Bioavailability and bioefficacy of polyphenols in humans. II. Review of 93 intervention studies[1–4]

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ABSTRACT

For some classes of dietary polyphenols, there are now sufficient intervention studies to indicate the type and magnitude of effects among humans in vivo, on the basis of short-term changes in biomarkers. Isoflavones (genistein and daidzein, found in soy) have significant effects on bone health among postmenopausal women, together with some weak hormonal effects. Monomeric catechins (found at especially high concentrations in tea) have effects on plasma antioxidant biomarkers and energy metabolism. Procyanidins (oligomeric catechins found at high concentrations in red wine, grapes, cocoa, cranberries, apples, and some supplements such as Pycnogenol) have pronounced effects on the vascular system, including but not limited to plasma antioxidant activity. Quercetin (the main representative of the flavonol class, found at high concentrations in onions, apples, red wine, broccoli, tea, and *Ginkgo biloba*) influences some carcinogenesis markers and has small effects on plasma antioxidant biomarkers in vivo, although some studies failed to find this effect. Compared with the effects of polyphenols in vitro, the effects in vivo, although significant, are more limited. The reasons for this are 1) lack of validated in vivo biomarkers, especially in the area of carcinogenesis; 2) lack of long-term studies; and 3) lack of understanding or consideration of bioavailability in the in vitro studies, which are subsequently used for the design of in vivo experiments. It is time to rethink the design of in vitro and in vivo studies, so that these issues are carefully considered. The length of human intervention studies should be increased, to more closely reflect the long-term dietary consumption of polyphenols. *Am J Clin Nutr* 2005;81(suppl):243S–55S.

KEY WORDS Polyphenols, flavonoids, procyanidin, bioavailability, isoflavone, quercetin, catechin

INTRODUCTION

It is clear that food components must, by definition, be bioavailable in some form to exert biological effects. There have been major advances in the past few years in our knowledge regarding polyphenol absorption and metabolism (1–4), and it is apparent that most classes of polyphenols are sufficiently absorbed to have the potential to exert biological effects. For example, quercetin after a meal containing onions, catechins after red wine consumption, and isoflavones after soy consumption reach micromolar concentrations in the blood (1, 2, 5–7). These findings demonstrate that polyphenols cross the intestinal barrier and reach concentrations in the bloodstream that have been shown to exert effects in vitro, in some studies.

There are thousands of articles on the effects of polyphenols on biological systems in vitro. However, many of those studies did not take bioavailability and metabolism factors into consideration, and the effects reported in those studies do not necessarily occur in vivo. Although most polyphenols are absorbed to some extent, this is very dependent on the type of polyphenol. The range of concentrations required for an effect in vitro varies from <0.1 μmol/L to >100 μmol/L. Because physiologic concentrations do not exceed 10 μmol/L, the effects of polyphenols in vitro at concentrations of >10 μmol/L are generally not valid, with the possible (but unproven) exception of the intestinal lumen. Furthermore, absorption is accompanied by extensive conjugation and metabolism, and the forms appearing in the blood are usually different from the forms found in food. This indicates that in vitro experiments with the form of polyphenols found in food (the aglycone) are not necessarily relevant to the in vivo situation (8).

There are now intervention studies in the literature, of varying quality, that demonstrate significant biological effects of polyphenol consumption among humans, with the use of many different biomarkers (**Tables 1–4**). This review examines the effects demonstrated in some of the intervention studies reported in the literature. It considers most of the reports on quercetin, catechins, and procyanidins and some of those on isoflavones. Some of the reports described intervention studies involving consumption of foods and, in many of those cases, it was not proved that the observed effects were attributable to the polyphenol component. This situation may improve in the future, for example, with the use of isogenic lines of onions that differ only in their quercetin contents, allowing comparisons between groups consuming the same food but with different polyphenol contents. The bioavailability issues for each group of polyphenols are discussed in the context of the intervention studies.

HUMAN INTERVENTION STUDIES WITH FLAVONOIDS AND BIOAVAILABILITY ISSUES

Flavonols (including quercetin)

Quercetin is found at high concentrations in onions, apples, tea, broccoli, and red wine and as a component of *Ginkgo biloba*.

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TABLE 1
Human intervention studies on flavonols or flavonol-containing foods[1]

Substances given	Principal polyphenol[2]	Dose per day	Days	No. of subjects per group[3]	Biomarkers significantly affected	Biomarkers not significantly affected	Ref
Ginkgo biloba extract EGb 761	Quercetin	120 mg extract	90	20	Decrease in bood pressure, increase in fasting plasma insulin and C-peptide	Liver function, coagulation tests, metabolic panel	9
Ginkgo biloba extract EGb 761	Quercetin	120 mg extract	90	6 hyperinsulinemic subjects	Enhanced hepatic extraction of insulin relative to C-peptide and reduction in plasma insulin	Plasma lipid profiles, blood cell counts	10
Ginkgo biloba extract EGb 761	Quercetin	160 mg extract	22	22 mountain climbers	Abolition of acute mountain sickness		11
Ginkgo biloba	Quercetin	320 mg extract	7	5 patients undergoing aortic valve replacement	Decrease in free radicals, delay in myoglobin release	Clinical improvement in outcome, but not significant	12
Ginkgo biloba extract EGb 761	Quercetin	320 mg extract	1	18 elderly subjects with slight age-related memory impairment	Increase in mental performance		13
"Phenol-rich diet"	Quercetin, kaempferol	21 mg quercetin, 9 mg kaempferol	6	19	Increase in erythrocyte superoxide dismutase activity, decrease in lymphocyte DNA damage (tail moment)	Plasma α-tocopherol and β-carotene	14
Onions	Quercetin	200 g onion	1	6	Increased resistance of lymphocyte DNA to strand breakage, decrease in urinary 8-hydroxy-2'-deoxyguanosine	Urinary malondialdehyde	15
Fried onions	Quercetin	50 mg	1	5	6% increase in plasma antioxidant capacity	Susceptibility of LDL to oxidation	16
Supplement	Quercetin	250 mg quercetin and other polyphenols	28	27		HDL/LDL cholesterol, platelet aggregation, plasma thromboxane B2, blood pressure, resting heart rate	17
Onions	Quercetin	114 mg	14	18		Platelet aggregation, thromboxane B2 production, other hemostatic variables	18
Parsley	Apigenin	114 mg	14	18		Platelet aggregation, thromboxane B2 production, other hemostatic variables	18
Onion and tea	Quercetin	76–110 mg quercetin and other flavonols	14	10 stable type 2 diabetic patients	Decrease in oxidative damage to lymphocyte DNA	Plasma vitamin C, α-tocopherol, urate, albumin, bilirubin, SOD, GPx, selenium	19
Supplement	Quercetin	30 mg	14	4	Decrease in TIMP-1 plasma protein and lymphocyte mRNA	TIMP-2 and matrix metalloprotein-2 lymphocyte mRNA or plasma protein	20
Supplement	Quercetin	100 mg [curcumin, 400 mg]	1	15 renal transplant patients	Improved renal function only in patients with elevated serum creatinine, improved urine output and lowered isoprostanes in patients with delayed graft function	Blood pressure, calcineurin levels	21

(*Continued*)

TABLE 1 (*Continued*)

Substances given	Principal polyphenol[2]	Dose per day	Days	No. of subjects per group[3]	Biomarkers significantly affected	Biomarkers not significantly affected	Ref
Supplement	Quercetin	2 × 500 mg	30	30 men with chronic pelvic pain syndrome	Improvement in NIH prostatitis symptom score		22
Supplement	Quercetin	30 mg	14	10	Improved oxidative resistance of LDL	Plasma triglycerides, total, HDL or LDL cholesterol, vitamin E or C, retinal and carotenoid	23
Supplement	Quercetin	2 × 500 mg	28	20	Improvement in cystitis symptoms	No side effects or adverse reactions	24

[1] SOD, superoxide dismutase; GPx, glutathione peroxidase; TIMP, tissue inhibitor of matrix metalloproteinase.

[2] The measured polyphenol; the dose is given in the next column. Ginkgo biloba also contains terpenes, and onions also contain high levels of biologically active sulfur compounds and other nutrients.

[3] Healthy subjects, unless otherwise stated.

Intervention studies with quercetin are shown in Table 1. Some diverse effects have been demonstrated for *Ginkgo biloba*, but these may also be attributable to the terpenoid component of these extracts. Other studies have shown effects on antioxidant biomarkers, such as increased resistance of lymphocyte DNA to strand breakage, decreased urinary 8-hydroxy-2'-deoxyguanosine concentrations, increased plasma antioxidant capacity, decreased tissue inhibitor of matrix metalloproteinase-1 expression, altered renal function, improved prostatitis symptoms, and improved oxidative resistance to LDL. However, there are also studies that show no effects on these biomarkers.

Despite the lack of convincing evidence for consistent effects of quercetin in vivo in humans, there are numerous studies on the properties of quercetin in vitro. The apparent discrepancy between in vitro and in vivo studies may be partly attributable to absorption and metabolism. Generally, quercetin is not found in the plasma as the free form or as the parent glucoside. At the doses used in the intervention studies noted in Table 1 (21–1000 mg), it would be found exclusively as methyl, sulfate, or glucuronic acid conjugates (102); when added together, these compounds would represent the equivalent of ~1–5 μmol/L aglycone equivalents at the highest dose. Lower doses of quercetin are more methylated than higher doses in humans (103). Quercetin has a relatively long plasma half-life of 11–28 h, and a 50-mg dose would lead to concentrations of up to ~0.75–1.5 μmol/L in plasma (1, 4). There is only limited information on the properties of the conjugates in vitro, although it can be concluded that the conjugates have quantitatively different properties and are generally less biologically active, compared with the aglycones (104, 105). However, deconjugation could occur in some tissues such as the liver (106) or at sites of inflammation (107), leading to reactivation of the conjugated quercetin. The bioavailability issues may partially account for the lack of biological activity in vivo, although the lack of activity may reflect the short-term nature of the studies of quercetin and the selection of inappropriate biomarkers.

Isoflavones (genistein and daidzein)

The intervention studies on isoflavones are the most advanced and sophisticated of those for all of the polyphenols. Studies of the consumption of isoflavones lasting up to 1 year have shown effects on bone biomarkers, such as significant increases in bone mineral density and bone mineral content and changes in bone biomarkers, such as reduced excretion of pyridinium cross-links and increased serum concentrations of bone-specific alkaline phosphatase and osteocalcin. Other effects include changes in LDL and HDL cholesterol concentrations, increases in LDL oxidation lag time, and changes in menopausal symptoms and hot flashes. Many, but not all, of the changes could be related to binding to the estrogen receptor, and this has been reviewed (108, 109).

Isoflavones occur in soy as glycosides, but some fermented products contain free aglycones (110). Consumption of isoflavone-rich foods or of the purified isoflavones themselves leads to appearance in the plasma, with a peak of absorption at 6–8 h (7). A dose of 50 mg of either daidzein or genistein, as typically used in intervention studies (the intervention studies noted in Table 1 used 37–128 mg per person per day), yields a peak plasma concentration of ~2 μmol/L at ~6 h (4). The glycosides are not present in plasma, and most of the isoflavones are conjugated as sulfates or glucuronides; some free aglycone is also present, and Setchell et al (7) found 8% of the total daidzein as unconjugated aglycone 2 h after consumption of a dose of 50 mg. This decreased to 3% at steady state, which would apply to the intervention studies, because the studies were conducted for 14–365 d. After 4 wk of 30 mg/d isoflavones, the peak plasma concentrations showed no significant changes at the measurement times of 2 and 4 wk (111), which indicates that the bioavailability of isoflavones does not decrease during long periods of intake. However, we have unpublished data showing that isoflavone bioavailability increases during an extended period of intake (C Manach, unpublished observations, 2004), and this issue is currently unresolved. When administered in intervention studies, the isoflavones are clearly active and affect several biomarkers, especially related to bone. It is not known whether this effect is derived from free aglycone in the plasma, whether the conjugated forms are also active, or whether active deconjugation occurs in the relevant tissues. Some of the isoflavone conjugates are active in vitro (112), although information is very limited.

Catechins [(+)-catechin, (−)-epicatechin, (−)-epigallocatechin, (−)-epicatechin gallate, and (−)-epigallocatechin gallate]

(+)-Catechin and (−)-epicatechin are widely distributed in foods. Catechin concentrations are especially high in broad beans, black grapes, apricots, and strawberries. (−)-Epicatechin is found at high concentrations in apples, blackberries, broad

TABLE 2
Human intervention studies of isoflavones or isoflavone-containing foods

Substances given	Principal polyphenols[1]	Dose per day of isoflavones	Days	No. of subjects per group[2]	Biomarkers significantly affected	Biomarkers not significantly affected	Ref
Soy foods	Genistein, daidzein	25–45 mg	30	6 female subjects (age: 20–29 y)	Follicular phase length increased, peak progesterone concentration delayed, midcycle peaks of luteinizing hormone and follicle-stimulating hormone suppressed, reduction in total cholesterol		25
Soy vs placebo	Genistein, daidzein	37 mg	70	20 postmenopausal women	Bone resorption	Bone stiffness	26
High, low, and control soy	Genistein, daidzein	73 mg	30	41 hypercholesterolemic men and postmenopausal women	Serum interleukin-6 increased in women	Serum C-reactive protein, serum amyloid A, serum α-tumor necrosis factor	27
Genistein	Genistein	54 mg	365	30 women (age: 47–57 y)	~3% increase in bone mineral density in femur and lumbar spine		28
Isoflavone concentrate	Genistein, daidzein	80 mg	14	29 healthy menopausal women		Endothelial function (flow-mediated dilation)	29
Isoflavone-rich soy	Genistein, daidzein	80 mg	168	24 perimenopausal women	Bone mineral density increased 5.6%, bone mineral content increased 10.1%		30
Isoflavone extract	Genistein, daidzein	100 mg	120	40 postmenopausal (2 y) women	Decrease in menopausal symptoms, decrease in total cholesterol and LDL cholesterol	Blood pressure, plasma glucose, HDL or triglyceride levels	31
Isoflavone-rich soy	Genistein, daidzein	56 mg	17	24	Plasma 8-epi-prostaglandin F2 decreased by ~20%, LDL oxidation lag time by 10%	LDL-tocophenol, plasma polyunsaturated fatty acids or malondialdehyde	32
Isoflavone tablet	Genistein, daidzein	80 mg	56	20 women (age: 50–70 y)		Blood pressure, plasma lipid, plasma lipoprotein, endothelium-(in)dependent dilation	33
Soy protein foods	Genistein, daidzein	86 mg	31	31 hyperlipidemic subjects	Decreased circulating oxidized LDL		34
Red clover	Genistein, daidzein, biochanin, formononetin	80 mg	35	17 women	Increase in arterial compliance by 23%	Plasma lipids	35
Soy protein	Isoflavones	90 mg	60	66 hypercholeserolemic postmenopausal women	Non-HDL cholesterol reduced, HDL cholesterol increased, mononuclear cell LDL receptor mRNA increased, increase in bone mineral content and density of lumbar spine		36
Soy protein powder	Isoflavones	128 mg	90	14 premenopausal women	Increase in plasma luteinizing hormone and follicle-stimulating hormone during preovulatory phase, decrease in free T_3 and dehydroepiandrosterone sulfate during early follicular phase, decreased estrone during midfollicular phase	Length of menstrual cycle	37

(*Continued*)

TABLE 2 (*Continued*)

Substances given	Principal polyphenols[1]	Dose per day of isoflavones	Days	No. of subjects per group[2]	Biomarkers significantly affected	Biomarkers not significantly affected	Ref
Genistein supplement	Genistein	54 mg	365	30 women (age: 47–57 y)	Reduced excretion of pyridinium cross-links, increased serum bone-specific alkaline phosphatase and osteocalcin, increase in bone mineral density in femur and lumbar spine		28
Supplement	Isoflavones	132 mg	84	32 postmenopausal women with diet-controlled type 2 diabetes mellitus	Lower fasting insulin, lower insulin resistance, lower LDL and total cholesterol, lower free thyroxine	HDL cholesterol, triglycerides, weight, blood pressure, creatinine, steroid hormones	38
Isoflavone-enriched soy protein	Isoflavones	90 mg	365	15 young healthy women		No changes in bone mineral density or other bone parameters	39
Supplement	Isoflavones	62 mg	28	23 healthy perimenopausal women	Reduced excretion of bone resorption biomarkers, LDL and serum cholesterol decreased	HDL or VLDL cholesterol, triglycerides, no liver cell damage and no tendency to diabetes	40

[1] The measured polyphenol; the dose is given in the next column.
[2] Healthy subjects, unless otherwise stated.

beans, cherries, black grapes, pears, raspberries, and chocolate. The gallates and the gallocatechins are found almost exclusively in tea, especially green tea. Deducing the intake of catechins during intervention studies is more difficult than for isoflavones, because the non-galloylated forms are widespread and can complicate intake estimates. This can lead to consumption of additional sources of catechins; furthermore, the amounts were not measured in some cases. The situation is clearer for the gallates or galloylated catechins, because they are almost exclusive to green tea. It is difficult to estimate the total flavonoid intake from black tea, because the fermentation process gives rise to a variety of structurally different oligomers. However, the amounts of remaining monomeric catechins can be readily estimated. Catechins are biologically active molecules that have a wide range of effects in vitro.

In human intervention studies (Table 3), catechins increased plasma antioxidant activity, as assessed with a variety of assays, decreased plasma lipid peroxide and malondialdehyde concentrations, increased plasma ascorbate concentrations, decreased nonheme iron absorption, and increased the resistance of LDL to oxidation. In addition, the green tea catechins, including the galloylated catechins, increased fat oxidation and energy expenditure and decreased the respiratory quotient and body weight. There were also some effects on vascular dilation.

The amounts of catechins administered in various intervention studies were highly variable, and administration was for short periods (1–28 d) (Table 1). With a dose of epigallocatechin gallate (EGCG) of 50 mg, peak plasma concentrations were ~0.15 μmol/L (4). Although the exact percentages vary among individuals, among different studies, and with time after consumption, a substantial amount of the EGCG in plasma is unconjugated. For example, EGCG given to volunteers in one dose of 2 mg/kg body wt yielded 77% of total EGCG as the unconjugated form at 1 h after consumption, with some individuals exhibiting values as high as 100% (113).

For the non-galloylated catechins, doses of 35 or 160 mg (+)-catechin (in red wine or chocolate) yielded plasma concentrations of 0.1–0.2 μmol/L (6, 114), and a similar dose of (−)-epicatechin yielded 0.2 μmol/L in plasma (114, 115). Both (+)-catechin and (−)-epicatechin are present in plasma exclusively as conjugates with methyl, sulfate, or glucuronic acid groups (6, 113, 116). Generally, catechins have short plasma half-lives (2–3 h).

In summary, the intervention studies with monomeric catechins give rise to plasma concentrations on the order of 0.1–0.5 μmol/L, but with rapid clearance. Substantial amounts of unconjugated forms of EGCG would be present in plasma, but all (+)-catechin and (−)-epicatechin is predicted to be conjugated. The percentage of catechins in the plasma that are sulfated or glucuronidated depends on the dose, but this is not usually measured in intervention studies.

Procyanidins [oligomeric (+)-catechin and (−)-epicatechin]

Procyanidins are oligomeric catechins, covalently linked together. Dimers and trimers are most common, but the degree of oligomerization can be quite high. Procyanidins are present at particularly high concentrations in cocoa, grapes/wine, and apples and are also found in many fruits, such as blackberries, cherries, figs, and plums (117). Purified procyanidins are weakly bioactive in vitro but exhibit numerous effects in vivo in intervention studies. It is important to note that procyanidins usually occur together with monomeric (+)-catechin and (−)-epicatechin; therefore, it is not clear whether the observed effects are

TABLE 3
Human intervention studies of monomeric catechins or (+)-catechin-containing foods[1]

Substances given	Principal polyphenols[2]	Dose per day	Days	No. of subjects per group[3]	Biomarkers significantly affected	Biomarkers not significantly affected	Ref
Black tea, green tea, polyphenol-rich extract	Catechins	900 mL	28	13–16		Serum interleukin-6, interleukin-1β, tumor necrosis factor-α, C-reactive protein, fibrinogen, plasminogen activator inhibitor-1, LDL oxidation, plasma cholesterol or triglycerides, plasma vitamin C or E	41, 42
Green tea drink	Catechins	5 g of green tea	1		Plasma ascorbic acid increased	Plasma β-carotene, α-tocopherol, uric acid	43
Green tea drink	Catechins	150, 300, 450 mL	1	10	Dose-dependent increase in plasma antioxidant activity		44
Green tea extract	Catechins	254 mg total catechins	1	18	40% decrease in plasma phospholipid hydroperoxide levels		45
Green tea, black tea	Catechins	8 cups	3	18		LDL oxidation ex vivo	46
Oolong tea	Catechins	0, 7.5, 15 g tea	3	12	Fat oxidation 12% higher		47
Green tea extract	Catechins	90 mg EGCG	1	10	Energy expenditure 4% higher, respiratory quotient 3% lower, 24-h urinary noradrenaline 40% higher		48
Green tea extract	Catechins	0.1 mmol	1	10	Decrease in nonheme iron absorption		49
Black tea	Catechins	5 cups	28	21	Endothelium-dependent dilation increased by 2.3%, independent dilation by 4.2%, lower soluble P-selectin	E-selectin, ICAM-1, VCAM-1, platelet aggregation, coagulation and fibrinolytic factors, F_2-isoprostane excretion	50–52
Green and black tea	Catechins	1 L	7	13	Trend to increasing ex vivo lag time for lipoprotein oxidation	F_2-isoprostane excretion	51, 53
Green tea extract	Catechins	375 mg catechins (270 mg EGCG)	90	70 Moderately obese patients	Body weight and waist circumference decreased		54
Green tea extract	Catechins	18 mg	21	8 smokers, 8 nonsmokers	Increased plasma antioxidant capacity	Plasma protein oxidation, plasma superoxide dismutase, glutathione peroxidase, catalase, urinary 8-oxodeoxyguanosine	55
Encapsulated green tea extract	Catechins	3 g extract (=10 cups)	28	20 female subjects on high-linoleic acid diet	Decrease in plasma malondialdehyde	Serum lipid, prostaglandins, nitric oxide metabolites, coagulation indicators	56
Green or black tea	Catechins	900 mL tea	28	45		Serum lipids, LDL oxidation	57
Black tea	Catechins	500 mL tea	1	10		Plasma antioxidant status	58
Black tea	Catechins	750 mL tea	28	14	Increase in LDL oxidation lag time ex vivo	Total cholesterol, triacylglycerol, apolipoprotein B	59

(*Continued*)

TABLE 3 (*Continued*)

Substances given	Principal polyphenols[2]	Dose per day	Days	No. of subjects per group[3]	Biomarkers significantly affected	Biomarkers not significantly affected	Ref
Black tea	Catechins	450 mL tea	1	49	Increased brachial dilation, not attributable to caffeine; small increase in systolic blood pressure	Nitroglycerin-induced endothelium-independent vasodilation, arterial diameter, diastolic blood pressure, heart rate, no effect on platelet aggregation	60, 61
Black tea	Catechins	900 mL	28	49	Increased brachial dilation, not attributable to caffeine	Nitroglycerin-induced, endothelium-independent vasodilation, arterial diameter, diastolic blood pressure or heart rate, no increase in systolic blood pressure; no effect on platelet aggregation	60, 61

[1] ICAM, intercellular adhesion molecule; VCAM, vascular cell adhesion molecule.
[2] The measured polyphenol; the dose is given in the next column.
[3] Healthy subjects, unless otherwise stated.

attributable to the procyanidin component, the monomeric component, or both. The predominant effects are on the vascular system and include substantial increases in plasma antioxidant activity, decreased platelet aggregation (both stimulated and unstimulated), decreased plasma concentrations of lipid peroxide and thiobarbituric acid-reactive substances, decreased LDL cholesterol concentrations, increased HDL cholesterol concentrations, decreased susceptibility of LDL to oxidation, endothelium-dependent blood vessel dilation and decreased blood pressure, beneficial effects on capillary fragility and permeability, increased plasma ascorbate concentrations, decreased P-selectin expression, increased concentrations of nitrosated/nitrosylated species, decreased serum thromboxane concentrations, increased diameters of microvessels, reduced serum thromboxane B2 concentrations, increased plasma homocysteine concentrations, increased plasma vitamin B6 concentrations, maintenance of endothelial function (compared with loss with a high-fat diet), increased platelet-derived nitric oxide production, decreased superoxide release, increased α-tocopherol concentrations, and decreased concentrations of circulating autoantibodies to oxidized LDL (Table 4).

The metabolic fate of procyanidins after consumption is still a mystery. After consumption of 2 g of high-procyanidin grape seed extract by volunteers, the plasma concentrations of procyanidin B1 reached only 10 nmol/L (118); after consumption of 0.375 g cocoa/kg body wt, the plasma concentrations of procyanidin B2 reached only 41 nmol/L (114). When administered in a purified form to rats, procyanidin dimer B3 was not found in the plasma (119). However, human intervention studies with procyanidin-rich foods, as discussed above, show biological effects (Table 4). Either the effects are attributable to currently unidentified metabolites of the procyanidins or the effects are attributable to another component, such as the monomeric catechins (or both).

Microbial metabolites of polyphenols

The data on the bioavailability of polyphenols presented above considered only the presence of intact polyphenols in the blood, ie, the ingested compound or its conjugates. The extensive microflora in the colon also plays a critical role in the metabolism of polyphenols. After microbial enzyme-catalyzed deconjugation of any polyphenol conjugates that reach the colon, there are 2 possible routes available, namely, absorption of the intact polyphenol through the colonic epithelium and passage into the bloodstream (as free or conjugated forms) or breakdown of the original polyphenol structure into metabolites. The absorption data presented above include the contribution of the absorption of intact polyphenols in the colon but do not include the breakdown contribution. Microbial metabolism deserves special consideration, because many of the diverse polyphenols are broken down into simpler phenolic compounds that are common to many different polyphenols. In addition, some of the microbial metabolites could have unique biological effects. For example, the isoflavone daidzein is converted to equol by gut microflora in $\sim$30–40% of the population, and the equol is absorbed into the bloodstream in these people. There is emerging evidence that "equol producers" demonstrate better effects on some biomarkers, such as bone mineral density, after isoflavone consumption, compared with nonproducers (109). This is an example in which microflora activate a polyphenol to a more potent biologically active compound. Although intervention studies demonstrate an effect for procyanidins, the identity of the active component (or components) is not clear. Although intact procyanidins have some biological effects, they are poorly absorbed in an intact form (114, 118, 119). The active species could therefore be metabolites. Some low-molecular weight metabolites were identified in humans in vivo after consumption of cocoa procyanidins (120), but the biological activities of these metabolites are not known and remain to be investigated.

In summary, there are now many human intervention studies in the literature that show biological effects, but the exact effects depend on the class of polyphenol used. There are clear gaps. Most of the studies were short term, and there is a real need for longer-term studies; very few studies demonstrated a dose-response relationship, and this is also needed for convincing evidence. In addition, most studies, with the exception of those

TABLE 4
Human intervention studies of procyanidins or procyanidin-containing foods

Substances given	Principal polyphenols[1]	Dose per day	Days	No. of subjects per group[2]	Biomarkers significantly affected	Biomarkers not significantly affected	Ref
Semisweet chocolate	Procyanidins, (−)-epicatechin	−420 mg procyanidins, 137 mg (−)-epicatechin	1	13	Increase in plasma antioxidant activity, decrease in plasma thiobarbituric acid-reactive substances		62
Chocolate chips	Procyanidins, catechins	220 mg flavonoids	1	18	Reduction in ADP/collagen-stimulated and adrenalin/collagen-stimulated, platelet-related primary hemostasis		63
Cocoa	Procyanidins, catechins	897 mg total	1	16	Inhibition of adrenalin-stimulated platelet activation and function		64
Cocoa powder/dark chocolate	Procyanidins, catechins	466 mg procyanidins	28	23	LDL oxidation lag time decreased by 8%, HDL cholesterol 4% higher	Thromboxane B2 or 6-keto-prostaglandin, plasma cholesterol, triglycerides	65
Cocoa	Procyanidins, catechins	70 mg (+)-catechin/(−)-epicatechin and 106 mg procyanidins (compared with equivalent with low polyphenols)	1	20	Endothelium-dependent dilation of brachial artery from 3.4% to 6.3%, plasma nitrosated/nitrosylated species increased by ~60%	Brachial artery diameter, forearm blood flow, blood pressure, heart rate, plasma nitrite, plasma nitrate	66
Cocoa tablets	Procyanidins, catechins	234 mg polyphenols	28	32	Increase in plasma ascorbic acid, lower P-selectin expression, lower ADP-induced platelet aggregation, lower collagen-induced platelet aggregation	Plasma antioxidant status, plasma oxidation biomarkers	67
Cocoa	Procyanidins, catechins	500 mg polyphenols	14	13	Lower systolic and diastolic blood pressure	Heart rate, plasma cholesterol, triglyceride, and glucose concentrations	68
Chocolate	Procyanidins, catechins	320 mg procyanidin, 104 mg (−)-epicatechin	1	20	Small increase in plasma antioxidant activity	Plasma 8-isoprostane	69
Cocoa	Procyanidins, catechins	100 g dark chocolate	1	12	Increase in plasma antioxidant activity by 20%	Addition of milk reduced the effect on plasma antioxidant activity	70
Pycnogenol (French pine bark extract)	Procyanidins, some (+)-catechin	75 mg	30	30 women with melasma	Decrease in average melasma area		71
Pycnogenol (French pine bark extract)	Procyanidins, some (+)-catechin	150 mg	42	25	Increase of plasma oxygen radical absorbance capacity, decrease in LDL cholesterol, increase in HDL cholesterol	LDL oxidizability, plasma lipid peroxides	72
Pycnogenol (French pine bark extract)	Procyanidins, some (+)-catechin	200 mg	56	11	Decrease in systolic blood pressure, decrease in serum thromboxane		73
Pycnogenol (French pine bark extract)	Procyanidins, some (+)-catechin	120 mg for 30 d, then 60 mg for 30 d	60	11	Decrease in serum reactive oxygen species, lymphocyte apoptosis and $p56^{lck}$, reduction in erythrocyte sedimentation rate		74

(*Continued*)

TABLE 4 (*Continued*)

Substances given	Principal polyphenols[1]	Dose per day	Days	No. of subjects per group[2]	Biomarkers significantly affected	Biomarkers not significantly affected	Ref
Pycnogenol (French pine bark extract)	Procyanidins, some (+)-catechin	300 mg	60	40 with chronic venous insufficiency	Disappearance of edema and pain, reduction in leg heaviness	Venous blood flow	75
Pycnogenol (French pine bark extract)	Procyanidins, some (+)-catechin	100 mg	1 dose	16 smokers	Decrease in smoking-induced platelet reactivity		76
Pycnogenol (French pine bark extract)	Procyanidins, some (+)-catechin	150 mg	28	60	Decreased platelet aggregation, increased diameter of microvessels	Blood glucose and lipids	77
Pycnogenol (French pine bark extract)	Procyanidins, some (+)-catechin	200 mg	56	19 smokers	Reduced platelet activity, reduced serum thromboxane B2	(No effect in non-smokers)	78
Pycnogenol (French pine bark extract)	Procyanidins, some (+)-catechin	200 mg	30	24 athletes	Increased endurance		79
Pycnogenol (French pine bark extract)	Procyanidins, some (+)-catechin	200 mg	90	4 sub-fertile men	Improved sperm morphologic features	Ham's F10 capacitated count, motility score	80
Pycnogenol (French pine bark extract)	Procyanidins, some (+)-catechin	1.1, then 1.7 mg/kg body weight	28 + 28	21	Decrease in ultraviolet light sensitivity		81
Pycnogenol (French pine bark extract)	Procyanidins, some (+)-catechin	30 mg	14 + 28	39 patients with endometriosis, severe menstrual pain or chronic pelvic pain	Reduction in menstrual cramps, abdominal pain, and tenderness		82
Cranberry juice	Procyanidins	50 mL juice	180	150 women with urinary tract infections	20% decrease in recurrence of urinary tract infections		83
Cranberry juice	Procyanidins	500 mL juice	1 dose	9	Plasma vitamin C increased by 30%, plasma antioxidant activity increased		84
Cranberry juice	Procyanidins	354 mL juice		112 men with prostate cancer undergoing radiotherapy		Urinary symptoms	85
Blueberry	Procyanidins, anthocyanins, quercetin	500 mL juice	1	9		Plasma vitamin C, plasma antioxidant activity	84
Black currant and apple juice	Procyanidins, anthocyanins, quercetin	1.5 L (9.6 mg quercetin)	5	7	Plasma malondialdehyde decreased, plasma 2-amino-adipic acid semialdehyde increased	Erythrocyte 2-aminoadipic semialdehyde, plasma trolox equivalent antioxidant activity, γ-glutamyl semialdehyde	86
Red wine	Procyanidins, anthocyanins, quercetin	500 mL	1	5		No effect on immune functions (tumor necrosis factor-α, interleukin-2, interleukin-4, lymphocyte proliferation, phagocytic activity)	87
Red wine	Procyanidins, anthocyanins, quercetin	375 mL	14	9	Decreased plasma lipid peroxides	LDL or HDL cholesterol, plasma triacylglycerol	88
Red wine	Procyanidins, anthocyanins, quercetin	4 glasses	21	11	Increased plasma homocysteine, increased plasma vitamin B6		89
Red wine	Procyanidins, anthocyanins, quercetin	240 mL wine	30	6	Maintenance of endothelial function, compared with loss with a high-fat diet		90

(*Continued*)

TABLE 4 (*Continued*)

Substances given	Principal polyphenols[1]	Dose per day	Days	No. of subjects per group[2]	Biomarkers significantly affected	Biomarkers not significantly affected	Ref
Purple grape juice	Procyanidins	7 mL/kg	14	20	Platelet aggregation inhibited, platelet-derived nitric oxide production increased, superoxide release decreased, α-tocopherol increased, plasma protein-independent activity increased		91
Purple grape juice	Procyanidins	2 × 8mL/kg body weight	56	22 (age: 64 ± 10 y) with severe endothelial dysfunction	Increased flow-mediated dilation of the brachial artery	Plasma glucose or insulin, nitroglycerin-mediated vasodilation	92
Grape juice	Procyanidins	400 mL juice	7–10	10	Platelet aggregation reduced by 77%	No effect of orange or grapefruit juice	93
Grape polyphenol extract	Procyanidins	300 mg	28	24 male smokers	Small decrease in plasma thiobarbituric acid-reactive substances and increase in LDL oxidation lag phase	HDL, LDL, or total cholesterol	94
Grape seed extract	Procyanidins	100 mg	60	40 hypercholesterolemic subjects	Decrease in circulating auto-antibodies to oxidized LDL	LDL/HDL cholesterol, triglycerides	95
Purple grape juice	Procyanidins	7.7 mL/kg	14	15 adults with coronary artery disease	Flow-mediated dilation of blood vessels, LDL lag time increased		96
Pomegranate juice	Polyphenol mixture	1.5 mmol total polyphenols	14	10 hypertensive patients	Increased serum paraoxonase activity, increased plasma total antioxidant activity, increase in resistance of LDL to oxidation, decreased serum angiotensin-converting enzyme, decreased cytochrome P450 activity, decreased LDL susceptibility to aggregation, increased serum paraoxonase	Total cholesterol, LDL cholesterol, triglycerides	97, 98
Endothelon[3]	Procyanidins	100 mg	14	37 elderly subjects with microcirculatory problems	Beneficial effect on capillary fragility and permeability		99
Endothelon[3]	Procyanidins	200 mg	35	50	Improvement of the visual performance after glare, of the visual adaptation to low luminances		100
Endothelon[3]	Procyanidins	150 mg	30–60	13 hypertensive and diabetic patients	Increase of capillary resistance		101

[1] The measured polyphenol; the dose is given in the next column.
[2] Healthy subjects, unless otherwise stated.
[3] Endothelon, standardized grape seed extract.

with isoflavones, administered food instead of pure compounds, and the effects noted may thus be attributable to some other component in that food. Finally, metabolism by microflora needs to be understood, because the gut microflora probably plays a major role in the biological activity of many polyphenols.

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