

Nutraceutical and antioxidant effects of a delphinidin-rich maqui berry extract Delphinol®: a review

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Anthocyanins represent water-soluble flavonoid species, commonly found in higher plants, the richest plant source representing berries. While all anthocyanins present with antioxidant activity, the delphinidins represent the most potent antioxidant anthocyanin species owed to largest number of hydroxyl groups in the B-ring. The richest known natural source of delphinidins is the maqui berry (*Aristotelia chilensis*) from which an extract Delphinol®, standardized to 25% delphinidin, is commercially available. Delphinol® significantly reduces oxidative stress (oxidized LDL and F2-isoprostane) and blood glucose in controlled clinical trials. In human umbilical vein endothelium delphinidins concentration-dependently decrease intracellular oxygen radicals. Furthermore, delphinidins increase endothelial nitric oxide synthase expression and decreases expression of vaso-constrictory endothelin-1. Delphinidins inhibit the expression of cell adhesion molecules ICAM and VCAM, thus counteracting vascular inflammatory situations. Furthermore, delphinidins decrease platelet activity and may contribute to thrombosis prevention. Research on delphinidins showed improved endothelial function with elevated endothelial NO generation, lowered platelet aggregability and anti-inflammatory vascular effects. Delphinidins dose-dependently inhibit NF-κB-, activator protein-1- as well as COX-2 expression in UV-exposed epidermis. Delphinidins are found to be internalized into keratinocytes and pre-clinical investigations show significant UV-photo-protective

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effects with topical application of 40 nM delphinidin, both when applied prior to UV exposure as well as after exposure. Delphinidins may counteract skin-aging due to inhibition of UV-induced expression of matrix metalloproteinase in fibroblasts. In a rodent osteoporosis model delphinidin was found to inhibit differentiation of osteoclasts, resulting in an inhibited bone demineralization, while other anthocyanins were ineffective. Future research on Delphinol® and delphinidins may be expected to identify further health benefits.

KEY WORDS: Anthocyanins - Dietary supplements - Antioxidants.

Regular consumption of polyphenols has repeatedly been suggested to lower the risk for developing chronic health problems later during life. A large number of epidemiologic studies suggest protective effects of fruits and vegetables against cardiovascular, inflammatory and other chronic diseases.^{1, 2} Recently, it was shown from analyses of the Nurses' Health Study that higher intake of anthocyanins, flavones and flavonols at midlife, significantly increases the likelihood of health and well-being at older age.³ Evaluations of the Nurses' Health Study

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(NHS) II with 93,600 women suggested a significant hypertension improvement and reduction of myocardial infarction related to regular consumption of berry anthocyanins, derived from food-frequency questionnaires, while no other flavonoid species significantly decreased the risk.^{4, 5}

More recently, two epidemiologic investigations found a positive association between total flavonoid, including anthocyanins, intake and bone mineral density in women, thus suggesting a lowered risk for women to develop osteoporosis.⁶

Though various hypotheses have been initially proposed to explain the health contributions from fruits and vegetables, plant polyphenols as natural antioxidants are now understood to be the main contributors. All plant phenols are derived from phenylalanine, or the close precursor shikimic acid. As common denominator, all flavonoids represent with a C15 structure with two benzene rings A and B, connected by a three-carbon chain. The chain is closed in most flavonoids, forming the heterocyclic C ring.⁷ Depending on the connection of the B-ring to the C-ring, flavonoids are classified into different subclasses. Furthermore, methylation, glycosylation, acylation, prenylation, sulfonation and hydroxylation generate the different subclasses: flavanols, flavanones, flavones, isoflavones, flavonols, dihydroflavonols and anthocyanidins. The latter species presents with an oxygen cation, making it well water-soluble.

Anthocyanidins occur naturally in plants in the form of sugar derivatives, which are then referred to as anthocyanins. An an-

thocyanin which has been deprived of the sugars is generally referred to as the aglycone, then again resembling an anthocyanidin. In older literature, especially on bilberry flavonoids, the term anthocyanoside was used synonymously for anthocyanins. There are six principal types of anthocyanidins: delphinidin, cyanidin, pelargonidin, peonidin, petunidin and malvidin (Figure 1). Anthocyanins strongly absorb light in the visible spectrum and lend prominent colors to most flowers, and the same holds true for berries. The hue of anthocyanins is related to the B-ring substitution pattern and color saturation intensifies with increasing numbers of hydroxyl groups, while it decreases with addition of methoxyl groups.⁸ The pH value greatly affects the color, with highly acidic conditions converting the anthocyanidin structure into the stable flavylium-cation, which presents with a bright red color. At neutral to alkaline conditions, anthocyanidins and anthocyanins are converted to unstable, colorless pseudo-bases.

The radical scavenging activity of anthocyanins in cellular systems was described to correlate with the total number of hydroxyl groups present in the molecule, with the presence in B-ring 3',4'-ortho-dihydroxyl and 3-hydroxyl, representing the main structural requirements for anthocyanins in suppressing oxidative stress.⁹ These prerequisites render delphinidin the most interesting anthocyanidin candidate for radical scavenging. Radical scavenging activity comparisons of anthocyanins and their aglycons have been carried out *in vitro*, testing the scavenging activity of

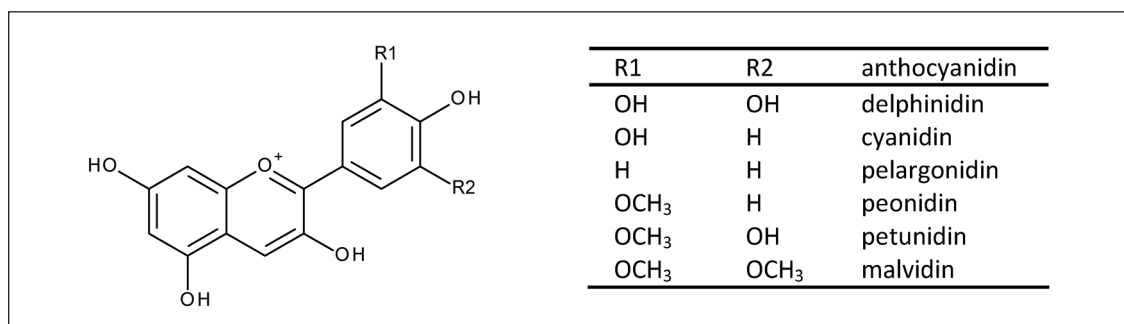


Figure 1.—The chemical structures of anthocyanidin species in edible plants [From Watson RR *et al.*].¹

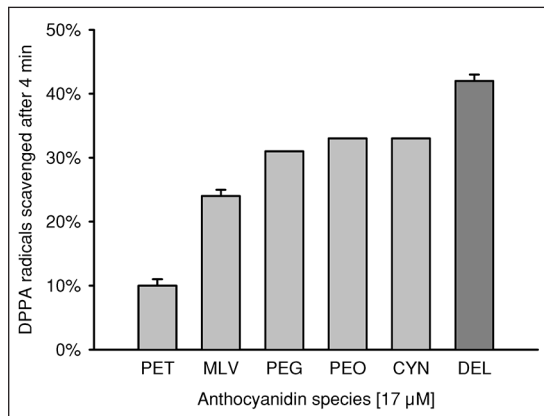


Figure 2.—Antioxidant activities of six anthocyanidin species petunidin (PET), malvidin (MLV), pelargonidin (PEG), petunidin (PEO), cyanidin (CYN) and delphinidin (DEL). Expressed in percentage of DPPH radicals scavenged after four minutes of reaction time, with anthocyanidins at concentration of 17 µM [Adapted from Kähkönen MP *et al.*].¹⁰

2,2-diphenyl-1-picrylhydrazyl (DPPH) radical.¹⁰ Among the aglycons delphinidin was shown to exhibit the highest DPPH neutralizing potency, followed by cyanidin and peonidin (Figure 2). The mono-glucosides of delphinidin and cyanidin were practically as active as the aglycons under the experimental conditions, whereas other anthocyanins showed decreased radical scavenging activity as compared to the corresponding aglycons. In general glycosylation decreases antioxidant activity and the nature of the carbohydrate has an impact, which was also found for delphinidin-rutinoside. The comparative radical scavenging activity at same concentration showed that delphinidin is more potent than ascorbic acid, catechin and quercetin.

Maqui berry (*Aristotelia chilensis*) as delphinidin-rich source for standardized extract Delphinol®

Delphinidin anthocyanins are present in many plants, though dietary meaningful quantities are predominantly found in berries. Owing to the strong light absorption in the visible wavelength range, delphinidin-rich berries typically present with bright hue and at higher abundance with increas-

ingly darker color. Typical examples of berries bearing delphinidins are blackcurrant, blueberries bilberries and maqui berries.⁸ The richest currently known natural source of delphinidins is the maqui berry (*Aristotelia chilensis*), indigenous to Chile.¹¹ The juice of maqui berries was found to be at least tri-fold richer in total polyphenols than in all other berries tested: red grapes, cranberries, strawberries, raspberries, blueberries, and blackberries. Correspondingly, the radical absorbance capacity (TRAP) of maqui berry juice was about three-times higher than corresponding values for other berries tested. Ripe maqui berries present with a deep black purple color. Maqui berries are commercially extracted to yield a polyphenolic extract, standardized to bear minimum 25% delphinidins, with other constituents representing further anthocyanins, phenolic acids, flavonols (quercetin, rutin, myrecitin and flavanols (catechins and epicatechins)).¹² The standardized aqueous maqui berry extract Delphinol® is distributed world-wide by the company Maqui New Life (MNL, trade mark owner), based in Santiago, Chile and Cham, Switzerland. Delphinol® has been investigated by Brunswick Laboratories (Southborough, Massachusetts, USA) to exhibit a total ORAC 5.0 antioxidant value of 30852 µM trolox equivalent per gram.¹³ Delphinol® was found to effectively neutralize peroxy-, hydroxyl-, superoxide anion- and peroxynitrite-radicals, as well as singlet oxygen. Owing to the nature of maqui berries and the extraction process employed, Delphinol® represents the richest natural dietary source of delphinidins.

Absorption, tissue distribution, metabolism and elimination of dietary delphinidin anthocyanins

The fate of anthocyanins in general, after oral administration, has been described to follow a unique pattern, rather different from those of other flavonoid species. Only recently a study demonstrated that anthocyanins may be more bioavailable than previously thought.¹⁴ The administration of 500

mg ^{13}C -isotope labeled cyanidin-3-glucoside to eight healthy volunteers allowed for tracing the absorption, metabolism and excretion, by examination of ^{13}C in blood, breath, urine and feces. Metabolites were identifiable up to 48 hours post intake, while the relative bioavailability was estimated to $12.4 \pm 1.4\%$.

The absorption of anthocyanins may occur in the stomach as well as the intestine.¹⁵ The rapid bioavailability observed for orally consumed anthocyanins led to the conclusion that a gastric transport system such as bilitranslocase, an organic anion carrier, may be involved in the absorption of intact glycosylated anthocyanidins.¹⁶ In plasma, anthocyanins can be found in their intact form.¹⁷ The aglycons exist in plasma, though with short half-life, as they are unstable and prone to degradation. It has been proposed that other active transporters, further to bilitranslocase, may be involved in the absorption of intact anthocyanins from the stomach as well as the small intestines. Investigations of rat jejunum mounted in Ussing chambers showed that the delphinidin aglycon interacts with sodium glucose co-transporter SGLT-1, causing inhibition of glucose transport. In human volunteers, supplemented with delphinidin-rich maqui berry extract Delphinol®, standardized to $\geq 25\%$ delphinidin anthocyanins, a significant inhibition of post-prandial blood glucose increase was observed after carbohydrate challenge.¹⁸ Interestingly, another group found that delphinidin anthocyanins enter human umbilical vein endothelium via the SGLT-1 transporter. While anthocyanin concentrations in blood may appear to be low, more research is required to investigate the tissue distribution of delphinidin anthocyanins. Anthocyanins present with varying binding affinities to plasma proteins with albumin accounting for most of the binding capacity in human blood. The affinity of anthocyanins to human albumin has been found to correlate with the number of hydroxyl groups, with delphinidin anthocyanins presenting with greatest affinity to human serum albumin.¹⁹ The glycosylation pattern of delphinidin may

define predominant target tissue destinations. The investigation of affinities of different delphinidin anthocyanins showed that delphinidin 3,5-O-diglucoside internalizes into acinar (lachrymal gland) cells by an order of magnitude greater quantities than delphinidin-3-glucoside or delphinidin-3-sambubioside.²⁰ Because delphinidin-3-glucoside internalizes into endothelium it may be taken for granted that this delphinidin anthocyanin species may cross the blood-brain-barrier. Several studies have confirmed the presence of anthocyanins in various brain regions of pigs and rats, such as the cortex, cerebellum, hippocampus and the striatum.^{21, 22, 23} The circumstance that intact delphinidin glycosides remain in the body is supported by the appearance of delphinidin-3-sambubioside in urine of healthy volunteers, who consumed a single portion of an aqueous *Hibiscus sabdariffa* extract.²⁴ Further to intact delphinidin anthocyanins, also metabolites, such as delphinidin monoglucuronide, were identified in urine in the range of few micrograms.

Antioxidant and photo-protective effects of delphinidins in dermatology

Human skin, the epidermis and dermis as barrier between external environment and body interior, is involved in a multitude of important tasks, such as managing mechanical and physicochemical insults as well as infections. Exposure of the skin to sunlight causes photo-oxidative reactions and affects the integrity of skin cells, keratinocytes and fibroblasts. UVA radiation represents approximately 95% of the total UV exposure, it can penetrate the skin deeper and induce reactive oxygen species which affect enzymes and may cause energy deficiencies, affecting mitochondria. Though UVA does not interact with the DNA, the pro-oxidant potency makes it an important source of oxidative stress in the dermis and plays a major role in photoaging.²⁵ UVB, in contrast, predominantly affects the skin surface, being absorbed by keratinocytes which leads to immune suppression, hy-

perpigmentation, hyperplasia, photo-aging, sunburn, and may cause mutations and malignancies. While UV radiation increases destruction of extracellular matrix proteins resulting from direct interaction with reactive oxygen species as well as up-regulated metalloproteinase expressions, fibroblasts are rendered less efficient for new collagen and elastin synthesis, causing skin elasticity loss.²⁶ Photodamage also results in accumulation of abnormal elastin in the dermis and several MMPs are involved in the process.²⁷ Exposure to UV radiation depletes antioxidants ascorbic acid, carotenoids, glutathione, coenzyme Q10, superoxide dismutase and catalase in all layers of UV-exposed skin.^{28, 29} Correspondingly, several studies have confirmed that UV-radiation induced oxidative processes in human skin which are preventable by antioxidants and especially combinations of antioxidants with phytochemicals.³⁰ Polyphenols have been extensively researched for photo-protective effects, green tea epigallocatechin gallate probably representing the most prominent species.³¹ The low molecular size of delphinidin, the high bioavailability and the expected good skin tissue distribution makes this anthocyanidin a promising candidate for skin-ageing protection. The currently available research on delphinidin points to significant photo-protective, antioxidant, anti-inflammatory and anti-aging virtues.

In murine epidermal cells the presence of delphinidin dose-dependently inhibited the expression of inflammatory cyclooxygenase 2 (COX-2) in response to UVB exposure, significantly from a dosage as low as 5 μM delphinidin, corresponding to 1.7 mg/L.³² UVB exposure activated pro-inflammatory nuclear factor- κB (NF- κB) and activator protein-1 in epidermal cells, however in presence of delphinidin this activation pathway was abolished. Mouse skin treated topically with delphinidin at concentrations of 0, 40 and 200 nM delphinidin in acetone, corresponding to 0, 13.6 and 68 $\mu\text{g/L}$ respectively, was subsequently exposed to 0.5 kJ/m² UVB radiation. The topical pre-treatment of mouse skin with delphinidin significantly inhibited the UV-induced inflammatory re-

sponse at both delphinidin concentrations, though radiation-induced COX-2 expression in murine skin was maximally inhibited already at lowest concentration of 40 nM topical delphinidin. Correspondingly, also prostaglandin E2 production was significantly inhibited with delphinidin. This anthocyanidin was found to directly bind to kinases MAPKK4 and PI-3K and the UV-induced activities of MAPKK4 or PI-3K in mouse skin were significantly suppressed by delphinidin pre-treatment.

Investigations of primary cultured human fibroblasts, exposed to UVB radiation, identified potent protection from reactive oxygen- and nitric oxide species in presence of delphinidin.³³ UVB irradiation (0.02 J/cm²) of human fibroblasts expressed matrix metalloproteinase type 1, which was totally abolished at both delphinidin concentrations tested, 10 and 20 μM . Treatment of human fibroblasts with delphinidin was discovered to significantly inhibit UVB-induced MAPKK and MAPK phosphorylation. With the potent inhibition of UV-induced expression of matrix metalloproteinase Delphinol® may counteract break-down of dermal collagen, which represents the pathophysiology of photo-ageing and visible skin ageing manifesting in loss of elasticity and wrinkling.

Another group has extensively investigated photo-protective effects of delphinidin on human keratinocyte skin cells and mouse skin.³⁴ Human HaCaT keratinocytes were demonstrated to internalize delphinidin with resulting dose-dependently increased intracellular antioxidant capacity (Figure 3).

Keratinocyte cell line HaCaT was found to be delphinidin dose-dependently in range 1-20 μM , protected from UVB-induced inhibition of cell growth. Delphinidin at concentration of 3.4 mg/L was found to provide substantial protection from UVB-mediated cell viability decrease and morphological changes. Delphinidin was found to inhibit keratinocyte apoptosis. While unprotected keratinocytes exposed to UVB radiation at dosages 15 and 30m J/cm² resulted in 11.9% and 25.7% of apoptotic cells, in presence

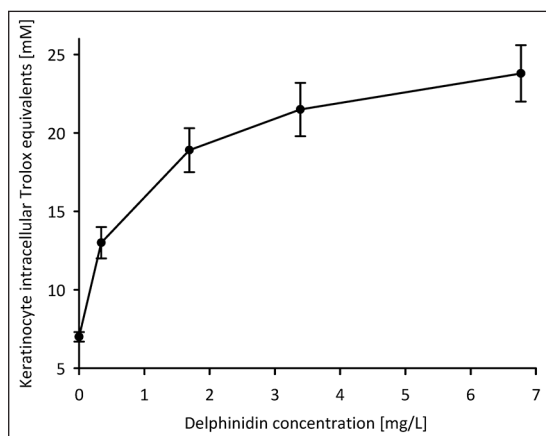


Figure 3.—Human keratinocytes (HaCaT) internalize delphinidins, which increases intracellular antioxidant capacity. Keratinocytes were exposed to different delphinidin concentrations for 24 hours and following media removal and washing, intracellular antioxidant capacity was estimated by ABTS radical de-colorization assay. [Adapted from Afaq F *et al.*].³⁴

of delphinidin (3.4 mg/L) the same radiation dosages resulted in only 4.5% and 7.7% apoptotic cells, respectively. HaCaT keratinocytes were shown to be delphinidin dose-dependently (1–20 μ M) protected from UVB (15–30 mJ/cm²) irradiation, inhibiting UVB-mediated development of lipid peroxidation, formation of 8-OHdG as sign of oxidative DNA-damage and apoptosis. The apoptosis protective effects were suggested to result from the potential to limit adverse effects of UV-radiation via modulation of Bcl-2 family members and inhibition of caspase activation. *In vivo* investigation with hairless (SKH1) mice showed that topical application of delphinidin (1 mg/ 100 μ L DMSO) inhibited UVB-mediated apoptosis (TUNEL staining) and DNA-damage markers pyrimidine dimers and 8-OHdG.

The concept of skin photo-protection with polyphenols requires sufficient amounts of active compounds delivered to the dermis and epidermis. In topical application delphinidin was found to be a very potent photo-protector. Moreover, the study by Fariq and co-workers suggests that delphinidin is incorporated and accumulated into human keratinocytes.³⁴ Pure delphinidin and enriched natural sources such as Delphinol® are expected to provide consider-

able photo-protection and anti-ageing skin benefits. It has been repeatedly reported that structurally different antioxidants show beneficial interactions for providing greater protection from oxidative stress.³⁵

Delphinidin activities in cardiovascular health

The endothelium represents a monolayer of cells constituting the inner surface of all blood vessels and also lymph vessels. The endothelium controls flow of nutrients and fluids and also senses physical and biochemical alterations occurring in the blood stream. The endothelium modulates coagulation and facilitates cellular trafficking such as in leukocyte transmigration during infection or injury. Endothelial cells orchestrate vaso-constriction, such as by endothelin-1 or, conversely vaso-dilation by nitric oxide. Endothelial dysfunction represents an inseparable situation with cardiovascular health issues. Endothelial dysfunction is inherently involved in pro-coagulative, pro-inflammatory and vaso-constrictive situations.

Epidemiological studies have pointed to an inverse association between dietary anthocyanin consumption and reduced occurrence of cardiovascular diseases. It has been proposed that specific anthocyanins exhibit anti-atherosclerotic effects by quenching oxidative stress, which is causal to endothelial dysfunction. Delphinidin anthocyanins such as delphinidin-3-glucoside were proposed to inhibit endothelial oxidative injury.³⁶

Delphinidin glucoside internalizes into endothelium via SGLT1 transporter; quenches oxidative stress and improves endothelial function

Preclinical experiments showed that delphinidin-3-glucoside is internalized into primary human umbilical vein endothelial cells (HUVEC) via the sodium-dependent glucose co-transporter (SGLT) type 1.³⁷ An increase of glucose competed with delphinidin, with the latter being glucose-dependently less

actively internalized into endothelium. The SGLT-1 inhibitor phlorizin abolished the internalization of delphinidin-3-glucoside by approximately 96%. SGLT was “knocked-out” by transfection of HUVECs with small interfering RNA (siRNA), which resulted in significant reduction of delphinidin uptake into human endothelial cells. *In vivo* delphinidin intravenously administered to mice showed sustained retention in blood vessels as compared to blood. The internalized delphinidin-3-glucoside was found to attenuate oxidized LDL-induced mitochondrial dysfunction, decreasing superoxide anion generation and reactive oxygen species.

Interestingly, delphinidin anthocyanins were described to interact with SGLT in rat small intestine, which has been suggested to be the responsible mechanism of action related to delayed dietary glucose absorption identified in healthy human volunteers.³⁸

The effects of delphinidin on expression of vasoconstrictor endothelin-1 and vasodilator nitric oxide synthase (eNOS) were shown in human umbilical vein endothelium.³⁹ Delphinidin was found to significantly and dose-dependently decrease secretion of endothelin-1 from endothelial cells. At lowest employed concentration of 50 μM delphinidin, endothelin-1 was reduced significantly by 58%, whereas cyanidin at same concentration showed negligible and insignificant effects. Further to decreased endothelin secretion with delphinidin, the study found significantly decreased pre-endothelin mRNA expression.

Endothelial nitric oxide synthase quantification in human endothelial cells (HUVEC) showed that 50 μM delphinidin significantly increased eNOS expression by 58% versus untreated control, whereas cyanidin at same concentration had minor effects. Higher concentrations of both anthocyanidins resulted in elevated expression of eNOS, with an increase of 78% over untreated control in presence of 100 μM delphinidin. These findings point to a potential for delphinidins to reduce vascular tension with corresponding benefits for improving systolic and diastolic blood pressure.

Another research group described a

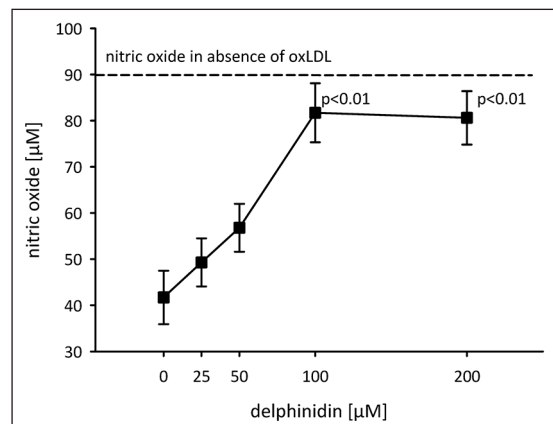


Figure 4.—HUVEC endothelial cells, exposed to oxidized LDL, release less nitric oxide into cell culture medium, dropping from 90 μM to 41 μM . Presence of delphinidin increases nitric oxide in dose-dependent fashion, reaching the greatest effect at 100 μM with no further increase at high concentrations [Adapted from Chen CY *et al.*].⁴⁰

significant delphinidin dose-dependent increase of nitric oxide release from human endothelial cells (HUVEC) exposed to 100 $\mu\text{g}/\text{mL}$ oxidized LDL.⁴⁰ In presence of oxidized LDL, nitric oxide release from endothelial cells decreased from 90 μM to 42 μM (Figure 4). The affected nitric oxide release from endothelial cells, as result of oxidized LDL exposure, was shown to be delphinidin-concentration dependently restored. In presence of 100 μM delphinidin, endothelial cells released nitric oxide into surrounding media at a concentration of 80 μM , which did no further increase at greater delphinidin concentrations.

Delphinidin was shown to concentration-dependently lower intra-cellular reactive oxygen species in HUVEC. Endothelial cells stained with dichlorofluorescein diacetate showed delphinidin dose-dependent reduction of intracellular reactive oxygen species generation when cells were challenged with oxidized LDL.⁴⁰ Furthermore, delphinidin was found to counteract oxidized LDL-mediated apoptosis of endothelial cells. More recently, research from the same group suggests that delphinidin glycoside protects HUVEC against oxidized LDL-induced injury by induction of autophagy.⁴¹ Oxidized LDL activates autophagy as a cellular self-protective measure against oxidized LDL-in-

duced injury and delphinidin glucoside amplifies this mechanism in human endothelial cells. Jin and co-workers discovered that delphinidin glucoside activates adenosine monophosphate-activated protein kinase (AMPK) and sirtuin-1. The sirtuin-1 is known to interact with several components orchestrating induction of autophagy. A comparative study on anthocyanin-species-related efficacy for protection of endothelium from oxidized-LDL triggered damage identified the molecular requirements to anthocyanins.⁹ The structure-activity relationship is described to correlate with the total number of hydroxyl-groups in the B-ring (maximally 3',4'-ortho-dihydroxyl) and the C-ring (maximal 3), with maximal number of hydroxyl groups in anthocyanidins presented in delphinidin.

Delphinidin was demonstrated to potentially inhibit vascular growth factor (VEGF) release from vascular smooth muscle.⁴² VEGF is strongly expressed in human atherosclerotic plaques and the number of VEGF-positive cells increases gradually with progression of atherosclerotic plaques. Whereas cyanidin also showed inhibitory effects on VEGF expression, malvidin, peonidin, catechin, epicatechin, resveratrol, quercetin and caffeic acid were found to be ineffective.

Delphinidin has been demonstrated to significantly and dose-dependently counteract oxidized-LDL triggered monocyte endothelium-adhesion and migration.⁴³ Exposure of endothelial cells to oxidized LDL caused rapidly rising intracellular oxidative stress and induced expression of adhesion molecules p-selectin and ICAM-1. Delphinidin at concentrations of 50 to 200 μ M significantly lowered endothelial intra-cellular oxidative stress and cell adhesion molecule (ICAM and P-selectin) expression and attenuated monocyte adhesion and migration. In presence of delphinidin the oxidized LDL-induced endothelial expression of p38MAPK protein expression, NF- κ B transcription activity and protein expression was inhibited. Because the adherence of monocytes to inflamed endothelium plays an important role in the pathophysiology of atherosclerosis, Chen *et al.* suggest a preventative role for delphinidin.

Delphinol® delphinidins reduce oxidative stress in humans

Delphinidins have been demonstrated to lower oxidized LDL plasma levels in humans.⁴⁴ Twenty seven women aged 51 ± 6 years with an average body mass index of 26.4 ± 2.7 kgm^{-2} , smoking in average 26.4 ± 2.7 cigarettes a day and 23 men aged 54 ± 9 years, BMI 26.9 ± 2.5 kgm^{-2} who regularly smoke cigarettes (15.3 ± 3.2 a day), were supplemented with 300 mg Delphinol® daily for four weeks in randomized, double-blind, placebo-controlled, cross-over fashion. Study participants were randomly assigned to one of two groups with 25 participants each.

Oxidative stress was estimated by measurement of plasma oxidized LDL (immuno-enzymatically), urine total F2a-isoprostanes (HPLC) and plasma phosphatidylcholine hydroperoxides (HPLC). The research out-

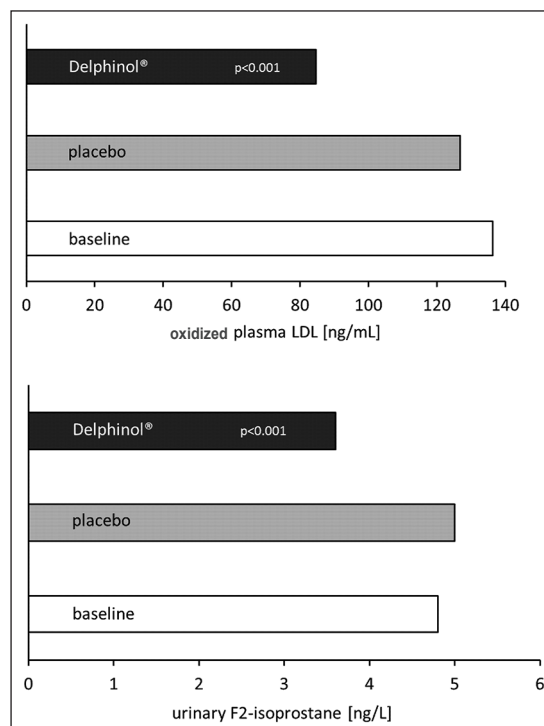


Figure 5.—Both, urinary F2-isoprostane and oxidized plasma LDL, as markers of oxidative stress, significantly decreased in response to four weeks supplementation with 300 mg Delphinol® as compared to four weeks with placebo, in double-blind, placebo-controlled, cross-over fashion. [Adapted from Scapagnini G *et al.*].^{44, 45}

come showed a statistical significantly decreased plasma level of oxidized LDL-cholesterol and F2-isoprotanes (Figure 5).⁴⁵ Urinary analyses suggest good bioavailability of Delphinol® anthocyanins, with significant increase of urinary polyphenol presence after four weeks of Delphinol® supplementation. The treatment was well tolerated with no side-effects reported. Routine safety analyses did not identify clinically significant alterations in biochemical and hematologic parameters.

Scapagnini *et al.* also found significantly lowered plasma PCOOH levels in their subjects supplemented with Delphinol®. The significantly decreased oxidized plasma LDL values, identified by Scapagnini *et al.* in humans, is in good confirmation with earlier described *in vitro* and *ex vivo* findings shown for delphinidin.¹⁰ *Ex vivo* experiments with human blood plasma investigated protective effects of Delphinol® for preventing unsaturated lipids from peroxidation, measuring thiobarbituric acid reactive substances (TBARS), which form as by-product of lipid peroxidation using thiobarbituric acid as a reagent.⁴⁶ Delphinol® dose-dependently lowered peroxidation of unsaturated lipids in human blood plasma, initiated by exposure to 2,2'-azobis-2-methyl-propanimidamide dihydrochloride (AAPH). The comparison of Delphinol® antioxidant effects in plasma with fat-soluble carotenoid-antioxidant astaxanthin (AstaReal®, Fuji Health Science, Burlington USA), suggests comparative antioxidant efficacy for prevention of human plasma lipid peroxidation at same concentration level of 10 mg/mL. Interestingly, the study identified synergistic antioxidant effects of both Delphinol® and AstaReal® at 1:1 ratio, for prevention of human plasma lipid peroxidation. Delphinol® did likewise show synergistic effects with vitamin E for protection of human plasma lipids from oxidation.⁴⁶

Delphinidins show potential to inhibit angiogenesis

The process of new blood vessel growth from existing capillaries is involved in both

physiological as well as pathological processes. Angiogenesis contributes to uncontrolled vessel growth in chronic inflammation, chronic wounds such as ulcers, retinopathy and other malignancies. *In vitro* investigations showed that low concentrations of delphinidin inhibit vascular endothelial growth factor (VEGF)-induced tyrosine phosphorylation of the VEGF receptor (VEGFR-2) in HUVEC endothelial cells, causing inhibition of downstream-signaling triggered by VEGFR-2.⁴⁷ The inhibition of VEGFR-2 by delphinidin then inhibits VEGF-induced activation of ERK-1/2 signaling with the consequence that human endothelial cells chemotactic motility as well as their differentiation into capillary-like tubular structures is inhibited. Lamy and co-workers found that all other anthocyanidin species did not display the angiogenic effects of delphinidin.⁴⁷

Delphinidin glycosides inhibit platelet activation and thrombosis

Platelets from human and murine blood were investigated for effects of delphinidin glucoside on aggregation and perfusion flow *ex vivo*.⁴⁸ Delphinidin-3-glucoside was shown to significantly and dose-dependently inhibit platelet aggregation of platelet-rich human blood plasma following stimulation with adenosine diphosphate (ADP), collagen or thrombin receptor activating peptide (TRAP). Delphinidin glucoside was described to markedly inhibit ADP-induced human platelet aggregation at a dosage as low as 0.5 µM, corresponding to 0.23 mg/L. Delphinidin-3-glucoside was demonstrated to inhibit thrombus growth and prolong the time period to vessel occlusion *in vivo*. Furthermore, a significantly delayed blood vessel occlusion time was identified in a carotid artery thrombosis model with 50 µM delphinidin-3-glucoside as compared to a control group.

Using gel-filtered human platelets, with other blood plasma molecules removed, delphinidin glucoside was found to directly inhibit platelet aggregation. Delphinidin at concentrations of 5 and 50 µM was further

shown to inhibit expression of adhesion molecule P-selectin in human thrombocytes, in response to ADP, collagen, TRAP, thrombin-stimulated P-selectin, CD63, CD40L. Delphinidin-3-glucoside was further described to suppress fibrinogen binding to platelets in both human platelet rich plasma and gel-filtered platelets.

The authors conclude that delphinidin glucoside can inhibit platelet activation independently from other components. The observation that delphinidin glucoside, applied at dosages of 0.5, 5 and 50 μ M intravenously to mice, was shown to not significantly affect bleeding time as compared to control with injected delphinidin-free buffer, suggests absence of side-effects. However, the mechanism by which delphinidin glucoside inhibits platelet activation remains unclear.

Delphinidins supports bone mineralization

Osteoporosis, the gradual loss of bone mass, occurs commonly in women following the menopause, while men are often affected at the age of 60 years and older. This skeletal disease is characterized by low bone mass and an impaired micro-architecture of bone tissue, as a result of which the bones are more prone to fractures than normal. Men at same age only have half the risk for suffering osteoporotic fractures than women. Studies have pointed to the circumstance that elevated oxidative stress represents an independent risk factor for osteoporosis.⁴⁹ Various flavonoids such as quercetin and hesperidin were found to have preventive potency for bone loss in ovariectomized- animal models.⁵⁰ An investigation of 3160 women, a cohort of female twins from the TwinsUK registry, elucidated the correlation between flavonoid consumption, distinguishing between subclasses (flavanones, anthocyanins, flavan-3-ols, oligomeric proanthocyanidins, flavonols and flavones), and women's bone density.⁵¹ Total flavonoid consumption was positively associated with bone mineral density, with

anthocyanins resulting in greater bone density of both the hip and spine.

Delphinidin was found to suppress the differentiation and function of osteoclasts, the cells resorbing bone. With the activity of osteoclast cells resorbing bone reaching equilibrium with osteoblast cells, synthesizing bone formation, osteoporotic processes were effectively limited. In a rodent osteoporosis model with ovariectomized mice the oral administration of delphinidin was shown to be a potent active ingredient to limit osteoporotic bone resorption as shown by computer tomography imaging.⁵² In cell culture experiments, anthocyanidin species were compared for inhibition of osteoclastogenic development from macrophage lineage, triggered by the NF-kB ligand (RANKL), which resulted in mild inhibition with cyanidin, insignificant effects with peonidin, yet dose-dependent and significant effects with delphinidin. Epicatechin was utilized as negative control because it does not possess osteoclastogenic activity. These investigations suggest a potential benefit from regular consumption of delphinidin-rich sources such as Delphinol® for preventing bone demineralization at higher age.

Conclusions

Both *ex-vivo* and clinical research suggest potent antioxidant virtues of delphinidin-rich Delphinol® maqui berry extract. The significant reduction of oxidized LDL and increased blood antioxidant capacity contributes to overall health improvement and especially supports cardiovascular health. Delphinol® will contribute to decrease the risk for developing atherosclerosis. The internalization of delphinidin glucoside into endothelium reduces intra-cellular oxidative stress, quenches pro-inflammatory stimuli, increases autophagy by up-regulated sirtuin-1 and restores activity of nitric oxide synthase, with consequences on enhanced flow-mediated vasodilation. Current research suggests considerable potential for improvement of endothelial function with Delphinol®, comprising blood pressure nor-

malization, micro-circulation enhancement, platelet activity normalization, thrombosis prevention and anti-inflammatory contributions. The internalization of delphinidin glycosides into endothelium in consequence will involve this anthocyanin passing the blood brain barrier, which suggests neuro-protective benefits for maintaining healthier cognitive function. The inhibition of sodium-glucose co-transporter inhibition in small intestine by Delphinol®, leads to significantly delayed glucose absorption from carbohydrate meals which improves metabolic profile and further supports cardiovascular health.

In addition, delphinidin has dermatological applications which emphasize the skin antioxidant and anti-aging capacity, as well as pronounced photo-protective contributions.

Finally, the discovery implying that delphinidins may present with particularly high efficacy for prevention of age-related bone demineralization warrants further research on this subject. In view of the rapidly increasing proportion of aging people in almost all parts of the developed world, the call for natural solutions to defy age-related ailments is mandated.

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Conflicts of interest.—Frank Schönlaui PhD is an employee of Maqui New Life AG, Switzerland.