anthocyanins, was given daily to rowers performing physical exercise for 1 mo. Before and after the supplementation period, the subjects performed an incremental rowing exercise test. Blood samples were taken from the antecubital vein before each exercise test, 1 min after the test, and following a 24-h recovery period. After the supplementation period, TBARS concentrations in the samples, collected 1 min after the exercise test and following a 24-h recovery period, were significantly lower in the subjects receiving chokeberry juice than in the control group. In the supplemented group, glutathione peroxidase activity was lower in the samples collected 1 min after the exercise and SOD activity was lower in the samples taken following a 24-h recovery, as compared to the subjects receiving a placebo. These findings indicate that an increased intake of aronia polyphenols limits the exercise-induced oxidative damage to red blood cells, most probably by enhancing the endogenous antioxidant defense system.

As demonstrated by Ohgami and others (2005), chokeberry extract exhibited a strong antiinflammatory effect on endotoxin-induced uveitis in rats. The authors observed that the number of inflammatory cells, the protein concentrations, and the levels of NO, PGE₂, and TNF- α in the aqueous humor in the groups treated with extract were significantly decreased in a dose-dependent manner. The antiinflammatory effect of 100 mg extract was as strong as that of 10 mg prednisolone. The tested extract also suppressed LPS-induced iNOS and COX-2 protein expressions in RAW 264.7 cells *in vitro* and dose-dependently. The results suggest that aronia extract reveals an antiinflammatory effect that is due to the direct blocking of the expression of the iNOS and COX-2 enzymes and leads to the suppression of the production of NO, PGE₂, and TNF- α . It was found by Pawlowicz and others (2001) that anthocyanins from chokeberry increased fructose levels in the seminal fluid of men with oligospermia and increased levels of antibodies anti-O-LDL, which is characteristic with an increased production of free radicals and enhanced oxidative stress.

Final remarks and future outlook

From this review it is evident that *Aronia melanocarpa* is an extremely rich source of polyphenols rendering it one of the highest for *in vitro* antioxidant activities among fruits. Ironically, the polyphenols which are present in the highest amounts in chokeberries (anthocyanins and proanthocyanidins) are the least well absorbed. The intake of monomeric flavonols and flavanols with chokeberries is very low and it is not very likely that they have significant influence on plasma concentrations of polyphenols. Chlorogenic and neochlorogenic acids are found in significant concentrations in chokeberries, but esterification decreases their intestinal absorption. Nevertheless, there is appreciable experimental evidence of the effectiveness of *Aronia* products in a broad range of pathological conditions mediated by uncontrolled oxidative processes. The first site of the antioxidant action of chokeberry polyphenols is the gastrointestinal tract where they (mainly proanthocyanidins and their metabolites released by the gut microflora) could act as radical scavengers. The mechanisms of the *in vivo* antioxidant activity of aronia polyphenols after absorption spreads out far beyond radical scavenging and includes suppressing the formation of ROS and RNS, inhibition of prooxidant, and restoration of antioxidant enzymes, and probably also cellular signaling to regulate the level of antioxidant compounds and enzymes. However, more research is needed to fully understand the exact mechanisms of chokeberry antioxidant activity *in vivo*.

Some potential investigations to reach this goal can be summarized as follows: investigation of plasma antioxidant activity after supplementation with aronia products; setting up detailed bioavailability studies beyond aronia anthocyanins; performance of detailed studies to understand the interaction between aronia polyphenols and microflora of the colon; use of more oxidative stress/oxidative defense biomarkers in animal and human studies; investigation of tissue accumulation with ^{14}C -labeled anthocyanins and other aronia polyphenols in animals; and investigation of the antioxidant activity of chokeberry polyphenols metabolites *in vitro* and *in vivo* and evaluation of their health benefits.

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