Dietary Polyphenols and the Prevention of Diseases

AUGUSTIN SCALBERT, CLAUDINE MANACH, CHRISTINE MORAND, and CHRISTIAN RÉMÉSY
Laboratoire des Maladies Métaboliques et Micronutriments, INRA, Centre de Recherche de Clermont-Ferrand/Theix, St-Genès-Champanelle, France

LILIANA JIMÉNEZ
Danone Vitapole Recherche, Palaiseau Cedex, France

Polyphenols are the most abundant antioxidants in the diet and are widespread constituents of fruits, vegetables, cereals, dry legumes, chocolate, and beverages, such as tea, coffee, or wine. Experimental studies on animals or cultured human cell lines support a role of polyphenols in the prevention of cardiovascular diseases, cancers, neurodegenerative diseases, diabetes, or osteoporosis. However, it is very difficult to predict from these results the effects of polyphenol intake on disease prevention in humans. One of the reasons is that these studies have often been conducted at doses or concentrations far beyond those documented in humans. The few clinical studies on biomarkers of oxidative stress, cardiovascular disease risk factors, and tumor or bone resorption biomarkers have often led to contradictory results. Epidemiological studies have repeatedly shown an inverse association between the risk of myocardial infarction and the consumption of tea and wine or the intake level of some particular flavonoids, but no clear associations have been found between cancer risk and polyphenol consumption. More human studies are needed to provide clear evidence of their health protective effects and to better evaluate the risks possibly resulting from too high a polyphenol consumption.

**Keywords** antioxidants, cancers, cardiovascular diseases, diabetes, flavonoids, neurodegenerative diseases, osteoporosis, polyphenols

INTRODUCTION

Polyphenols are common constituents of foods of plant origin and major antioxidants of our diet. The main dietary sources of polyphenols are fruits and beverages. Fruits like apple, grape, pear, cherry, and various berries contain up to 200–300 mg polyphenols per 100 g fresh weight. Typically, a glass of red wine or a cup of tea or coffee contains about 100 mg polyphenols. Cereals, chocolate, and dry legumes also contribute to the polyphenol intake. The total dietary intake is about 1 g/d. It is much higher than that of all other known dietary antioxidants, about 10 times higher than that of vitamin C and 100 times higher than those of vitamin E and carotenoids.

Several hundreds of different polyphenols have been identified in foods. The two main types of polyphenols are flavonoids and phenolic acids. Flavonoids are themselves distributed among several classes: flavones, flavonols, flavanols, flavanones, isoflavonoids, proanthocyanidins, and anthocyanins. Some of the most common flavonoids are quercetin, a flavonol abundant in onion, tea, and apple; catechin, a flavanol found in tea and several fruits; hesperetin, a flavanone present in citrus fruits; cyanidin, an anthocyanin giving its color to many red fruits (blackcurrant, raspberry, strawberry, etc.); daidzein, the main isoflavone in soybean; proanthocyanidins, common in many fruits, such as apple, grape, or cocoa and are responsible for their characteristic astringency or bitterness (Figure 1). One of the most common phenolic acids is caffeic acid, present in many fruits and vegetables, most often esterified with quinic acid as in chlorogenic acid, which is the major phenolic compound in coffee. Another common phenolic acid is ferulic acid, which is present in cereals and is esterified to hemicelluloses in the cell wall.

As antioxidants, polyphenols may protect cell constituents against oxidative damage and, therefore, limit the risk of various degenerative diseases associated to oxidative stress. As
compared to other antioxidants, research on their health effects started more recently. This late interest for polyphenols is largely explained by the complexity of their chemical structures. Numerous studies on animal models have shown that, when added to the diet, they limit the development of cancers, cardiovascular diseases, neurodegenerative diseases, diabetes, and osteoporosis (see4 for a review and references cited in the following sections). The purpose of this article is to critically review first the antioxidant effects of polyphenols and their relevance for health, and secondly the experimental evidence supporting a protective role of polyphenols against the main degenerative diseases affecting Westernized populations. Emphasis is placed on clinical and epidemiological studies. Evidence based on animal experiments is also presented and discussed with regards to the doses of polyphenols administered which may widely differ from those experienced by humans with their diet. The mechanisms of action as suggested by animal and in vitro studies are also briefly summarized. Lastly, the possible risks associated to high polyphenol consumption are discussed.

**ANTIOXIDANT PROPERTIES OF POLYPHENOLS**

Degenerative diseases, such as cancers, cardiovascular diseases, osteoporosis, and degenerative diseases, are associated with aging. Oxidative damage to cell components, DNA, proteins, and lipids accumulates with age and contributes to the degeneration of the somatic cells and to the pathogenesis of these diseases. Antioxidants present in food can help limit this damage by acting directly on reactive oxygen species or by stimulating endogenous defence systems. The phenolic groups in polyphenols can accept an electron to form relatively stable phenoxyl radicals, thereby disrupting chain oxidation reactions in cellular components.5

The antioxidant potency of polyphenols has been evaluated in vitro by measuring their ability to trap free radicals and reduce other chemicals. Their potency is compared to that of a reference substance, usually Trolox (a water-soluble derivative of vitamin E), gallic acid, or catechin. In all cases, the reaction studied is the reduction of an oxidant by polyphenols. The most commonly used oxidants are listed below:

1. ABTS°⁺ formed from ABTS (2,2'-azonobis(3-ethylbenzothiazoline-6-sulphonic acid) in the presence of metmyoglobin and hydrogen peroxide.6 The reaction is monitored by colorimetric determination of the colored ABTS°⁺ radical. This method is often referred to as the TEAC method (Trolox Equivalent Antioxidant Capacity).
2. Radicals formed by heating AAPH (2,2'-azobis (2-amidinopropane) dihydrochloride) or ABAP (2,2'-azobis (2-amidinopropane) hydrochloride). The radical concentration is usually monitored by following the degradation of phycoerythrin by fluorimetry. This technique is known as the ORAC (Oxygen Radical Absorbance Capacity)7 or TRAP method (Total Radical-trapping Antioxidant Parameter).8
3. Ferric ions: This approach is known as the FRAP method (Ferric-Reducing Ability of Plasma).9
4. Radicals formed by autoxidation of linoleic acid.10
Polyphenols with catechol groups (aromatic rings with two hydroxyl groups in the ortho position) have greater antioxidant potency than those with simple phenol groups (aromatic rings with a single hydroxyl group). Quercetin and epigallocatechin, for example, have been shown to be excellent traps for ABTS$^+$ and are almost five times more active than Trolox at equivalent molar concentrations.$^{11,12}$

However, the value of such measurements for comparing the potential health benefits of isolated polyphenols or plant extracts is limited for a number of reasons:

1. Quite different results are observed when the antioxidant properties of different polyphenols are compared using different assays. There is, thus, no clear correlation between the ability of 13 different phenolic compounds to reduce ABTS$^+$ on the one hand$^{11}$ or radicals derived from AAPH on the other hand$^{13}$ (Figure 2).

2. Polyphenols are extensively metabolized in the body, and the majority of catechol nuclei are methylated, dehydroxylated, or conjugated by O-glucuronidation and formation of sulphate esters.$^{1,3}$ These reactions modify their antioxidant capacity.$^{14}$ O-Conjugation of the catechol hydroxyl group prevent their oxidation and transformation into toxic quinones. The quinones formed from by oxidation of catechol substrates, such as plant polyphenols, oestrogens, and catecholamines, participate to redox cycles that generate superoxide radicals or react with nucleophilic cell components.$^{15-17}$ The catechol polyphenols with the greatest antioxidant capacity may, thus, also be the most toxic. Polyphenol metabolism could account for the absence of carcinogenicity of quercetin despite its well established mutagenic properties in vitro.$^{18}$

3. Antioxidant properties of polyphenols largely depend on their chemical and physico-chemical environment, which varies according to tissues and physiological conditions and a fortiori from the conditions of the in vitro antioxidant assays. The major influence of the presence of plasma proteins, such as albumin,$^{19}$ and the presence of other antioxidants,$^{20}$ has been described.

4. The impact of polyphenols on antioxidant protection of tissues has been previously determined by their bioavailability, which differs considerably from one polyphenol to the other.$^{1,3}$

It should, therefore, not be a surprise that no correlation between the antioxidant potency of various polyphenols measured in vitro and their biological activity determined in vivo or at the cellular level has ever been published.

In summary, health benefits of polyphenols cannot be merely reduced to their antioxidant potency as measured in vitro. Antioxidant assays applied to different polyphenols or to food extracts are of poor predictive value to compare their health benefits. A precise examination of the type and content of the different polyphenols in a given plant extract or food should clearly be preferred. However, this does not exclude redox properties of polyphenols from being key factors that may trigger various cell responses at the origin of their biological effects.$^{21}$

When ingested, polyphenol-rich foods and beverages increase the antioxidative capacity of plasma. The antioxidative capacity is measured by the same methods as those used to compare different food extracts in vitro, chiefly ORAC and FRAP, or by a chemiluminescence assay, where the decomposition of free radicals formed by horseradish peroxidase from sodium perborate or hydrogen peroxide is followed by their reaction to luminol, which emits light on decomposition.$^{22,23}$ Increased antioxidative capacity is systematically observed over the hours following the intake of polyphenol-rich beverages, such as tea,$^{24-27}$ wine,$^{7,27-31}$ and beer,$^{32}$ as well as fruit and vegetables rich in polyphenols, such as strawberries and spinach.$^7$ This increase in the plasma antioxidative capacity of plasma following the consumption of polyphenol-rich food may be explained either by the presence of reducing polyphenols and their metabolites in plasma, by their effects upon concentrations of other reducing agents (sparring effects of polyphenols on other endogenous antioxidants), or by their effect on the absorption of pro-oxidative food components, such as iron (see section Risks Associated to Polyphenol Consumption).

If it is well established that polyphenol ingestion results in an increase of the plasma antioxidant capacity, there is still some uncertainties about its efficiency to enhance the protection of cellular components, such as lipids or DNA, against oxidative stress in humans. In some studies, polyphenol intake reduced the plasma concentration of lipid oxidation products. The ingestion of tea catechins (250 mg) by healthy volunteers decreased the plasma concentrations of phospholipid peroxides.$^{33}$ Similarly, the consumption of polyphenol-rich blackcurrant and apple juice by healthy volunteers (1.5 l/d for 7 d) significantly reduced the plasma concentrations of malondialdehyde (MDA).$^{34}$ The consumption of chocolate (80 g) also resulted in decreased...
concentrations of oxidised lipids, as measured by reaction with thiobarbituric acid. In contrast, the consumption of red wine (200 mL per d for 10 d) had no significant effect on plasma MDA levels. The consumption of 55 mg of isoflavones for 8 wk had no effect upon concentrations of F2-isoprostane, a marker of lipid peroxidation.

The effects of polyphenols on the stability of deoxyribonucleic acids (DNA) have also been examined. In vitro, polyphenols can have either harmful or protective effects. In the presence of transition metals, such as Cu(II) and Fe(III), phenolic compounds induce the breakage of DNA. Such effects are caused by a reduction of these transition metals that, once reduced, catalyze the formation of hydroxyl radical (OH−) (Fenton reaction). These reactions have also been noted in cultured cells. Such breakage of DNA has been considered both beneficial (cytotoxic and apoptotic effects on tumor cells) and toxic (mutagenic effects on normal cells).

Conversely, polyphenols may also protect DNA against degradation induced by cytotoxic agents. In vitro, they can inhibit the formation of adducts between activated polyyclic hydrocarbons and DNA. In particular, the antimutagenic properties of ellagic acid have been clearly established, at least in Salmonella typhimurium, and have been explained by the formation of inactive adducts between activated carcinogens and ellagic acid, or by inhibition of aryl hydrocarbon hydroxylase responsible for the activation of polyyclic hydrocarbons.

A number of in vivo studies have demonstrated the protective effect of polyphenol consumption against DNA damage. In the rat, wine polyphenols added to the diet protected hepatic DNA against oxidative damage induced by 2-nitropropane. Similar effects were observed with ellagic acid, but not epigallocatechin gallate (EGCG). Wine polyphenols added to the diet of rats also reduced basal DNA oxidative damage (without induction) to colonic mucosa. Supplementation of diet with tea theaflavins and thearubigins protected DNA in the colonic mucosa against oxidative damage induced by 1,2-dimethylyhydrizine or by inhibition of aryl hydrocarbon hydroxylase responsible for the activation of polyyclic hydrocarbons.

In humans, the consumption of antioxidants or of fruit and vegetables has been associated with reduced levels of oxidative damage to lymphocytic DNA. Similar observations have been made with polyphenol-rich foods or beverages. The consumption of 240 mL wine per d for one mo resulted in pronounced reduction in levels of oxidized DNA bases (8-OHdG) in blood leukocytes, in particular, when the volunteers were fed a high-fat diet. The consumption of onions (rich in quercetin) with meals helped increase resistance of lymphocytic DNA to ex vivo induced oxidation and reduced urinary excretion of 8-OHdG. The consumption of 400 g of onions and 6 cups of tea per d for 2 wk by diabetic patients also reduced the oxydizability of lymphocytic DNA, as assessed by the Comet assay. However, other authors have observed no effects on lymphocytic DNA of a similar diet in healthy volunteers, and no effects on urinary excretion of 8-OHdG following consumption of an extract of green tea for 3 wk. These contradictory data make difficult the evaluation of the importance of DNA protection by polyphenols for disease prevention, particularly since the relationship between oxidative damage to DNA and disease risk (in particular, risk of cancer) has not been established.

These difficulties in unravelling antioxidant effects of polyphenols in vivo may be due to some variations in the status of other antioxidants. It is likely that polyphenols act in synergy with other antioxidants. It has been shown in several in vitro studies that the relatively polar polyphenols regenerate or spare lipophilic antioxidants, such as vitamin E. Antioxidants effects of polyphenols may be more easily observed when the status in other antioxidants is low.

**POLYPHENOLS AND CARDIOVASCULAR DISEASE**

A number of animal studies have demonstrated that the consumption of polyphenols limits the development of atherosomatous lesions. Supplementation of drinking water with de-alcoholized wine, pomegranate juice, catechins, or quercetin reduced the size of these lesions in apoE-deficient mice. These effects are associated with reduced low density lipoprotein (LDL) uptake by macrophages, lower oxidation of isolated LDL (TBARS method), and decreased susceptibility of LDL to aggregation. Similar results were obtained through supplementation of a cholesterol-enriched diet with an extract of grape seeds rich in proanthocyanidins and administered to rabbits.

An abundant literature has shown that polyphenols can inhibit oxidation of LDL in vitro; this type of oxidation is considered to be a key mechanism in atherosclerosis. These antioxidant effects result in the decreased oxidation of LDL lipids and of α-tocopherol. However, evidence in humans is contradictory. Certain studies have shown that the consumption of beverages and foods rich in polyphenols (red wine, cocoa, tea, or pomegranate juice) resulted in reduced susceptibility of LDL to oxidation induced ex vivo by Cu(II). The lower levels of oxidation products of phosphatidylcholine (the main lipid found in LDL) observed after consumption of green tea catechins in man suggest that polyphenols effectively protect LDL against oxidation. However, several other studies have shown no effects of polyphenol consumption on the ex vivo oxidation of LDL. In the plasma, polyphenols are largely conjugated with glucuronide and sulfate groups. They are, therefore, polar and, most probably, largely eliminated during the isolation of LDL preceding the ex-vivo oxidation test. This could explain the lack of protection observed in these last studies. This does not exclude the association of some polyphenol aglycones or of some esters with fatty acids to LDL, as has been suggested in an experiment where [3H]-genistein was added to human plasma.

These antioxidant effects are doubtlessly insufficient in themselves to account for the suggested protective effects of polyphenols against cardiovascular diseases. Tea catechins were shown to inhibit the invasion and proliferation of the smooth muscle cells in the arterial wall, a mechanism that may contribute to slow down the formation of the atheromatous lesion. Polyphenols
may also modify lipid metabolism, but the data are, again, contradictory. Grape seed polyphenols have a hypocholesterolemic effect on rats fed a high-cholesterol diet. The consumption of flavonones by normolipemic or hyperlipemic rats during 2–6 wk reduced total plasma cholesterol, LDL-cholesterol, and triglycerides. In contrast, in rabbits, dietary supplementation with cocoa polyphenols for 10 d had no effect upon the plasma lipid profile. In apoe-deficient mice, catechins derived from tea did not affect plasma lipid concentrations, but induced a decrease in cholesterol and triglyceride levels in the aorta. In man, acute consumption of one cup of black tea or consumption of 6 cups of green or black tea over a 4-wk period had no effect on plasma concentrations of cholesterol and triglycerides. Several other clinical trials also failed to show any effects of isoflavones on plasma lipids.

Polyphenols may exert antithrombotic effects. They inhibit platelet aggregation in vitro. They were also shown to inhibit platelet aggregation in several animal models: the consumption of red wine (rich in polyphenols), rather than white wine or alcohol, in the rat prevented the platelet rebound effect (measured by ex vivo thrombin-induced platelet aggregation), otherwise observed in the hours following alcohol withdrawal. The consumption of red wine or non-alcoholic red wine reduces bleeding time and platelet aggregation induced by collagen in the rat. Thrombosis induced by stenosis of the coronary artery in the dog is inhibited when red wine or grape juice, but not white wine, is administered by gastric intubation. Platelet aggregation induced ex vivo by collagen is inhibited in the monkey by consumption for 7 d of grape juice, but not by orange juice or grapefruit juice. Evidence in humans is more limited. The consumption of a procyanidin-rich cocoa beverage by human subjects inhibited the activation of platelets when stimulated ex vivo by epinephrine or ADP in the 2–6 h following ingestion. However, no effects were observed with other flavonoid-rich foods, such as onion, parsley, soy, citrus juices, or tea.

Polyphenols can improve endothelial dysfunction, an early event in atherogenesis. Endothelial dysfunction is associated with different risk factors for atherosclerosis before the plaque is formed; its use as a prognostic tool for coronary heart disease has been proposed. The endothelial-dependent vasorelaxing activity of isolated polyphenols, such as wine anthocyanins, soy isoflavones, resveratrol, quercetin, and cocoa procyanidins, has been observed on isolated rat or rabbit aorta and in female macaques. These effects could be mediated by the protection of the vasorelaxant factor nitric oxide against oxidation. In human subjects, endothelial dysfunction can be assessed by measuring the brachial artery flow-mediated dilation. The consumption of black tea (450 mL) increased artery dilation 2 h after intake by coronary patients. A similar improvement of endothelial function was observed when the same patients consumed 900 mL of tea/d during 4 wk. The consumption of 240 mL red wine during 30 d also counteracted the endothelial dysfunction induced by a high-fat diet.

Associations between polyphenol intake or the consumption of polyphenol-rich foods were examined in several epidemiological studies. Both the consumption of tea and a moderate consumption of wine have been regularly associated to a lower risk of myocardial infarction in both case-control and cohort studies. However, an increased risk was observed in two studies carried out in the UK, where the consumption of tea is particularly high. This could be explained by an insufficient adjustment for lifestyle factors. An inverse association between flavonol and flavone intake and the risk of coronary death or non-fatal myocardial infarction has been observed in several cohort studies. Catechin intake has also been associated to a lower risk of coronary death but not to stroke. These results of molecular epidemiology are still fragmentary, as flavonols, flavone, and catechins do not account for more than one tenth of the total polyphenol intake. Reliable food composition data are needed to estimate the consumption of other polyphenols and to study its association with disease risk.

### POLYPHENOLS AND CANCER

Anticarcinogenic effects of polyphenols are well documented in animals. Polyphenols, when given to rats or mice before and/or after the administration of a carcinogenic agent or the implantation of a human cancer cell line, are most often protective and induce a reduction of the number of tumors or of their growth. These effects have been observed at various sites, including mouth, stomach, duodenum, colon, liver, lung, mammary, or skin. Many polyphenols, such as quercetin, catechins, isoflavones, lignans, flavanones, ellagic acid, red wine polyphenols, resveratrol, or curcumin, were tested; all of them showed protective effects in some models. Different mechanisms have been suggested to explain their anticarcinogenic effects. First, polyphenols may act as blocking agents at the initiation stage. They influence the metabolism of procarcinogens by modulating the expression of cytochrome P450 enzymes involved in their activation to carcinogens. They may also facilitate their excretion by increasing the expression of phase II conjugating enzymes. This induction of phase II enzymes may have its origin in the toxicity of polyphenols. Polyphenols can form potentially toxic quinones in the body that are, themselves, substrates of these enzymes. The intake of polyphenols could then activate these enzymes for their own detoxication and, thus, induce a general boosting of our defenses against toxic xenobiotoxins. Polyphenols may also limit the formation of initiated cells by stimulating DNA repair.

Secondly, polyphenols can act as suppressing agents, and inhibit the formation and growth of tumors from initiated cells; they inhibit cell proliferation in vitro. It was also shown that some polyphenols can affect growth-related signal transduction pathways through inhibition of protein kinase C and AP-1-dependent transcriptional activity. They inhibit oncogene expression and the activity of ornithine decarboxylase, a key enzyme in the synthesis of polyamines associated to cell proliferation. They may also inhibit cell proliferation through their effect on the metabolism of arachidonic acid.
Both green tea polyphenols and curcumin were found to inhibit cyclooxygenase-2 activity and arachidonic metabolism in the colonic mucosa of rats. Phenolic phytoestrogens could influence the growth of hormone responsive tumors through their estrogenic properties or their capacity to affect the response to endogenous estrogens. This may explain the protective effects of isoflavones against mammary and prostate cancers observed in different animal models.

Polyphenols can induce apoptosis of tumor cells and, therefore, reduce the growth of tumors. Evidence has been given both in vitro and in vivo. However, the significance of this mechanism in cancer prevention is not clear, as polyphenols may also have opposite effects. They were shown to inhibit apoptosis of some non-tumorigenic cells when induced by hydrogen peroxide. Lastly, polyphenols, such as those of green tea, can also inhibit angiogenesis and, therefore, limit the growth of the tumors. Prevent tumor invasion through inhibition of the matrix metalloproteinases. However, in an experiment with nude-mice inoculated sub-cutaneously with a human colon carcinoma cell line, the growth of the tumors was inhibited by EGCG added in the drinking water from d 45, after inoculation and thereafter. The authors concluded that an effect on angiogenesis starting earlier in the development of the tumor was unlikely; they showed that an inhibition of telomerase was involved.

The anticarcinogenic properties of polyphenols could, thus, be explained by many different mechanisms. To explain their protective effect by their antioxidant properties and inhibition of DNA oxidative damage is certainly an oversimplification. However, various antioxidants, including polyphenols, inhibit NF-kB activation, probably through triggering a redox-sensitive signal in the cells. The inhibition of such transcription factors by polyphenols may play an essential role in the prevention of cancers. Beyond these, many hypotheses on mechanisms of action, the most difficult task, remain to demonstrate their anticarcinogenic effects in humans.

Often, the doses used in animal or cell experiments largely exceed those that can be expected in humans on a regular diet. All the corresponding literature should clearly be re-examined to take into account the doses applied, as well as the mode of administration. More credit should be given to the effects observed at low doses. Intravenous administration of very high doses of quercetin (up to 2000 mg/m²) at a 3-wk interval to 51 patients having a cancer resistant to standard therapies induced a decrease of some tumor markers. These doses are clearly far beyond what could be expected from dietary exposure, and such results are of low value to elucidate the role of dietary polyphenols in cancer prevention. A clear distinction between cancer treatment at pharmacological doses and cancer prevention at dietary levels of exposure should be made when discussing experimental results obtained on animal models or on cell lines grown in vitro. The confusion is often maintained on purpose, to communicate on the beneficial health effects of polyphenol-containing food products. For example, resveratrol has interesting anticarcinogenic properties that may lead to the development of new drugs. Its presence in wine and absence in any food sources has stimulated the interest of the industry to promote the health properties of wine. However, its very low concentration in wine (0.3–2 mg/L in red wines) makes the attribution of the wine health benefits to this molecule unlikely.

The question of doses is essential, as opposite effects have been observed at different exposure levels. Caffeic acid induces hyperplasia and tumors in the forestomach and kidney when administered in the diet of rats or mice at a dose of 0.5–2% of the diet, whereas it shows anticarcinogenic effects at doses of 0.05–0.15 %. On the contrary, genistein at high doses (50–100 µM) inhibits the growth of human breast cancer cells in vitro, whereas it induces proliferation at lower doses (0.01–10 µM), effects that were explained by their estrogenic properties at low doses and cytotoxicity at higher doses. A similar influence of the dose of genistein was observed on the expression of prostate-specific antigen by prostate cancer cells. Neurodegeneration induced in mice by N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine was prevented by low doses of green tea (0.5–1 mg/kg i.p.), whereas higher doses (5 mg/kg) increased the toxicity of the drug.

In the future, more attention should be paid to those studies showing effects at doses compatible with exposure experienced by humans with their diet, or to in vitro studies using polyphenol concentrations close to documented plasmatic or tissular concentrations. When calculating the dose to be administered to animals, extrapolation from human dietary intake should be made, preferentially at a constant content in the diet rather than at a constant intake per body weight unit, as has been recently discussed. For example, an intake of 40 mg/d isoflavone, an average value for Japanese populations, would correspond to a dose of about 0.01% in the diet, well below most of the doses tested in animal experiments.

More credit should also be given to the studies where polyphenols were administered per os, or to in vitro studies using polyphenol metabolites (largely conjugated metabolites), and not polyphenols in their ingested form. Indeed, as stressed above, polyphenols are essentially present in blood and tissues as conjugated metabolites, and there is still limited evidence that they can be deconjugated in vivo. Thus, studies where dietary polyphenols are administered by injection are of limited value to understand their health effects, and in vitro studies with non-conjugated polyphenols should be re-evaluated using their conjugated metabolites.

The final evidence on the prevention of cancers by polyphenols will come from clinical and epidemiological studies. Tumor biomarkers are useful tools for prognosis, for the monitoring of therapy in various cancers, and for the evaluation of the influence of diet on the disease. Some polyphenols have been shown to reduce the levels of tumor biomarkers in different cancer cell lines. Genistein decreased the expression of protein-specific antigen (PSA) in prostate cancer cells, and EGCG, epicatechin gallate, or genistein significantly reduced in a human lung cancer cell line, the levels of heterogeneous nuclear ribonucleoprotein B1, a new biomarker for early clinical diagnosis of lung cancer. However, clinical evidence of an effect of polyphenols
on tumor biomarkers is still very limited. The comparison of a 6-wk consumption of an isoflavone-rich and isoflavone-poor soy beverages providing 69 and 3 mg/d isoflavones, respectively, failed to show any impact of isoflavones on two tumor biomarkers in 34 elderly men with elevated PSA. \(^{159}\) On the other hand, the consumption of a soy-containing diet providing an average of 154 mg/d isoflavones by 10 women during 1 mo resulted in a significative decrease of estradiol and progesterone plasmatic levels, two biomarkers for breast cancer risk. \(^{160}\) More studies are needed to establish dietary recommendations for cancer patients.

Polyphenol supplements might be useful as adjuvants in chemotherapy or radiotherapy treatments. Some polyphenols were shown to reinforce the antiproliferative activities of anticancer drugs. EGCG showed synergistic effects with sulin-dac or tamoxifen on apoptosis of the lung cancer cell line PC-9, \(^{161}\) and quercetin potentiated the growth inhibition of ovarian cancer cells and leukemia cells by cisplatin. \(^{162},^{163}\) The oral administration of green tea to Ehrlich ascites carcinoma tumor-bearing mice enhanced the anti-tumor activity of doxorubicin. \(^{164}\) However, such adjuvant effects vary widely between polyphenols. Galangin, when tested on leukemia cells, showed opposite effects to quercetin and inhibited the anti-apoptotic effects of cisplatin. \(^{165}\) Tangeretin, a citrus polymethoxylated flavonoid, when added to the diet of nude mice, inhibited the cytotoxic effects of tamoxifen on MCF-7 breast cancer cell inoculated subcutaneously. \(^{166}\) Clinical trials will be needed to establish adjuvant effects of the most promising polyphenols in cancer patients.

The associations between the consumption of coffee, tea, and wine and the risk of cancer have been studied in different epidemiological studies. The consumption of coffee has been associated to a reduced risk of colorectal cancer but not with cancers at other sites. \(^{166}\) Experimental evidence on tea strongly suggests a protective role of tea consumption against cancers, but the epidemiological evidence is inconclusive. Although some inverse associations between stomach or colon cancer risk and tea consumption were observed in some case-control and prospective cohort studies, when tested on leukemia cells, showed opposite effects to quercetin and inhibited the anti-apoptotic effects of cisplatin. \(^{165}\) Tangeretin, a citrus polymethoxylated flavonoid, when added to the diet of nude mice, inhibited the cytotoxic effects of tamoxifen on MCF-7 breast cancer cell inoculated subcutaneously. \(^{166}\) Clinical trials will be needed to establish adjuvant effects of the most promising polyphenols in cancer patients.

The associations between the consumption of coffee, tea, and wine and the risk of cancer have been studied in different epidemiological studies. The consumption of coffee has been associated to a reduced risk of colorectal cancer but not with cancers at other sites. \(^{166}\) Experimental evidence on tea strongly suggests a protective role of tea consumption against cancers, but the epidemiological evidence is inconclusive. Although some inverse associations between stomach or colon cancer risk and tea consumption were observed in some case-control and prospective cohort studies, \(^{167},^{168}\) the majority of ecologic, cohort, or case-control studies suggest that tea drinking has no clear effect on cancer, possibly due to the much lower intake compared to the animal experiments. \(^{169}–^{172}\) The possible existence of confounding factors in epidemiological studies has also been proposed to explain such discrepancies.

A prospective study has suggested that wine polyphenols may protect against the deleterious effects of alcohol on cancers of the upper digestive tract. The consumption of alcoholic beverages, such as beer or spirits, increased the risk of upper digestive tract cancer in a Danish cohort, whereas a moderate consumption of wine did not increase this risk. \(^{173}\) Another study on the same cohort also suggested the protective effects of wine consumption against lung cancer. \(^{174}\) However, the existence of some confounding factors cannot be excluded, as a positive correlation between esophageal cancer risk and wine consumption was observed in a case-control study carried out in Italy, where wine is the most common alcoholic beverage. \(^{175}\) An increase of esophageal cancer risk was observed in people consuming 3 glasses of wine or more per d, as compared to those consuming less than 3 glasses. If wine may appear less toxic with regard to cancer risk than other alcoholic beverages, it should be, in the present state of our knowledge, regarded as a risk factor rather than a protective factor, as it was found to increase the risk of gastric, breast, and lung cancer in several epidemiological studies. \(^{176}–^{179}\) Furthermore, encouraging a moderate wine consumption may also result in an increase of alcoholism. \(^{180}\)

Different attempts have been made to directly relate the intake of some polyphenols to the risk of cancer. Flavonol intake (quercetin and kaempferol) was inversely associated to the risk of lung cancer in 5 case-control and cohort studies, \(^{181}–^{185}\) but no association was found in 3 other studies. \(^{186},^{187}\) For cancers at sites other than the lung, no association was found with flavonol intake in 3 large prospective studies, \(^{189},^{182},^{188}\) but 2 case-control studies showed a lower risk of cancer of the stomach and of the upper-aerodigestive tract at high flavonol intake. \(^{188},^{189}\)

Catechin intake was not significantly associated to cancer in a cohort of Dutch men, \(^{190}\) but an inverse association was found with rectal cancer in a large American cohort of postmenopausal women. \(^{191}\) The intake of catechin originating from fruits, but not from tea, was also associated to a lower risk of cancer of the upper-digestive tract in the same American cohort, \(^{191}\) whereas the urinary excretion of epigallocatechin was inversely associated to gastric and esophageal cancer in a nested case-control study carried out in Shangai. \(^{167}\)

The consumption of dietary sources of phytoestrogens has been repeatedly associated to a lower cancer risk. Epidemiological studies have suggested a protective role of the consumption of soy products, rich in isoflavones, against various cancers and, more particularly, hormone-related cancers. \(^{192}\) The consumption of whole-grain cereals, a major source of lignans, has also been associated to a reduced risk of various cancers. \(^{193}\) Several attempts were made to relate the exposure to dietary phytoestrogens to cancer risk. Three case-control studies showed a lower urinary excretion of isoflavones or lignans in breast cancer patients compared to controls, \(^{194}–^{196}\) but no significative association with genistein and enterolactone excretion was found in another study. \(^{197}\) Other authors found more a complex relationship with a higher risk of breast cancer associated to both the lowest and the highest plasma levels of enterolactone. \(^{198}\) Similar studies on prostate cancer suggested either a protective effect \(^{199},^{200}\) or no association \(^{201}\) with isoflavone or lignan exposure.

Altogether, the epidemiological data on polyphenols and cancer do not appear conclusive. It is possible that protective effects are limited to sub-groups of the population with particular genotypes or at a higher risk of developing disease. \(^{202}\) The causative role of dietary polyphenols, when a protective role is suggested, in observational studies must also be questioned. An association of myricetin or kaempferol intake with a lower prostate or gastric cancer risk has been reported, \(^{181},^{188}\) but such an association could be explained by some unknown confounding factors, as average daily intakes of myricetin and kaempferol do not
of cultured neuronal cells when challenged by a peptide, 6-hydroxydopamine, or oxidized LDL.213 Polyphe-
nols reduced the neurodegenerative changes induced by chronic oxidative stress. Dietary supplemen-
tation with grape polyphenols, such as proanthocyanidins, flavanones, phenolic acids, and anthocya-
nmins, are needed.

POLYPHENOLS AND NEURODEGENERATIVE DISEASES

Neurodegenerative diseases represent an increasing burden to our aging societies. About 15% of the population over 65 are afflicted by Alzheimer’s disease and 1% by Parkinson’s disease, not including other type of dementia resulting from ischemic injury.204 Such diseases are dependent of oxidative stress, which particularly affects brain tissues,205 and antioxidants may, therefore, contribute to their prevention.204 Feeding aging rats a diet supplemented with aqueous extracts of spinach, strawberry, or blueberry rich in polyphenols improved their cognitive functions and neuronal signal transduction.206,207 Blueberries rich in anthocyanins were particularly effective. These effects were not explained by a sparing of vitamins E and C in the brain.208 A direct implication of polyphenols as antioxidants is, therefore, suspected.

Intravenous injection of epicatechin or catechin to mice improved the memory impairment induced by cerebral ischemia.209 Polyphenols also protect experimental animals against some neurotoxic drugs whose toxicity is linked to a stimulation of oxidative stress. Dietary supplementation with grape polyphenols reduced the neurodegenerative changes induced by chronic ethanol consumption, and improved the synaptic function measured on isolated synaptosomes.210 The oral administration of EGCG restored the dopaminergic neurotransmission in rats injected with N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), a drug used to reproduce a parkinsonian syndrome, and prevented the increase of SOD and catalase induced by this drug.150 The chronic consumption of ferulic acid with the drinking water protected mice from the deleterious effects of an intracerebral injection of β-amyloid peptide, a component of senile plaques postulated to be involved in the pathogenesis of Alzheimer’s disease.211 It prevented the drop of learning and memory performance and the increase in the level of inflammation markers in the brain induced by the β-amyloid peptide, possibly via a transitory activation of hippocampal astrocytes. Similar protective effects were observed with curcumin in an Alzheimer transgenic mouse model.212

In vitro experiments showed that catechins improve the survival of cultured neuronal cells when challenged by a β-amyloid peptide, 6-hydroxydopamine, or oxidized LDL.213–215 These effects appear to be mediated by restoration of protein kinase C activity or the inhibition of NF-κB translocation, both mechanisms involved in the regulation of cell proliferation and apopto-
sis. These effects are dose dependent. At low doses (0.1–10 μM), epigallocatechin gallate protects neuronal cells against oxidative damage and improves cell survival, whereas at higher doses (50 μM), it appears pro-oxidant and toxic.213 Therefore, low polyphenol concentrations would be more effective to prevent neurodegenerative diseases. Little is known about the polyphenol concentrations in the brain. The concentrations reached in the brain after feeding rats with genistein are much lower (0.04 nmol/g tissue) than those reached in the plasma (2 μmol/L) and other tissues.216 The poor permeability of the blood-brain barrier to polyphenols was confirmed in other studies with naringin or quercetin.217,218 The glucuronide conjugate of epicatechin was unable to protect cortical neurons against oxidative stress induced by H2O2.154 However, the low amounts of polyphenols present in the brain may be only aglycone, as has been shown for genistein, and due to the poor permeability of the blood-brain barrier, to anionic conjugates.216,219

The relationship between polyphenol or wine consumption and neurodegenerative risk has been studied in a few epidemiological studies. A moderate wine consumption was negatively associated to the risk of dementia in 3 prospective studies carried out in France, Denmark, and Canada.220–222 Such an association was not observed for beer or spirits in the Danish cohort. This indicates a possible contribution of polyphenols to the prevention of dementia. Cognitive impairment was also found to be lower in moderate alcohol drinkers compared to non-drinkers in an Italian cohort, but the probability of cognitive impairment was increased in heavy alcohol drinkers.223 An inverse association between the intake of flavonols and flavones and the risk of dementia has also been observed in a French cohort.224

POLYPHENOLS AND DIABETES

Many plants have been traditionally used in the treatment of diabetes. Polyphenols contained in these plants may explain some of their therapeutic activity.225,226 The acute or chronic administration of polyphenols to experimental animals influences glycemia. Caffeic acid and isofurfural acid, when administered intravenously to rats, reduce the fasting glycemia and attenuate the increase of plasma glucose in an intravenous glucose tolerance test.227–229 These effects were observed in a genetic model of insulin-dependent diabetes of rats or in streptozotocin-treated rats, but are less pronounced in normal rats. More interestingly, some hypoglycemic effects were also observed with polyphenols administered orally, shortly before consumption of the glucose source. A diacylated anthocyanin reduced the peak of glycemia induced by maltose consumption in normal rats.230 An ill-defined leucodelphinidin (probably a mixture of prodelphinidins) reduced fasting glycemia in rats and lowered the plasma glucose peak in a glucose tolerance test.231 Similar effects were observed with 4-hydroxybenzoic acid.232 Catechin improved the tolerance to glucose induced by starch or sucrose ingestion in rats.233 A fermented tea extract showed hypoglycemic effects in mice.234 These effects were also
Polyphenols may affect glycemia through different mechanisms, including the inhibition of glucose absorption in the gut or of its uptake by peripheral tissues. The hypoglycemic effects of diacetylated anthocyanins at a 10 mg/kg diet dosage were observed with maltoose as a glucose source, but not with sucrose or glucose. This suggests that these effects are due to an inhibition of α-glucosidase in the gut mucosa. Inhibition of α-amylase and sucrose in rats by catechin at a dose of about 50 mg/kg diet or higher was also observed. The inhibition of intestinal glycosidases and glucose transporter by polyphenols has been studied in vitro. An IC₅₀ of 60–200 μM was reported for a rat intestinal α-glucosidase. Quercetin (10–20 μM) inhibited glucose transport by GLUT2 in a transfected oocyte model and also inhibited glucose absorption in Zucker fa/fa rats. At much higher concentrations (1 mM), quercetin 3-O-glucoside, tannic acid, and chlorogenic acid inhibited the Na⁺-dependent hexose uptake in a rat everted gut sac model, or isolated rat brush border vesicles; catechin, ferulic acid, and caffeic acid were inactive. Polyphenols could not only inhibit the glucose absorption in the small intestine, but they could also limit their reabsorption in the kidney, as has been shown for phlorizin.

Several in vitro studies on cultured cells have shown that polyphenols may increase glucose uptake by peripheral tissues. Caffeic acid increases glucose uptake by rat adipocytes and mice myoblasts. Black and green tea extracts and EGCg also increased glucose uptake by rat epididymal adipocytes, both in the presence or absence of insulin. Isoferulic acid increased glucose uptake by soleus muscle isolated from streptozotocin-diabetic rats. However, opposite results were also reported for quercetin and genistein, which both inhibited glucose uptake when induced by insulin on rat adipocytes or hydrogen peroxide on leukemic cell lines. Genistein, but not daidzein, also inhibited glucose uptake by HL-60 cells and erythrocytes. Active polyphenol concentrations differed widely (1 to 115 μM) according to the studies. It is still difficult to explain these contradictory results. They could be explained by the different nature of the polyphenols tested or by the concentrations used in the assays.

Polyphenols may exert different actions on peripheral tissues that would diminish glycemia. They include the inhibition of gluconeogenesis, adrenergic stimulation of glucose uptake, or the stimulation of insulin release by pancreatic β-cells. The involvement of such mechanisms is still hypothetical. p-Hydroxybenzoic acid, which shows hypoglycemic effects in diabetic rats when submitted to a glucose tolerance test, had no effect on insulinemia and hepatic glycogen. On the contrary, some polyphenols might have opposite effects and decrease glucose uptake in peripheral tissues by inhibiting the GLUT1 glucose transporter, as shown on transfected Chinese hamster ovary cells overexpressing this transporter, or by inhibiting the response to insulin.

In humans, evidence of the effects of polyphenols on glycemia or diabetes risk is still very limited. The consumption of decaffeinated coffee did not affect glycaemia or insulinemia when ingested with glucose, but it decreased the secretion of glucose-dependent insulinotropic polypeptide and increased that of glucagon-like peptide 1 in a manner consistent with a delayed intestinal glucose absorption. The effects of the consumption of polyphenol supplements were also evaluated in diabetic patients. No effect on glycaemia was observed in type II diabetic patients after consumption for 2 mo of 50 mg/d of a red orange supplement containing anthocyanins, flavanones, and phenolic acids. In another clinical trial, type I diabetic patients ingested larger doses of diosmin (1800 mg/day) and hesperidin (200 mg/day) as tablets for 3 mo. Although the doses administered per body weight unit were similar to many of the animal studies previously described, such a supplementation had no effect on glycaemia, but it significantly reduced the level of glycated hemoglobin (HbA₁c). Polyphenols could, thus, limit the risk of diabetic complications, as advanced glycation end (AGE) products are known to generate oxidative stress. This effect on AGE products could explain the reduction of renal damage by curcumin observed in streptozotocin-treated rats.

Epidemiological evidence is also very limited. The consumption of coffee (rich in chlorogenic acid) has recently been associated with a decreased risk of type II diabetes. The consumption of decaffeinated coffee (20 g/d solids for 14 d, i.e., the equivalent of about 10 cups of coffee per d) lowered fasting glycaemia in healthy volunteers. The consumption of 400 mL decaffeinated coffee containing 25 g glucose by healthy volunteers reduced the postprandial level of glucose-dependent insulinotropic polypeptide (GIP) and enhanced that of glucagon-like peptide 1 (GLP-1), suggesting that chlorogenic acid decreases the rate of intestinal absorption of glucose. Chlorogenic acid could, therefore, counter the known hyperglycemic effects of caffeine.

**POLYPHENOLS AND OSTEOPOROSIS**

Estrogen deficiency in postmenopausal women is an important cause of osteoporosis, and hormone replacement therapy is often recommended to prevent bone loss. However, many women have been reluctant to follow such a treatment because of the fear of possible side effects and long-term risks. Isoflavones with weak estrogen-like activity have attracted much attention as a possible alternative treatment to prevent osteoporosis. Their osteoprotective effects have been evaluated in mice or rats in which an estrogen deficiency has been induced by ovariectomy. The supplementation of the diet with genistein, daidzein, or their glycosides during several weeks prevents the loss of bone mineral density and trabecular volume caused by the ovariectomy. These effects were observed at daily doses of 10–50 mg/kg body weight. The highest doses also induced uterine hypertrophy, but the lowest protective doses did not affect the uterine weight. Feeding soy proteins with either normal or reduced isoflavone content to ovariectomized rats also suggested that the osteoprotective effects of soy proteins were due...
to their isoflavones. The restoration of the bone mineral density was not observed when the isoflavones were administered 80 d after the ovariectomy of the rats, suggesting that they may prevent bone loss, but not reverse an established osteopenia.

The mechanisms responsible for these protective effects are still poorly understood. In vitro, daidzein was shown to inhibit the differentiation of osteoclasts developing on dentine slices and to diminish the dentine resorption. On the other hand, the daily subcutaneous injection of genistein to ovariectomized rats increased the number of osteoclasts associated to bone formation, but had no effects on bone resorption. Structurally related isoflavones may protect bones through different mechanisms of action, and with different magnitude.

Evidence of protective effects in humans is based on observational and intervention studies. The consumption of soybeans, a major source of calcium in Japan, was associated to a higher bone mineral density in Japanese women. A soy-rich diet followed by postmenopausal women stimulated their bone osteoblastic activity. Such effects of soy food consumption could also be explained by the intake of isoflavones, as soy foods are their main dietary sources. However, no correlation between isoflavonoid urinary excretion and rate of bone loss was observed in postmenopausal women in the Netherlands. Higher intake levels may be required to prevent bone loss. The consumption of soy proteins providing 80 mg/d isoflavones during 24 wk was more effective than isoflavone free soy protein to improve bone mineral density and content in perimenopausal women. Furthermore, in 3 other studies, isoflavone supplementation at a dose of 37–62 mg/d during 4 wk–1 yr significantly improved the urinary excretion level of several biomarkers of bone resorption. Femur and lumber spine mineral density was also improved in the longer study.

Much less is known on the possible impact of other polyphenols on osteoporosis. Rutin, a glycoside of quercetin, added to the diet of ovariectomized rats restored the loss of bone mineral density induced by the ovariectomy and was even more efficient than isoflavones. It also reduced the urinary excretion of deoxyxypyrindoline, a marker of bone resorption, and increased osteocalcineny, a marker of osteoblastic activity. Such an effect of rutin likely explains the inhibition of bone resorption observed in rats fed a diet rich in onions (the main source of quercetin in Western diets), whereas several other vegetables were without effects.

The consumption of tea has been associated with a higher bone mineral density in a cohort of English older women. Catechins abundant in tea could possibly counteract the effects of tea caffeine, known for its adverse effects on bone metabolism. No effect of the consumption of coffee was observed in the same study. A rat experiment also showed no effect of coffee on bone metabolism. Feeding rats during 140 d with a diet supplemented with instant coffee containing both chlorogenic acid and caffeine had no influence on bone histomorphometry, deoxyxypyrindoline urinary excretion, and osteocalcineny. The effects of catechins or chlorogenic acid on bone resorption in animal models so far have not been examined.

**Risks Associated to Polyphenol Consumption**

The scientific data summarized above on the effects of polyphenols on diseases have led to the marketing of new polyphenol dietary supplements and polyphenol-rich food products. Although no precise claims are attached to these products, different polyphenols have been proposed to limit oxidative stress and aging, and isoflavones have been recommended to limit hot flushes and to improve bone health in post-menopausal women. The consumption of such products leads to increase the intake of particular polyphenols beyond common levels of exposure associated to the diet.

Cases of acute toxicity have been reported in animals consuming plants rich in tannins. In humans, no such acute toxicity has ever been reported after the consumption of dietary polyphenols. However, a high consumption of polyphenols could increase the risk of some diseases. The large majority of published studies were focussed on health benefits, rather than on disease risks. Our knowledge on risks is, therefore, very limited.

It is often said that polyphenols consumed in high amounts could have pro-oxidant effects. They can reduce iron (III) to iron (II) and, thus generate hydroxyl radicals through the Fenton reaction. Such pro-oxidant effects have never been demonstrated in vivo. Most dietary polyphenols have catechol groups that can be oxidized to quinone in vivo and generate free radicals through redox cycling. However, quinone reductase, catechol-O-methyltransferase, and other conjugating enzymes limit the formation of such quinones in endogenous tissues. Similarly, a number of polyphenols, including quercetin, were shown to be mutagenic in cultured cells. A pro-carcinogenic effect of quercetin in rat models of nitrosomethylurea-induced pancreatic cancer or azoxymethane-induced colon cancer have been reported. But the majority of the studies carried out with quercetin in rodents showed anticarcinogenic effects. Conjugating enzymes likely plays an essential role to detoxify polyphenols and limit their mutagenicity in vivo.

Phytoestrogens, acting as estrogen agonists, may stimulate the proliferation of estrogen responding cells and increase the risks of some cancers. Genistein was shown to stimulate the growth of breast cancer cells implanted in ovariectomized mice. The consumption of soy proteins by premenopausal women increased the level of plasma estradiol and stimulated the appearance of hyperplastic epithelial cells in nipple aspirate fluid. On the contrary, in other models or conditions, phytoestrogens were shown to act as antiestrogens and antiproliferative agents and could, therefore, affect reproductive functions. Problems of infertility in sheep grazing on pastures or fodders rich in phytoestrogens were at the origin of their discovery. Supplementation of the diet of rat dams during the first 21 postnatal d with coumestrol (0.1 g/kg diet) produced a persistent estrus state in female offspring and a deficit in the sexual behavior of male offspring.

Flavonoids may also influence the thyroid function and have goitrogenic effects. A reduction of thyroid peroxidase activity...
was observed in rats fed a diet supplemented with genistein. These effects of genistein on the thyroid function are more pronounced when iodine is deficient. Other flavonoids, such as daidzein, quercetin, kaempferol, or naringenin, were also shown to irreversibly inhibit thyroid peroxidase. These data are of particular concern for soy-fed babies exposed to particularly high doses of isoflavones. Vitexin, a C-glycosylflavones abundant in millet, is another inhibitor of thyroid peroxidase. When administered to rats, it increased thyroid weight and decreased the plasma levels of thyroid hormones. It is thought to contribute to the genesis of endemic goiter in West Africa, where millet is a staple food.

Most dietary polyphenols have catechol group in their structures and, thus form very stable chelates with ferric ions. This fundamental property explains the inhibition of non-heme iron absorption by polyphenols and polyphenol-containing beverages, such as coffee, wine, or tea, as shown in several clinical trials. As these effects involve direct chelation of iron by polyphenols in the gut, they are only observed when the source of polyphenols is ingested together with the source of iron. This is why it is often recommended for people at risk of developing iron deficiencies to drink tea and other polyphenol-rich beverages between the meals rather than during the meals. The effects of polyphenols on iron absorption have been well demonstrated in clinical studies; however, the impact of dietary polyphenols on iron status is not as firmly established in free-living populations. In contrast, for other people with a high iron status, the inhibition of iron absorption by polyphenols may be rather beneficial, as high levels of plasma ferritin have been associated to a higher risk of cardiovascular diseases and colon cancer.

Adverse effects of polyphenol consumption on cardiovascular diseases have recently been suggested. High polyphenol intake could increase the risk of cardiovascular diseases through an effect on homocysteinemia, an independent risk factor of cardiovascular diseases. The consumption of 2 g chlorogenic acid/d during 1 wk by volunteers significantly increased the plasma levels of thyroid hormones. It is thought to contribute to the genesis of endemic goiter in West Africa, where millet is a staple food.

A protective role of polyphenols against degenerative diseases is supported by many studies carried out on animals, and different mechanisms of action have been proposed to explain such protective effects. Much progress has been made on the evaluation of their bioavailability. The significance for human health of all in vitro data obtained on cultured cells or isolated enzymes and receptors will have to be properly re-evaluated in the light of this new knowledge on bioavailability.

More human studies are needed to provide definitive proofs of the protective role of polyphenols. Conclusive evidence will largely come from clinical and epidemiological studies. The number of clinical studies so far published is still limited, and the results are often contradictory. If it is clear that the consumption of polyphenols improves some oxidative stress related parameters, but the associations between these parameters and disease risk are still not well clarified, we need to identify proper markers of disease risk and demonstrate that they are influenced by the consumption of polyphenols.

First, epidemiological data on polyphenols were published approximately 10 yr ago and since then progress has been relatively slow because of the lack of food composition tables and lack of validated biomarkers of polyphenol intake. Such tables or biomarkers are needed to evaluate the consumption of polyphenols in populations, but their obtention/identification is rendered difficult because of the large number of phenolic compounds present in food. A table for flavonoids based on the surveys of 97 literature sources is now available. More research is still needed to cover missing polyphenols and food sources.

It is impossible to conclude today that some particular polyphenols offer more health benefits than others and research efforts continue at an increasing pace for all polyphenol classes. All polyphenols share common properties as reducing agents. Most of the dietary polyphenols can chelate iron and other metal ions. As such, they may all trigger common cell responses through mechanisms that have been partly unravelled. On the other hand, some polyphenols, like phytoestrogens, show specific properties and may be more indicated for the prevention of specific diseases. More intervention studies are needed to clarify the impact of the most active polyphenols on specific diseases or disease biomarkers.

When developing new polyphenol-containing products for specific effects on a given pathology, we will also have to make sure that they do not increase risks for other pathologies. For example, resveratrol shows promising anticarcinogenic properties, but it also increases the risk of cardiovascular diseases in mice. Quercetin may be effective to prevent different diseases,
but it also reduces the life-span of mice, when added to the diet on a long term basis.  

It can be anticipated that the most protective polyphenols and the most appropriate levels of intake will be determined in the near future, for both the general population and populations at risk of developing particular diseases. Before this goal is reached, caution is necessary before recommending to increase their consumption. This is particularly important for dietary supplements, as their promotion may result in increases of polyphenol intake, sometimes far-exceeding the common intake of polyphenols. The most appropriate levels of intake will be determined at risk of developing particular diseases. Before this goal is reached, caution is necessary before recommending to make sound dietary recommendations for an effective health benefit.

REFERENCES


300 A. SCALBERT ET AL.


