

Fruits and vegetables in the prevention of cellular oxidative damage¹⁻⁵

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ABSTRACT Numerous studies have demonstrated in vitro effects of flavonoid components from fruits and vegetables on various measures of oxidative cellular damage. However, the questions that have not been answered satisfactorily deal with the absorption/metabolism of antioxidant components in fruits and vegetables and whether they are absorbed in sufficient quantities and in a form in which effects on in vivo measures of oxidative cellular damage could be observed. The focus of this review is on clinical studies that provide information about possible in vivo changes in antioxidant status with fruit and vegetable consumption. Two classes of flavonoids as antioxidants are considered in detail (anthocyanins and flavonols). Absorption of anthocyanins appears to be much less than that of the flavonol quercetin, perhaps as little as one tenth. Relatively high dietary levels of anthocyanins appear to be necessary to observe antioxidant effects in vivo. Metabolism of cyanidin 3-glucoside and quercetin by methylation or conjugation with glucuronide or sulfate will decrease antioxidant activity. However, quercetin metabolites seem to maintain at least part of their antioxidant activity in vivo. A potential role for food flavonoids and polyphenolics as antioxidants is discussed. *Am J Clin Nutr* 2003;78(suppl):570S-8S.

KEY WORDS Anthocyanin, quercetin, flavonols, flavonoids, antioxidant, cyanidin

INTRODUCTION

Reactive oxygen species from both endogenous and exogenous sources may be involved in the etiology of diverse human diseases, such as coronary artery disease, stroke, rheumatoid arthritis, and cancer. Diets rich in fruits and vegetables are associated with a reduced risk for these pathologies (1-3), and protection has often been attributed to antioxidant vitamins such as vitamin C, vitamin E, and β -carotene. Although fruits and vegetables are primary sources for these "nutrient" antioxidants, other dietary components may also be important protective agents. Flavonoids are plant polyphenolic compounds ubiquitous in fruits, vegetables, and herbs. Flavonoids are primarily categorized into flavonols, flavones, flavanols, flavanones, and anthocyanidins. The daily intake of flavonoids in Western countries has been estimated to be between 0.5 and 1.0 g (4) but likely is much lower than this. Flavonoids and other plant phenolics have been reported to have multiple biological effects, including antioxidant activity, antiinflammatory action, inhibition of platelet aggregation, antimicrobial activities, and antitumor activities (5).

This review will focus on in vivo studies that relate to antioxidant effects of these flavonoids. It is clear, however, that there are

numerous other cellular effects besides antioxidant effects that may be totally separate or act in concert with antioxidant effects. Other reviews (4-6) have dealt with other biological effects of flavonoids. This review will not attempt to cover all flavonoids but will focus on compounds from 2 flavonoid classes (anthocyanins and flavonols, particularly quercetin) that have relevance to diet and health.

ANTIOXIDANT CAPACITY OF FRUITS AND VEGETABLES

The hydrophilic antioxidant capacity of fruits and vegetables has been determined using the oxygen radical absorbance capacity assay (ORAC) (7-9). In general, the hydrophilic antioxidants account for more than 85% of the total antioxidants in fruits and vegetables, and antioxidant capacity of different fruits and vegetables may differ by a factor of 20-fold or more. This might suggest, from a standpoint of protecting against oxidative events in the body, that fruits or vegetables that have a higher antioxidant capacity should be more effective. However, as we will see, mechanisms of metabolism/absorption may affect this effectiveness.

In some fruits, anthocyanins make a major contribution to the total antioxidant capacity. Dietary intake of anthocyanins may exceed 200 mg/d in individuals consuming several servings of fruit, but the "usual" intake is likely much less. The flavonols, and in particular quercetin, are ubiquitous in fruits and vegetables and contribute to antioxidant capacity. However, in some fruits or vegetables, there may be more than 100 compounds that can be separated by HPLC that can contribute to the measured antioxidant capacity (10). Thus, by narrowing our focus to a few compounds in this review, we may not be considering the full potential of fruits and vegetables.

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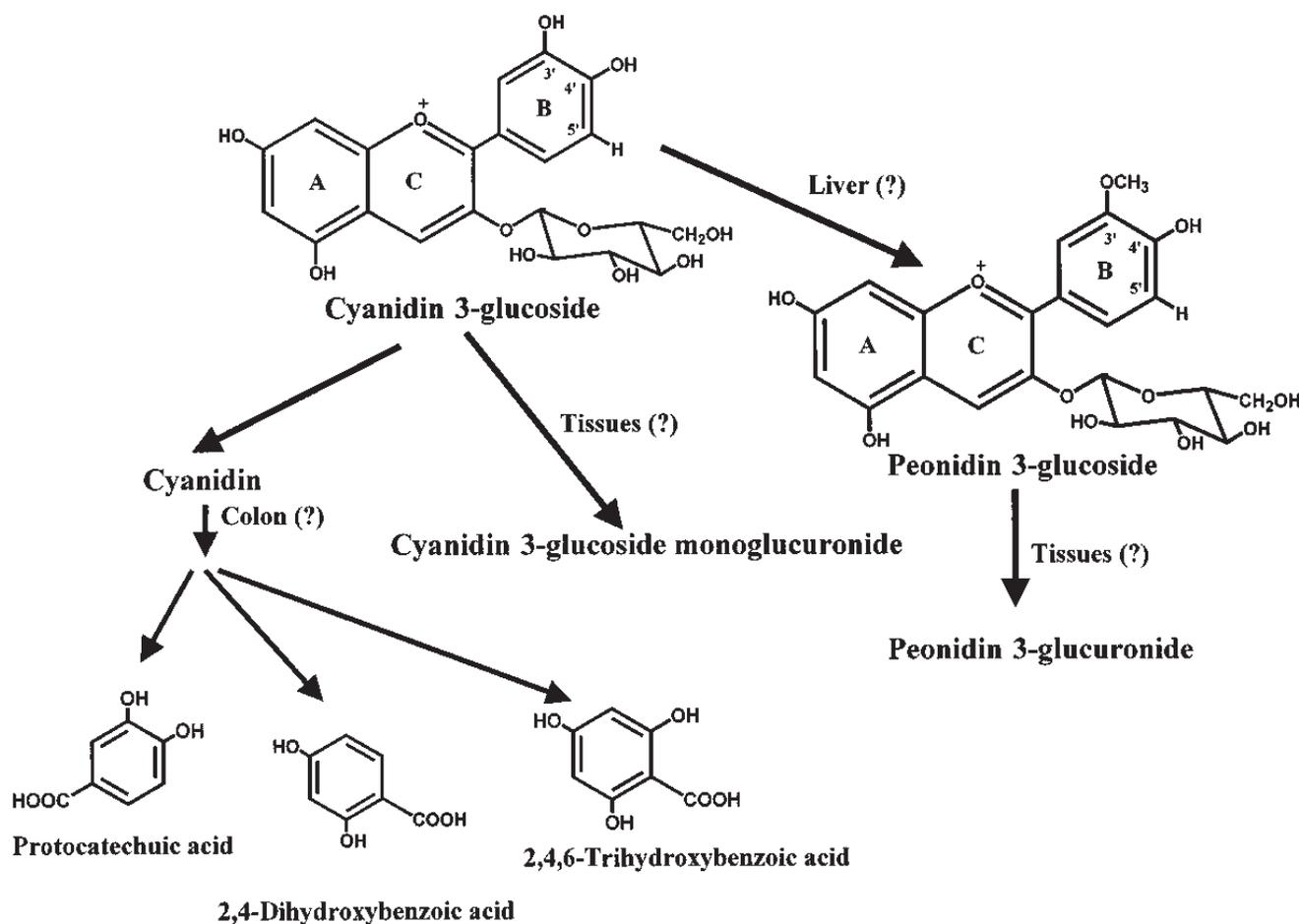


FIGURE 1. Proposed pathways of anthocyanin (cyanidin 3-glucoside) absorption/metabolism. Data from Cao et al (13), Bub et al (14), and Wu et al (15). Protocatechuic acid has been shown to be formed in rats (16). Other phenolic acids have been shown to be formed in vitro from anthocyanins at neutral pH (17). Formation of glucuronide conjugates in vivo in humans has been shown for the first time by Wu et al (15).

Anthocyanins as antioxidants

Anthocyanins are secondary plant metabolites responsible for the blue, purple, and red color of many plant tissues and occur primarily as glycosides of their respective anthocyanidin chromophores. The chemistry and distribution of anthocyanins have been reviewed (11). Like other flavonoids, anthocyanins and anthocyanidins (the aglycone form) have antioxidant properties (12). The phenolic structure of anthocyanins (**Figure 1**) conveys marked antioxidant activity in model systems via donation of electrons or transfer of hydrogen atoms from hydroxyl moieties to free radicals. The common anthocyanidin aglycones are cyanidin, delphinidin, malvidin, pelargonidin, peonidin, and petunidin. The differences in chemical structure occur at the 3', 4', and 5' positions (**Figure 1**). Cyanidin is the most common anthocyanidin, present in 90% of fruits (18). Anthocyanin levels [mg/100 g fresh weight (FW)] range from 0.25 in the pear to 500 in the blueberry (18), and the fruits that are richest in anthocyanins (> 20 mg/100 g FW) are very strongly colored (deep purple or black) berries. The cyanidin glycosides tend to have higher antioxidant capacity than peonidin or malvidin glycosides (12), likely because of the free hydroxyl groups on the 3' and 4' positions of cyanidin.

Anthocyanin absorption/metabolism

Anthocyanins can be absorbed intact as glycosides (**Figure 1**) (13, 14, 19–21). However, the proportion of anthocyanins absorbed and excreted in the urine as a percentage of the intake seems to be quite small (15), perhaps much less than 0.1% of intake. **Table 1** summarizes the known publications on anthocyanin absorption and excretion in humans. Maximum plasma levels of total anthocyanins were in the range of 1–120 nmol/L, with doses of 0.7–10.9 mg/kg in human studies (**Table 1**) (13, 20, 22). The clearance of anthocyanins from the circulation is sufficiently rapid that by 6 h, very little is generally detected in the plasma (13, 14).

In rats administered cyanidin 3-glucoside (C3G) orally (0.9 mmol/kg body wt), C3G rapidly appeared in the plasma, but the aglycone of C3G (cyanidin) was not detected, although it was present in the jejunum (24). Protocatechuic acid (PC), which may be produced by degradation of cyanidin, was present in the plasma at concentrations 8-fold higher than that of C3G in the rat but has not been detected in the plasma of humans following anthocyanin consumption (RL Prior, unpublished data, 2002). Although there are no data on the exact amount of anthocyanins that are absorbed, the plasma kinetic profile and the recovery of anthocyanins in the urine suggest

TABLE 1

Comparison of published anthocyanin data in plasma and urine of human subjects after consumption of food anthocyanins¹

Source of anthocyanins	Intake	Plasma max ²	Urine recovery	Reference
	mg/kg	nmol/L		
Elderberry and black currant (juice)	2.95	29 ± 10 ³	NA	Miyazawa et al (20)
Black currant concentrate	3.58	115	0.11%/8 h	Matsumoto et al (22)
Elderberry extract	10.9	97.4	0.055%/8 h	Cao et al (13)
			0.077%/14 h	Wu et al (15)
Blueberry (whole)	10.0	ND	0.004%/6 h	Wu et al (15)
Blueberry powder	15.0	42	NA ⁴	Mazza et al (16)
Red wine	0.90	1.4	0.024%/6 h	Bub et al (14)
Dealcoholized red wine	0.74	1.5	0.022%/6 h	Bub et al (14)
Red grape juice	1.50	2.8	0.023%/6 h	Bub et al (14)
Wine	218 (total)	NA	1.5–5.1%/12 h	Lapidot et al (23)

¹NA, data not available; ND, not detected.²Maximum concentration after ingestion. Baseline concentrations can be assumed to be zero, because no anthocyanins are detected in plasma before a meal.³ $\bar{x} \pm SD$.⁴Estimated that <0.003% of the dose was present in serum 3 h after ingestion (16).

that relatively small proportions are absorbed. The kinetic curves for plasma cyanidin glucosides from elderberry (13) compared with those for quercetin from onion (25) are presented in **Figure 2**. The data have been adjusted for the amounts consumed. What is remarkable is that the maximum concentration is an order of magnitude smaller for the anthocyanins than for quercetin.

In studies by Cao et al (13), the 2 major anthocyanins in elderberry (cyanidin 3-glucoside and cyanidin 3-sambubioside) were detected as glycosides in both plasma and urine. The maximum plasma concentration of total anthocyanins, which was reached within 71 min, varied from 55.3 to 168.3 nmol/L. Most anthocyanins were excreted in urine during the first 4 h. Wu et al (15) identified 4 additional anthocyanin metabolites from elderberry in

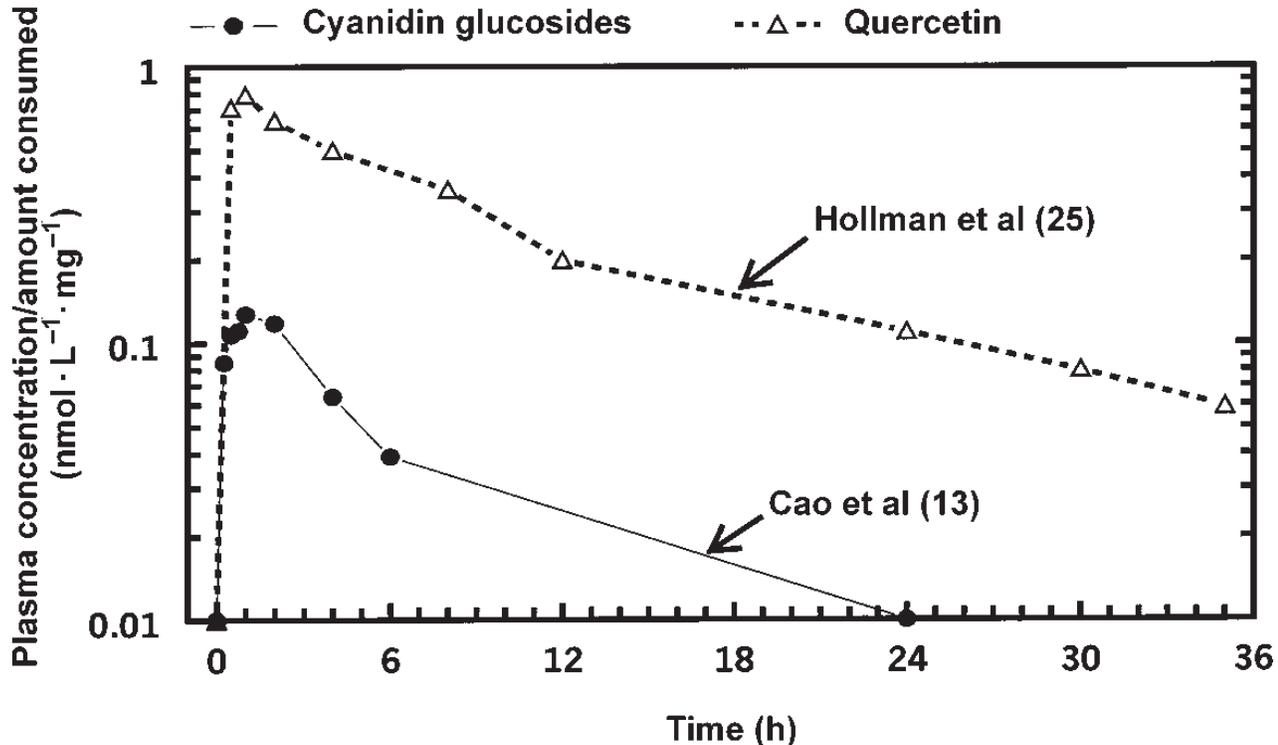


FIGURE 2. Plasma pharmacokinetics of quercetin and cyanidin 3-glucoside plus cyanidin 3-sambubioside. Data adapted from Hollman et al (25) for quercetin and from Cao et al (13) for cyanidin anthocyanins. Plasma quercetin and anthocyanin concentrations were divided by the amount consumed (68 mg for quercetin from 150 g onions and 720 mg for cyanidin 3-glucosides from an elderberry extract).

the urine: peonidin 3-glucoside, peonidin 3-sambubioside, peonidin monoglucuronide, and cyanidin 3-glucoside monoglucuronide. Total elderberry anthocyanin excretion in the first 4 h was $554 \pm 90 \mu\text{g}$ and accounted for only 0.077% of dose. The formation of the peonidin metabolites likely takes place in the liver through the catechol *O*-methyltransferase reaction. Delphinidin would be the only other anthocyanidin that might undergo this reaction, as malvidin and petunidin already are methylated in the 3' position.

In an additional study reported by Wu et al (15), 6 women were given 189 g lowbush blueberry (BB), which provided a total of 690 mg anthocyanins. In 5 of 6 subjects fed BB, urine samples contained 5–8 different anthocyanins, all of which were identified as being present in the blueberries consumed. Plasma anthocyanin levels were below detection limits ($\approx 5 \text{ ng}$) using 2 mL plasma. Total urinary anthocyanin excretion during the first 6 h was $23.2 \pm 4.8 \mu\text{g}$ or 0.004% of dose. In both of these studies no significant changes were observed in plasma antioxidant capacity. Matsumoto et al (22) reported that the cumulative excretion of the 4 compounds from black currant (delphinidin 3-*O*- β -rutinoside, cyanidin 3-*O*- β -rutinoside, delphinidin 3-*O*- β -glucoside, and cyanidin 3-*O*- β -glucoside) in urine in the first 8 h after intake was $0.11 \pm 0.05\%$ of the dose ingested (Table 1) (22).

Anthocyanins as in vivo antioxidants

Animal studies. C3G feeding significantly suppressed changes caused by hepatic ischemia-reperfusion (I/R) in rats fed 2 g/kg diet of C3G for 14 d. I/R treatment elevated the liver thiobarbituric acid-reactive substance concentrations (TBARS) and the serum activities of aspartate aminotransferase (glutamic oxaloacetic transaminase), alanine aminotransferase (glutamic pyruvic transaminase), and L-lactate dehydrogenase, marker enzymes for liver injury, and lowered the liver reduced glutathione concentration. Although liver ascorbic acid concentrations were also lowered by hepatic I/R, concentrations were restored more quickly in C3G-fed rats compared with control rats. Feeding C3G also resulted in a significant decrease in generation of TBARS during serum formation. The serum from the C3G-fed group also showed a significantly lower susceptibility to further lipid peroxidation provoked by AAPH or Cu^{2+} than that of the control group (26). Under these feeding and oxidative stress conditions, C3G functions as a potent in vivo antioxidant (27, 28).

In rats fed a vitamin E-deficient diet for 12 wk and then repleted with a diet containing a highly purified anthocyanin-rich extract (1 g/kg diet), a significant improvement ($P < 0.01$) in plasma antioxidant capacity and a decrease ($P < 0.001$) in the vitamin E deficiency-enhanced hydroperoxides and 8-oxo-deoxyguanosine concentrations in liver were observed (29). The anthocyanin extract consisted of a mixture of the 3-glucoside forms of delphinidin, cyanidin, petunidin, peonidin, and malvidin. Thus, there are 2 animal experiments that indicate that anthocyanins can be effective in vivo antioxidants when included in the diet at 1 or 2 g/kg diet. These levels in the diet provide 20–40 mg/d, which are much higher amounts than found in the typical diet of humans.

Lietti (30) demonstrated significant vasoprotective and anti-edema properties in experimental animals given an extract from bilberry that contained 25% anthocyanins. In rabbits, the increase in skin capillary permeability because of chloroform was reduced after both intraperitoneal (25–100 mg/kg) and oral administration (200–400 mg/kg) of anthocyanins. Anthocyanins from

Vaccinium myrtillus were effective both in a skin capillary permeability test and in a vascular resistance test in rats fed a diet devoid of rutin (quercetin rutinoside). In the former test, effective doses were in the range of 25–100 mg/kg (by oral route). Anthocyanins were 2-fold more active when compared with rutin. Orally administered anthocyanins from *V. myrtillus* inhibited carrageenan paw edema in rats showing a dose-response relationship. In the rat, elimination of anthocyanins occurs mainly through urine and bile, but the liver extracts a small amount of the anthocyanins (31). Anthocyanins were found to possess a greater affinity for kidneys and skin rather than for plasma or other tissues. Interestingly, long-lasting activity of anthocyanins on capillary resistance was observed even when plasma levels of the anthocyanins were no longer detectable (31). Early work of Mian et al (32) suggested that anthocyanins protect capillary walls by 1) increasing the endothelial barrier-effect through stabilization of the membrane phospholipids, and 2) increasing the biosynthesis of the acid mucopolysaccharides of the connective ground substance. This may explain the marked increase of newly formed capillaries and collagen fibrils induced by the anthocyanins. Whether these vasoprotective effects of anthocyanins are due to antioxidant effects is not clear.

Human clinical studies. Studies in humans of antioxidant effects following consumption of anthocyanins are less definitive. Much of the early work on anthocyanins has resulted from studies of bilberry or concentrated forms of anthocyanins from bilberry (33, 34). Much of the health-related effects reviewed in these publications focused around effects on the vascular system (vasorelaxant and vasomotor effects), effects on the eyes, antioxidant effects, and platelet aggregation effects.

Bub et al (14) compared changes in plasma malvidin 3-glucoside (M3G) and its urinary excretion after ingestion of red wine, dealcoholized red wine, and red grape juice in 6 healthy male subjects who consumed 500 mL of each beverage on separate days. M3G was poorly absorbed and seemed to be differentially metabolized compared with other red grape polyphenols. Bub et al (14) suggested that some unidentified anthocyanin metabolites and/or other polyphenols, other than the anthocyanin M3G may be responsible for the observed antioxidant and health effects in vivo in subjects consuming red wine.

We observed a small but significant increase in plasma hydrophilic and lipophilic antioxidant capacity following the consumption of a single meal of 189 g blueberries (X Wu and RL Prior, unpublished data, 2002) (15). However, Mazza et al (16) demonstrated a more consistent increase in plasma antioxidant capacity after the consumption of $\approx 1.2 \text{ g}$ anthocyanins from blueberries. An increase in antioxidant activity in plasma was observed in elderly subjects who consumed 1 cup blueberries/d for 30 d (J Bagnulo, D Cook, and RL Prior, unpublished observations, 2002). What is not known is whether anthocyanins are accumulated in tissues if consumed over an extended period of time.

Factors that will affect in vivo antioxidant effects of anthocyanins and other flavonoids include quantities consumed, quantities absorbed or metabolized, and plasma and/or tissue concentrations. Seeram et al (17) demonstrated that cyanidin glycosides from tart cherries spontaneously degraded to protocatechuic acid, 2,4-dihydroxybenzoic acid, and 2,4,6-trihydroxybenzoic acid in solution at pH 7 (Figure 2). Anthocyanins exist as the flavylium cation at pH < 3 , but at pH 3–6 they may exist as a quinoidal base, and at pH 7–8 they may convert to the chalcone. Thus, in any cell

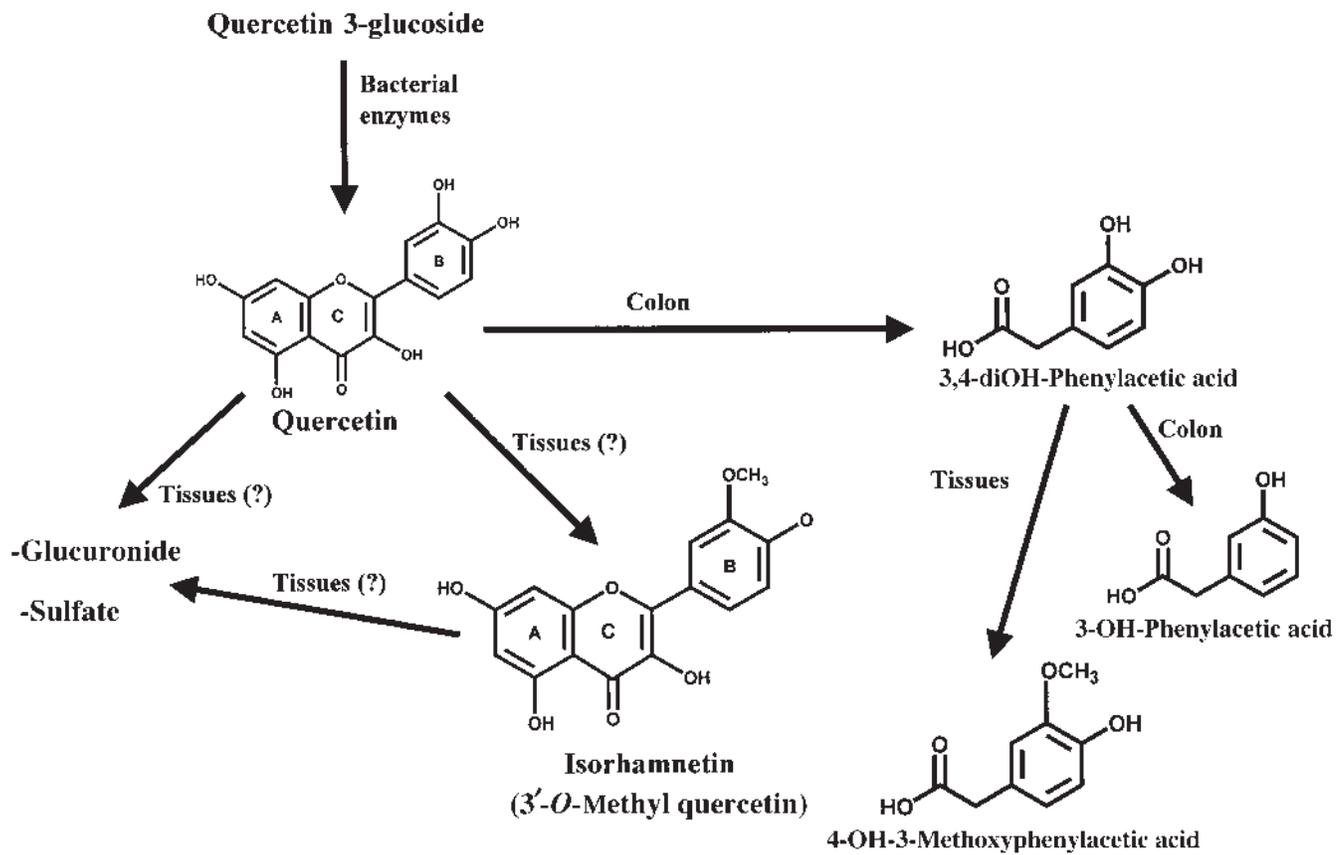


FIGURE 3. Proposed pathways of quercetin absorption/metabolism. Data from Hollman et al (38), Morand et al (39), Manach et al (40), Crespy et al (41), Walle et al (42, 43), and Graefe et al (44).

or tissue culture study using anthocyanins, one must be aware that at pH 7, the anthocyanins may degrade. What happens to anthocyanins during the absorption process once they are inside the cell and in plasma, where the pH will be above 7, is unknown. This instability of anthocyanins in tissue culture and in the body often tends to be overlooked and makes interpretation of *in vitro* data difficult, as one does not know whether the effects observed are due to the anthocyanins or due to some breakdown product. Although anthocyanins can have antioxidant effects in cell culture and other *in vitro* systems at relatively high concentrations, it is not clear whether concentrations can be reached *in vivo* at the tissue level to produce antioxidant effects. Because of the instabilities of anthocyanins in the neutral pH range, it is not clear whether anthocyanins remain intact in tissues long enough to act as antioxidants.

Quercetin and other flavonols as antioxidants

The flavonol quercetin (3,3',4',5,7-pentahydroxyflavone) is one of the most abundant dietary flavonoids and has been one of the most frequently studied flavonoids. Data on the quercetin content of foodstuffs are limited, but the available data suggest a range of 2–250 mg quercetin/kg wet weight in fruits; 0–100 mg/kg in vegetables, with onions being especially high (200–600 mg/kg); 4–16 mg/L in red wine; 10–25 mg/L in tea; and 2–23 mg/L in fruit juices (35, 36). The average dietary intake of quercetin in the Netherlands was estimated to be 16 mg/d (35). However, the extent of absorption of flavonoids such as quercetin is a critical issue relative to the many alleged health effects.

Quercetin and other flavonoids have been shown to modify eicosanoid biosynthesis (antiprostanoic and antiinflammatory responses), protect low-density lipoprotein (LDL) from oxidation (prevention of atherosclerotic plaque formation), prevent platelet aggregation (antithrombotic effects), and promote relaxation of cardiovascular smooth muscle (antihypertensive, antiarrhythmic effects). In addition, flavonoids have been shown to have antiviral and anticarcinogenic properties (6).

Quercetin absorption/metabolism

Quercetin was originally assumed to be absorbed from the small intestine following cleavage of the β -glucoside linkage by colonic microflora (37). Hollman et al (38) found that humans absorb quercetin but concluded that absorption was enhanced by conjugation with glucose (Figure 3). Morand et al (39) demonstrated in the rat that the major circulating metabolites of quercetin were glucuronide-sulfo conjugates of isorhamnetin (3'-O-methyl quercetin) and of quercetin. Excretion of quercetin or its conjugates in urine in 4 separate studies ranged from 0.07% to 17.4% of intake (Table 2). Crespy et al (41) found that quercetin, but not its glycosides, was absorbed from the rat stomach. Walgren et al (47, 48) demonstrated, from *in vitro* studies of Caco-2 cells, a complete lack of absorption of the glucosides of quercetin, mainly because of effective efflux by the multidrug resistance protein 2 (MRP2) transporter, but quercetin was readily absorbed. In subsequent studies in human subjects, Walle et al (42) found that quercetin glucosides were hydrolyzed in the small intestine by

TABLE 2

Urinary quercetin excretion after consumption of quercetin or quercetin glycosides from different sources

Source	Component	Intake	Urine excretion		Reference
			mg	% of intake	
Tea	Quercetin	49	0.5 ± 0.3 ¹		de Vries et al (45)
	Kaempferol	27	2.5 ± 1.3		
Onion	Quercetin	13	1.1 ± 0.5		Aziz et al (46)
Onion	Quercetin 4'-glucoside	102	0.2 ± 0.1		
	Conjugated quercetin	139	0.8 ± 0.1		
	Isorhamnetin 4'-glucoside	10.4	17.4 ± 8.2		
Onion	Quercetin	89	0.31 ± 0.14		Hollman et al (38)
Q 3 R	Quercetin 3-rutinoside	100	0.07 ± 0.19		Graefe et al (44)
Q-aglycone	Quercetin aglycone	100	0.12 ± 0.08		
Onion	Quercetin	100	4.4 ± 1.6		
Q 4' R	Quercetin 4'-glucoside	100	3.3 ± 1.3		
Tea	Quercetin	200	0.8 ± 0.6		
Q 3 R	Quercetin 3-rutinoside	200	0.7 ± 0.6		

¹ $\bar{x} \pm SD$.

bacterial enzymes. In recent studies by Graefe et al (44), only quercetin glucuronides, but not free quercetin, could be detected in plasma. Walle et al (43) found that as much as 23.0–81.1% of a [¹⁴C]quercetin dose was recovered as ¹⁴CO₂ in the expired air. Absorption was quite high, ranging from 36% to 53% based on total radioactivity, and absorption of quercetin aglycone was calculated to be as high as 65–81% (42). Hollman et al (49) found that absorption of quercetin in ileostomists was 52 ± 5% for quercetin glucosides from onions, 17 ± 15% for quercetin rutinoside, and 24 ± 9% for quercetin aglycone.

Quercetin as in vivo antioxidant

Animal studies. Intragastric quercetin (10 or 50 mg/kg) raised plasma concentrations of quercetin glucuronide and/or sulfate conjugates and the plasma was more resistant to copper sulfate-induced lipid peroxidation than the control plasma on the basis of the accumulation of cholesterol ester hydroperoxides and the consumption of α -tocopherol (50). These results suggest that some conjugated metabolites of quercetin can act as effective antioxidants. Antioxidant activity of conjugated metabolites has also been observed in vitro (39, 51).

Chronic administration of quercetin (10 mg/kg) to spontaneously hypertensive (SH) rats and normotensive Wistar Kyoto rats (52) decreased liver glutathione peroxidase activity, increased liver total glutathione levels, and increased hepatic and plasma malondialdehyde (MDA) concentrations in SH rats, compared with Wistar Kyoto rats. In SH rats, treatment with quercetin for 5 wk reduced blood pressure, increased glutathione peroxidase activity, and reduced both plasma and hepatic MDA levels. None of these effects were observed in Wistar Kyoto rats. Thus, in this genetic model of hypertension, quercetin showed both antihypertensive and antioxidant properties.

Lysosomal pellet and supernatant fractions obtained from the livers of the ddY strain of male mice fed a diet containing 0.2% quercetin for 7 d showed more inhibition of chemiluminescent intensity than those obtained from nontreated mice (53). Quercetin feeding also resulted in the inhibition of lipid peroxidation. Plasma from rats adapted to a diet containing 0.2% quercetin exhibited a total antioxidant status markedly higher than that of control rats (+60%) (38), suggesting that even though quercetin was conjugated in vivo, the metabolites exhibited antioxidant properties.

Anticarcinogenic effects of quercetin against cervical neoplasia in virgin Swiss albino mice (54) were attributed to its antioxidant properties, which were reflected in lower lipid peroxides and their role in the host detoxification system, as expressed in liver glutathione level, glutathione transferase, glutathione peroxidase, catalase, and superoxide dismutase activity.

Human clinical studies. Boyle et al (55) reported that after 6 wk of rutin supplementation (500 mg), the plasma levels of 3 flavonoids (quercetin, kaempferol, and isorhamnetin) were elevated but there was no significant change in plasma antioxidant status or other measures of oxidative stress (lymphocyte DNA damage, urinary MDA, 8-hydroxy-2-deoxyguanosine, and 8-iso-prostaglandin F_{2 α}). Human subjects supplemented with quercetin-containing capsules (1.0 g quercetin/d) exhibited a markedly enhanced plasma quercetin concentration (23-fold), but there were no effects on serum total, LDL, or high-density-lipoprotein cholesterol or triacylglycerol levels or other cardiovascular or thrombotic risk factors, including platelet aggregation, platelet thromboxane B₂ production, blood pressure, or resting heart rate (56). These authors suggested that any protective effect of foods containing quercetin was mediated by factors other than those they measured or that the protective effect was due to factors other than quercetin in those foods.

In human subjects, the lag phase for LDL oxidation was 44 ± 11 and 40 ± 5 min for the baseline and placebo, respectively, and increased significantly to 51 ± 7 with in vivo quercetin supplementation (30 mg/d for 2 wk) (57). The inhibition of LDL oxidation after in vivo supplementation was unrelated to changes in antioxidant vitamin and carotenoid concentrations.

Young et al (58) used a crossover design with 3 doses of black currant/apple (1:1) juice (750, 1000, and 1500 mL) in 5 subjects for 1 wk corresponding to an intake of 4.8, 6.4, and 9.6 mg quercetin/d. Urinary excretion of quercetin increased significantly with dose and with time. The fraction excreted in urine was constant (0.29–0.47%). Plasma quercetin did not change with juice intervention. Total plasma MDA decreased with time during the 1500-mL juice intervention. Plasma protein 2-adipic semialdehyde residues increased with time and dose, and glutathione peroxidase increased with juice dose, whereas other selected markers of oxidative status did not change. Because of the inconsistent effects on different markers of oxidative stress, the authors suggested that

these might be related to several components of the juice and not solely to their quercetin content. Knekt et al (59) concluded from epidemiologic evidence from 9208 Finnish men and women that the intake of apples was related to a decreased risk of thrombotic stroke, but this association apparently was not due to the presence of quercetin.

Fruits and vegetables as sources of antioxidants

Boyle et al (60) determined uptake as well as in vivo antioxidant effects of flavonoids from foods. Flavonoid glucosides (quercetin 3-glucoside and isorhamnetin 4-glucoside) were significantly elevated in plasma following ingestion of an onion meal, and the increases were associated with an increased resistance of lymphocyte DNA to DNA strand breakage. A significant decrease in the level of urinary 8-hydroxy-2'-deoxyguanosine was evident at 4 h following ingestion of the onion meal. After a combined tomato and onion meal, quercetin was detected in plasma and endogenous base oxidation was decreased, but resistance to strand breakage was unchanged. There was no significant change in the excretion of urinary MDA following either meal. Both meals containing onions, or onions and tomatoes, led to transient decreases in biomarkers of oxidative stress.

Marniemi et al (61) conducted a study to evaluate the long-term (8 wk) and short-term (5 h) effects of increased intake of 3 berries on antioxidant potential and lipid peroxidation. The berry treatment group members ate, in addition to their normal diet, a 100-g portion of deep-frozen berries (bilberries, lingonberries, or black currants) daily for 8 wk. The other groups ingested daily 100 mg α -tocopherol and 500 mg ascorbic acid (supplement group) or 500 mg calcium gluconate (control group). In the short-term experiment, 6 men ate 80 g of each of the 3 berries at one time. Subjects in the berry group had a slightly lowered LDL diene conjugation and slightly increased serum total radical-trapping antioxidant parameter (TRAP). In the short-term experiment, LDL TRAP showed a small 10% increase during 5 h after the intake of 240 g berries. Although there were effects of consumption of berries on antioxidant potential and diene conjugation in LDL particles in vivo, the effects were small. Cao et al (62) found that following the consumption of a single meal of either strawberries, spinach, or dealcoholized red wine, all of which are rich in antioxidant phenolic compounds, serum antioxidant capacity was increased in humans as assessed by 3 different methods: oxygen radical absorbing capacity (ORAC) assay, trolox equivalent antioxidant capacity assay, and ferric reducing ability assay (63). Furthermore, when the daily servings of fruits and vegetables were increased, plasma antioxidant capacity was increased in humans (62). Nagyova et al (64) observed in vegetarians that TBARS in LDL were reduced and total plasma antioxidant capacity was increased. In subjects who consumed a phytochemical-rich diet, Bruce et al (65) interpreted a decrease in erythrocyte superoxide dismutase of 69% and of glutathione peroxidase of 35% to indicate a decreased need for oxidative defense mechanisms.

CONCLUSIONS

It is clear that under in vitro assay conditions, both anthocyanins and flavonols clearly can function as antioxidants. However, in vivo, anthocyanin absorption appears to be at least an order of magnitude lower than for the flavonol quercetin. Whether anthocyanins get into cells or into an appropriate subcellular compartment in sufficient concentrations to affect metabolic processes

is not known. In animal models, dietary anthocyanins at relatively high doses (1–2 mg/kg diet) are protective against oxidative stress. In humans, anthocyanins appear to have some vasoprotective effects, but whether these are the result of antioxidant mechanisms is not clear.

Some common pathways of metabolism of flavonoids are emerging that can affect in vivo antioxidant capacity. Methylation in the 3' position of both cyanidin 3-glucoside and quercetin will decrease the antioxidant capacity of the metabolite. Further conjugation with glucuronide or sulfate may also affect antioxidant capacity depending on the position that is conjugated. Even though quercetin is conjugated during the absorption process, the conjugates still seem to retain antioxidant activity. Measurement of in vivo antioxidant effects of a single flavonoid compound appears to be difficult except at fairly high consumption rates. With whole foods, antioxidant effects may be more easily demonstrated, and the mixture or synergy between compounds in foods may have added benefit. 

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