Tea Flavonoids and Cardiovascular Diseases: A Review

L. B. M. Tijburg,¹ T. Mattern,² J. D. Folts,² U. M. Weisgerber,¹ and M. B. Katan³

¹Unilever Research Laboratorium, Vlaardingen, The Netherlands; ²University of Wisconsin Medical School, Madison Wisconsin; ³Wageningen Agricultural University, Division of Human Nutrition and Epidemiology, Wageningen, the Netherlands

* Address for correspondence: L. B. M. Tijburg, Unilever Research Laboratorium, P.O. Box 114, 3130 AC Vlaardingen, The Netherlands

I. INTRODUCTION

A. Cardiovascular Diseases and Their Causes

Cardiovascular diseases (CVD) are diseases of the heart and the blood vessels. In this article we limit the discussion to those cardiovascular diseases that are major causes of death in adults worldwide, namely, coronary heart disease (CHD) and cerebrovascular disease.

The number one cause of death in most industrialized countries is coronary heart disease, also known as ischemic heart disease. In coronary heart disease, blood flow in one or more branches of the coronary arteries is reduced because atherosclerotic plaques protrude from the inner surface of the arteries, narrowing the lumen. Such a reduction of blood flow may result in angina pectoris, that is, chest pain after exertion. Blood flow may also be blocked completely by a blood clot (thrombus) so that the heart muscle tissue downstream from the obstruction is no longer provided with oxygen and nutrients. The ensuing damage to the heart muscle may lead to death of the affected tissues (infarction) or be so severe that the patient dies. The underlying atherosclerotic disease process is thought to develop in different stages, beginning with lipid deposition and possibly lipid oxidation in the subendothelium of the arteries. Later, there is impaired vasodilation and inflam-

matory reactions. When narrowed by atherosclerotic plaque, the artery is susceptible to acute occlusive platelet-mediated thrombosis.

The two main forms of cerebrovascular disease are ischemic and hemorrhagic stroke. The former is caused by blockage of an artery in the brain by mechanisms similar to those in ischemic heart disease. This is the most common type of stroke in North America and most of Europe. Hemorrhagic stroke is caused by rupture of a blood vessel and is the most common type of stroke in Japan and China.

Cardiovascular diseases are recognised as multifactorial diseases. The most firmly established risk factors are high blood pressure, smoking, or high blood cholesterol level, diabetes, and family history. High blood low-density lipoprotein (LDL) cholesterol levels are strongly associated with the risk of coronary heart disease but only weakly with the risk of stroke (Iso et al., 1989).

Oxidation of LDL is thought to play an important role in the current concept of atherogenesis. In the initial stages of atherosclerosis, LDL accumulates in the intima of lesion-prone sites of the arterial wall (Schwartz et al., 1991). LDL itself is not considered atherogenic but appears to contribute only to lesion formation after the lipoprotein has been modified by oxidation (Witztum and Steinberg, 1991). Lipoprotein oxidation may occur in the artery wall where lipoproteins are exposed to oxidative stress and antioxidants can become depleted.

Oxidized LDL is thought to become entrapped in the subendothelial space. As a result of oxidative modification of LDL, monocytes are recruited to the arterial wall and monocyte-derived macrophages accumulate excessive amounts of oxidized LDL and become lipid-laden foam cells. These foam cells may progress from fatty streaks into more advanced atherosclerotic plaques. Inflammatory reactions, which are considered an important part of the atherosclerotic process, are also stimulated by oxidized LDL.

The possible involvement of oxidative processes early in atherogenesis has stimulated the idea that dietary antioxidants may have a preventive effect in cardiovascular disease (Gey, 1995). Epidemiological data and randomized trials indicate a protective role of dietary oxidants, including vitamin E and β -carotene, against CVD (Stephens et al., 1996; Gey, 1995). In addition, antioxidants such as probucol and vitamin E decrease atherosclerosis in experimental animals (Frei, 1995). Flavonoids derived from tea have been demonstrated to efficiently scavenge a variety of free radicals (Wiseman et al., this issue). Indeed, the potent antioxidant properties of tea polyphenols are thought to be one of the mechanisms underlying their putative protective action against CVD.

When the complex nature of atherosclerosis is considered, tea may have a protective role against CVD via a number of different mechanisms, one of which is its antioxidant activity. In this section, the possible mechanisms of action are evaluated. For example, the potential of tea to lower blood lipid levels and to inhibit LDL oxidation and inflammatory reactions is examined. In addition, the effect of tea on thrombotic and hemostatic factors, which link the atherosclerotic process with its clinical endpoints of myocardial infarction and stroke, are discussed.

B. Green and Black Tea Flavonoids

Green tea and black tea originate from the same plant, the *Camellia sinensis*. Green tea is produced by drying and cutting the fresh shoot of the tea plant. Green tea is a rich source of flavonoids, namely, catechins and flavonols (Figure 1). Black tea is subjected to more extensive processing than green tea. Following drying, disruption of the leaves causes phenolic compounds and polyphenol oxi-

dases to come into contact, a step that is usually — but incorrectly — called fermentation (Graham, 1992). During the fermentation process, tea catechins are converted to complex condensation products, namely, theaflavic acids, theaflavins, and thearubigin polymers (Figure 1).

II. EPIDEMIOLOGICAL STUDIES ON TEA, FLAVONOIDS, AND CARDIOVASCULAR DISEASES

A. Epidemiological Studies on Tea and Cardiovascular Disease

Ecological data indicate that tea consumption is at best one of many factors to affect the risk of coronary heart disease. If the effect of tea was overwhelming then populations with a high intake of tea should have uniformly low rates of heart disease. This is not the case, however, as exemplified by the U.K., a country in which both tea intake and heart disease mortality are high. However, this does not exclude a modest contribution of tea to the prevention of CVD, which could be overwhelmed by other unfavorable risk factors such as smoking in a country such as the U.K.

Probably the first analytical data to indicate a protective effect of a high consumption of black tea on CHD came from a case-control study of the Boston Collaborative Surveillance Program (1972). This study showed a non-significant risk reduction for a myocardial infarction (MI) of 34% for subjects consuming more than six cups of tea per day vs. those who did not drink any tea, but no adjustment was made for potentially confounding life style and dietary factors. The Scottish Heart Health Study also examined the relation between tea consumption and prevalence of CHD determined by recent medical diagnosis of nonfatal agina or MI (Brown et al., 1993). There was a positive association between tea consumption and CHD, which was abolished after adjustment for coffee consumption. Tea and coffee consumption were negatively correlated in this study. In cross-sectional studies as well as case control studies, clinical or preclinical symptoms can bring about life style and dietary changes so that cause and effect are confused. This seems relevant because tea is generally perceived as “less irritating” than coffee

FIGURE 1. Composition of flavonoids in green and black tea. The distribution flavonoids in green and black tea is expressed as percentage of total flavonoids. Catechins: R, R1 = H: EC; R1 = OH, R = H: EGC; R1 = H, R = H: ECG. Theaflavins, R = H or gallate. Thearubigins: hypothesized structure.

and may be chosen as an alternative to average by heart disease patients.

Prospective cohort studies circumvent these problems by measuring dietary intake prior to occurrence of disease. Recent prospective studies investigating the association between tea intake and subsequent CVD do not provide a consistent answer (Table 1). In two separate analyses from the same Californian cohort, tea intake was related neither to incident MI nor to mortality from CVD (Klatsky et al., 1990, 1993). In contrast, in a Norwegian cohort the risk of dying from CHD was 36% lower for men who consumed more than one cup of tea per day than men drinking no tea; however, this difference was not statistically significant (Stensvold et al., 1992). In a long-term study of a Dutch cohort, the risk of dying from CHD was significantly lower in men with a high intake of tea; this effect was independent of the major established risk factors (Hertog et al., 1993). In the same cohort, tea consumption was also associated with a lower incidence of stroke (Keli et al., 1996). The Dutch population provides a wider range of tea

consumption than the Norwegian or Californian population, which makes it more likely for an association to be identified. Interestingly, in a Welsh population of heavy tea drinkers the risk of death from CHD increased with tea intake (Hertog et al., 1997b). The authors of this study put forward the argument that the addition of milk to tea, which in this population is common, may lead to binding of tea components to milk protein and thereby prevent their absorption. However, adding milk to black tea did not influence the absorption of the tea flavonoids quercetin and catechins in human volunteers (Van het Hof and Hollman, personal communication).

Table 1 summarizes the above-described prospective studies on tea and CVD. Use of a prospective study design may eliminate certain forms of bias, but confounding remains a problem in any observational epidemiological study. Insufficient control for confounding provides the most likely explanation for the discrepancy in results between the studies presented in Table 1, although all of them made some attempt to include other risk

TABLE 1
Prospective Studies on Black Tea, Flavonols, and Cardiovascular Disease (CVD)

Ref.	Cohort, country	No. of endpoints	Endpoint	RR ^a	95% CI
Black tea					
Stensvold et al.	Oppland County Study, Norway	141	CHD mortality	0.64	0.38–1.07
Klatsky et al. ^b	Kaiser Permanent Hospital Cohort (Oakland and San Francisco), U.S.	1762	CVD mortality	0.98	NS
Hertog et al. ^c	Zutphen Elderly Study, NL	43	CHD mortality	0.45	0.22–0.93
Keli et al., 1996 ^c	Zutphen Elderly Study, NL	42	Stroke incidence	0.31	0.12–0.84
Hertog et al. 1997 ^d	Caerphilly Study, Wales	131	CHD mortality	2.3	1.0–5.1
Flavonols					
Rimm et al.	Male health professionals, U.S.	486	MI incidence	1.08	0.81–1.43
			CVD mortality in men without previous CVD	1.31	0.42–3.05
			CVD mortality in men with previous CVD	0.63	0.33–1.20
Knekt et al.	Finnish Mobile Clinic Health Examination Survey, Finland	324	CHD mortality, men	0.67	0.44–1.00
		149	CHD mortality, women	0.73	0.41–1.32

^a Relative risk for highest versus lowest category of intake in the fully adjusted model.

^b Tea intake also not related to MI incidence in the same cohort (Klatsky et al., 1990).

^c Negative association also found between flavonol intake and CHD mortality (Hertog et al., 1997)

^d No association found between flavonols intake and CHD mortality.

factors. Tea drinking tends to be associated with a healthy life style in continental Europe (Schwarz et al., 1994). In contrast, in the Welsh study men with the highest intake of tea smoked more, consumed less alcohol, and ate more fat (Hertog et al., 1997b). These and other confounders were adjusted for. However, factors such as smoking and social class were adjusted for only in broad categories so that confounding may not have been eliminated completely. Furthermore, measurements of dietary intake and alcohol consumption were necessarily crude, and no data were available on physical activity or psychosocial factors. Hence, the most plausible explanation for the higher mortality in heavy tea drinkers in Wales is residual confounding by unmeasured or imperfectly measured coronary risk factors that clustered with tea intake.

B. Epidemiological Studies on Flavonoids and Cardiovascular Disease

Prospective epidemiological studies have examined the relation between intake of flavonols and CHD or stroke. There is quite some overlap between studies of tea and those of flavonols. Flavonols are a subclass of flavonoids, and in some countries tea is a major source of flavonols; for instance, tea accounted for 81% of the flavonol intake in Wales (Hertog et al., 1997b) and 61% in the Netherlands (Hertog et al., 1993). On the other hand, flavonols make up only 10% of the total flavonoids in tea, and in some populations tea is a minor source of flavonols. The association of CVD with flavonol intake therefore merits analysis separate from that with tea intake.

Prospective studies with individual assessment of dietary intake are listed in Table 1. The Zutphen cohort in the Netherlands mentioned above also demonstrated an adjusted relative risk of 0.32 (95% CI 0.15 to 0.71) of dying from CHD in men with high flavonol intake when compared with those with low intake (Hertog et al., 1993). This was expected because a high intake of tea and a high intake of flavonols coincided. In a subcohort of men without history of heart disease, neither tea nor flavonol intake was related to incidence of fatal or non-fatal first MI. A recent update of this analysis confirmed the inverse association with CHD mortality,

whereas an association with first MI was suggestive (Hertog et al., 1997a). There was also no association of flavonol intake with CVD mortality or incidence of non-fatal MI in a large cohort of U.S. male health professionals (Rimm et al., 1996). However, in the same study Rimm and co-workers did find a suggestion of a risk reduction in men with previous CVD. This could point to an effect on thrombotic tendency in men with a history of coronary heart disease rather than an effect on early events such as LDL oxidation and lipid deposition. An inverse association of tea as well as flavonol intake with risk of what was mainly thrombotic stroke seems to support this theory (Keli et al., 1996). Additional indications for a beneficial role of flavonols come from a recent Finnish cohort study, where intake in men of more than 5.5 mg flavonols per day produced a relative risk for CHD of 0.67 (CI 0.44 to 1.00) when compared with intakes lower than 2.4 mg (Knekt et al., 1996). As tea consumption in Finland is low, the main sources of flavonol intake in this study were onions and apples.

In conclusion although the evidence from epidemiological studies for a protective effect of tea drinking on CHD and stroke risk is not conclusive, although several studies have demonstrated a significant and substantial risk reduction in tea drinkers.

III. EFFECT OF FLAVONOIDS AND TEA ON LDL OXIDIZABILITY

The exact nature and source of the oxidants that initiate oxidation of LDL *in vivo* is unknown, but several mechanisms have been suggested (Berliner and Heinecke, 1996). Cultured vascular cells — endothelial cells, smooth muscle cells, or macrophages — are capable of oxidation of LDL in the presence of transition metal ions, as are transition metal ions alone. The myeloperoxidase-derived intermediates, hypochlorous acid and tyrosyl radicals, and lipoxygenase-derived products have also been suggested to play a role in LDL oxidation. Finally, nitric oxide — a major regulator of vascular tone — may promote LDL oxidation via peroxynitrite, which is formed by the reaction of

nitric oxide with superoxide (Frei, 1995; Berliner and Heinecke, 1996).

Tea flavonoids have been shown to effectively scavenge free radicals (Salah et al., 1995), inhibit lipid peroxidation (Yoshino et al., 1994), and chelate free transition metal ions via the ortho-diphenolic structure (Miller et al., 1996). The effect of various catechins and flavonols on oxidative modification of LDL has been investigated in cultured vascular cells and in cell-free systems, mainly using copper as prooxidant.

A. Effect of Tea Flavonoids on LDL Oxidizability *In Vitro*

Brewed green tea is an effective inhibitor of copper-mediated LDL oxidation *in vitro*. This is probably due to the radical scavenging activity of catechins rather than to their copper-chelating capability (Luo et al., 1997). Catechin inhibits the formation of lipid peroxidation products in LDL exposed to copper (Mangiapane et al., 1992). Gallate esters — epigallocatechin gallate (EGCG) and epicatechin gallate (ECG) — were more active than the respective free form epigallocatechin (EGC) and epicatechin (EC) (Miura et al., 1994; Vinson et al., 1995). Various catechins prolonged the lag time before significant LDL oxidation in the order EGCG>ECG>EC>catechin (C)>EGC (Miura et al., 1994). Vinson et al. (1995) reported that EGCG was more effective than EGC and ECG and that catechin was the least effective in inhibiting Cu²⁺-induced LDL oxidation. In a copper-free LDL system with metmyoglobin as prooxidant, catechin and epicatechin were as effective as ECG and EGCG as chain-breaking antioxidants; EGC was the least efficacious (Salah et al., 1995). Theaflavins were also effective inhibitors of copper-mediated LDL oxidation *in vitro*, although less efficacious than the monomeric catechins and catechin gallate esters (Miura et al., 1995; Miller et al., 1996).

There are several lines of evidence that tea flavonoids are capable of inhibiting oxidation of LDL mediated by vascular cells. Isolated polyphenols from green tea were able to inhibit the oxidation of LDL by cultured macrophages (Zhenhua et

al., 1991), but there is limited information on the effectiveness of individual tea flavonoids. Catechin and quercetin protected LDL from oxidation when incubated with various cultured cells, for example, human monocyte-derived macrophages, human umbilical vein endothelial cells, or lymphoid cells (De Whalley et al., 1990; Mangiapane et al., 1992; Nègre-Salvayre et al., 1991). It has been suggested that this is due to the ability of flavonoids to inhibit lipoxygenase activity. Indeed, there are indications that flavonoids inhibit lipoxygenase activity *in vitro* (Robak et al., 1988) and *in vivo* (Katiyar et al., 1992).

In addition to inhibiting cell-mediated LDL oxidation, catechin and quercetin also protected lymphoid cells against cytotoxic effects of previously oxidized LDL. This effect is probably due to an increased antioxidant status of the cells in the presence of flavonoids (Nègre-Salvayre et al., 1991; Nègre-Salvayre and Salvayre, 1992). Interestingly, catechin also inhibited the uptake and degradation of previously oxidized LDL by human macrophages, but the mechanism of this phenomenon is not clear (Mangiapane et al., 1992).

Taken together, green tea infusion, catechins, and flavonols can effectively protect LDL from oxidation in various *in vitro* systems and also diminish the deleterious effects of oxidized LDL on vascular cells. There is little information as yet on the effects of black tea flavonoids, in particular thearubigins, on LDL oxidation *in vitro*.

B. Effect of Tea on LDL Oxidizability *Ex Vivo*

Flavonoids can inhibit LDL oxidation in man only if they are absorbed and able to partition into LDL and/or the subendothelium where oxidation occurs. Consumption of six cups per day of green or black tea (900 ml/d) for 4 weeks had no significant effects on the resistance of LDL to copper-mediated oxidation *ex vivo* in non-smokers (Van het Hof et al., 1997) or in smoking subjects (Princen, H., personal communication). In contrast, Ishikawa et al. (1997) showed a small but significant prolongation of the lag time of LDL oxidation *ex vivo* compared with baseline measurement following 4 weeks of

black tea consumption (600 ml/d). It was not clear, however, whether this change was statistically significant from the change in the control group. Whether the differences between these studies can be attributed to differences in the intake of tea flavonoids, which was higher in Ishikawa's study, or to other variables remains to be established.

In conclusion, tea flavonoids strongly inhibit copper-induced and cell-mediated oxidation of LDL *in vitro*; whether tea consumption inhibits LDL oxidation *in vivo* by increasing the intrinsic resistance of the LDL particles to oxidation remains to be investigated further.

IV. EFFECT OF TEA ON PLASMA LIPIDS

A. Effect of Tea on Plasma Lipids in Experimental Animals

An elevated level of LDL is a major risk factor for CHD, and some animal studies suggest that flavonoids lower plasma levels of (LDL) cholesterol. Consumption of mixed catechins (Muramatsu et al., 1996), catechin (Valsa et al., 1995), or EGCG (Matsuda et al., 1986; Chisaka et al., 1988) decreased serum cholesterol levels in rats and mice fed an atherogenic diet. Dietary catechins at levels of 1 to 2 g/100 g diet inhibited the absorption of dietary cholesterol (Muramatsu et al., 1986; Chisaka et al., 1988). This effect may be due to precipitation of cholesterol in mixed micelles in the presence of high levels of catechins (Ikeda et al., 1992).

B. Effect of Tea on Plasma Lipids in Humans

The relation between green tea consumption and plasma lipid levels has been assessed in several epidemiological studies (Table 2). In a cross-sectional study in Japanese men, Kono et al. (1992) found an inverse association between the consumption of green tea and serum total cholesterol levels (Table 2). Similar and highly significant results were found elsewhere in Japan (Imai and Nakachi, 1995). However, in Japan high intake of

green tea is often associated with a traditional low-fat Japanese diet, which in itself has lipid-lowering effects. Therefore, residual confounding by dietary factors cannot be excluded. This was supported by the recent finding that after appropriate adjustment for dietary and health habits, green tea consumption was not associated with plasma lipid levels (Tsubono et al., 1997).

Cross-sectional studies on black tea consumption and serum lipid levels have yielded inconclusive results (Table 2). Most studies on the relation between black tea consumption and serum cholesterol levels showed no significant association. Black tea consumption was found to be inversely associated with serum cholesterol levels in a Norwegian cohort after adjustment for several risk factors, including coffee intake (Stensvold et al., 1992). In a cross-sectional study in Israeli men, a high intake of black tea was also associated with lower levels of plasma cholesterol, while serum level of triglycerides were increased (Green and Jucha, 1986). In most countries tea consumption is inversely associated with coffee consumption. In populations drinking unfiltered coffee, including those in Norway and Israel, coffee intake is positively associated with serum cholesterol levels (Urgert and Katan, 1997). In the Norwegian study that showed an inverse association between black tea consumption and cholesterol, coffee was only adjusted for in broad categories, so that residual confounding cannot be excluded (Stensvold et al., 1992).

The effect of black tea consumption on blood lipids has been assessed in several short-term controlled trials. Consumption of 1.8 l black tea per day for 4 weeks had no effect on total serum cholesterol, LDL, or high-density lipoprotein (HDL) cholesterol or triglyceride levels when compared with water (Bingham et al., 1997). Similar findings were reported when smaller amounts (0.75 to 0.9 l per day) were consumed for 4 weeks (Van het Hof et al., 1997; Ishikawa et al., 1997). Data on green tea and blood lipids in humans are limited, but consumption of 0.9 l of green tea for 4 weeks did not significantly change the serum levels of LDL or HDL cholesterol when compared with water (Van het Hof et al., 1997).

Taken together, epidemiological studies and controlled trials indicate that consumption of black

TABLE 2
Association between Tea Consumption and Blood Lipid Levels

Author	Country, number of subjects	Tea	Total cholesterol	HDL cholesterol	LDL cholesterol	Triglycerides	Adjusted for
Tsubono, 1997	Japan (1000)	Green tea	0	0	0	0	Age, smoking, alcohol, BMI, PAL, education, coffee, diet
Imai, 1995	Japan (1371)	Green tea	-($p < 0.001$)	+($p = 0.02$)	-($p = 0.02$)	-($p = 0.02$)	Age, smoking, alcohol, relative body weight
Kono, 1992	Japan (1306)	Green tea	-($p = 0.03$)	0	0	0	Employment, rank, alcohol, BMI, smoking, physical activity
Wei, 1995	U.S. (2000)	Black tea	0	0	0	0	Age, sex, BMI, alcohol, PAL
Carson, 1993	U.S. (1035)	Black tea	0	0	0	0	Age, smoking, BMI, alcohol, PAL
Stensvold, 1992	Norway (20,199)	Black tea	-($p < 0.05$)				Age, smoking, BMI, physical activity, coffee
Green, 1992	Israel (6016)	Black tea	0	-(NS)	0	0	Age, smoking, BMI, coffee, alcohol, PAL, ethnic origin
Green, 1986	Israel (658)	Black tea	-($p = 0.004$) ^a -(NS) ^b	0 ^a 0 ^b	-($p = 0.0015$) ^a 0 ^b	+($p = 0.01$) ^a 0 ^b	Age, smoking, BMI, alcohol, PAL
Klatsky, 1985	U.S. (42,627)	Black tea	0	0			Age, smoking, marital status, education, birthplace

^a Age 20 to 39 years.

^b Age 40 to 69 years.

Note: -- = inverse association; + = positive association; 0 = no association; BMI = body mass index; PAL = physical activity level; NS = not significant.

tea does not induce significant changes in blood lipid levels in humans. Moderate amounts of green tea do not seem to have an effect either. The discrepancy between animal and human experiments may be due to the relatively large amounts of tea flavonoids used in animal studies or to the inappropriateness of animal models.

V. EFFECT OF TEA ON EXPERIMENTAL ATHEROSCLEROSIS IN ANIMAL MODELS

The first study indicating a protective effect of black tea on atherosclerosis in experimental animals was published in 1967 (Young et al., 1967). In this small study with five animals per group, consumption of black tea seemed to reduce the extent of atherosclerosis in hypercholesterolemic rabbits. However, in a more extensive study with 20 New Zealand white rabbits that were fed an atherogenic diet containing 0.15% cholesterol, black tea consumption for 6 months had no significant effect on aortic atherosclerotic plaque formation (Tijburg et al., 1997).

Consumption of green tea polyphenols for 4 months significantly reduced aortic atherosclerosis in hypercholesterolemic rabbits (Fu-quing et al., 1989). Similarly, green tea reduced atherosclerotic plaque formation by 30% in New Zealand white rabbits fed a high-fat diet supplemented with 0.15% cholesterol, but this effect lacked statistical significance (Tijburg et al., 1997). The potential of green and black tea to inhibit experimental atherosclerosis will be clarified when more studies are published.

VI. EFFECTS OF FLAVONOIDS AND TEA ON INFLAMMATORY REACTIONS

Atherosclerosis is considered a disease with a strong inflammatory component. The effects of flavonoids on inflammatory processes have been studied almost exclusively in isolated cells or in experimental animals.

The possible mechanisms of the antiinflammatory action of flavonoids have been reviewed recent-

ly (Formica and Regelson, 1995). Tea infusions have been shown to have antiinflammatory actions in laboratory animals (Jolles, 1966; Man et al., 1995). Black tea polyphenols reduced the ulcerous effects of aspirin and the bleeding from capillaries in the stomach mucosa caused by aspirin (Man et al., 1995). Also, the theaflavins and thearubigins from black tea have a pronounced antagonism to bradykinin, which is released during some antiinflammatory responses (Natajara et al., 1972). The constituents responsible for the antiinflammatory and capillary strengthening properties of tea include the catechins and rutin (quercetin-3-rhamnoglucoside).

The antiinflammatory effects of flavonoids, including some of those found in tea, are explained in part by their inhibitory effect on arachidonic acid metabolism (Ferrandiz and Alcaraz, 1991). Inflammation is known to be accompanied by the release of prostaglandins, which by chemotaxis attracts leukocytes to the source of the inflammatory response. In addition, many flavonoids interfere with histamine metabolism and inhibit migration of murine neutrophils *in vitro* (Alcaraz and Villar, 1989). Catechins and quercetin were shown to inhibit three aspects of human neutrophil function *in vitro* that are considered to contribute to inflammation: the release of lysosomal enzymes, the chemiluminescence response, and the production of free radicals (Kenny et al., 1990). Quercetin and kaempferol have also been demonstrated to block the expression of adhesion molecules on cultured human endothelial cells, for example, ICAM, VCAM, and E-selectin, and thus may inhibit monocyte recruitment to the arterial wall (Gerritsen et al., 1995).

Interleukin-1 is involved in inflammatory responses and damages endothelial cells in several models. *In vitro*, EGCG has been shown to stimulate interleukin-1 release from monocytes at concentrations around 0.1 mM (Sakagami et al., 1992). However, this concentration is much higher than concentrations that can be achieved *in vivo* (Hollman et al., 1997). In contrast, consumption of green tea polyphenols by SKH-1 hairless mice has been shown recently to offer protection against ultraviolet B radiation-induced inflammatory responses (Agarwal et al., 1993). Pretreatment of mice with green tea poly-

phenols inhibits the activity of interleukin-1 in skin (Katiyar et al., 1995). It is interesting to speculate that green tea polyphenols may be useful in inhibiting a wide range of inflammatory responses, including that proposed as a mediator in coronary artery disease (Middleton and Kandaswami, 1993). Inhibition of these reactions might be potentially useful in delaying the progression of the atherosclerotic process. However, the relevance of *in vitro* studies, which used unphysiologically high concentrations of flavonoids that cannot be achieved *in vivo*, remains to be established in man.

VII. EFFECT OF TEA FLAVONOIDS ON THROMBOSIS AND HEMOSTASIS

Platelet activity is thought to be increased in patients with coronary risk factors such as elevated LDL cholesterol (Renaud et al., 1986), hypertension (Hamet et al., 1985), diabetes (Fuster, 1994), and cigarette smoking (Folts and Bonebrake, 1982). Platelets are known to contribute to the development and progression of the atherosclerotic narrowing process, in part by releasing growth factors that cause the multiplication and migration of vascular smooth muscle cells from the media up into the intimal space (Fuster, 1994). Platelets also help to add layers of clot to the developing atherosclerotic plaque, which leads to fatal or non-fatal myocardial infarction (Fuster, 1994).

A. Antiplatelet Effects of Tea Flavonoids *In Vitro* and in Experimental Animals

A variety of flavonoids have been demonstrated to have modest antiplatelet potency in platelet-rich plasma *in vitro* (Gryglewski et al., 1987). A hot-water extract of green tea inhibited the collagen-induced aggregation of rabbit platelets (Sagesaka-Mitane et al., 1990). The gallate esters of catechins (EGCG and ECG) were more efficacious than EC and EGC. When human platelets were incubated with various pure catechins, only EGC inhibited ADP-induced aggregation, whereas other catechins were not active (Chang and Hsu, 1991).

The flavonols quercetin and myricetin were strong inhibitors of ADP- and arachidonic acid-induced aggregation of human platelets but were almost ineffective when collagen was used to induce aggregation (Landolfi et al., 1984). Rutin was not effective. In an *ex vivo* aggregation model quercetin and rutin, but not catechin, were capable of dispersing preformed platelet thrombi that were adhering to rabbit aorta endothelium (Gryglewski et al., 1987).

Some of the flavonols including quercetin and rutin, were potent antiplatelet and antithrombotic agents *in vivo* when given intravenously to experimental animals (Gryglewski et al., 1985; 1987; Slane et al., 1994). An *in vivo* animal model for studying the antiplatelet/antithrombotic effects of drugs, foods, and beverages has been developed (Folts, 1982; Folts, 1991). In this model, damage is produced in a coronary artery in dogs, and the lumen diameter of the damaged artery is reduced. This causes periodic acute platelet-mediated thrombus formation followed by embolization, which produces cyclical reductions in coronary blood flow and transient ischemia. In this model, aspirin and red wine or grape juice, which have a high content of polyphenolic flavonoids, inhibited *in vivo* platelet (Folts et al., 1976; Demrow et al., 1995).

Intragastric administration of Earl Grey black tea (10 ml/kg), the equivalent of three cups for a human, also inhibited *in vivo* platelet activity and prevented experimental coronary thrombosis in dogs (Folts, 1996). This means that either the original flavonoids or the additives in Earl Grey tea, or their metabolites were absorbed by the gut and were present in the bloodstream (Folts, 1996). Green tea given by stomach tube also abolished the *in vivo* platelet activity (Folts, unpublished observations). Coffee did not affect platelet activity. Thus, tea but not coffee significantly inhibits *in vivo* platelet activity in dogs when given by stomach tube.

B. Effect of Flavonoids on Thrombosis and Hemostasis in Humans

Few human trials have been published that assessed the effects of dietary flavonoids on platelet aggregation *ex vivo*. Consumption of more

than 100 mg/d quercetin-3-glucoside from onions for 7 d had no effect on collagen- or ADP-induced platelet aggregation *ex vivo* (Janssen et al., 1997). In the same study it was shown that quercetin-3-glucoside at concentrations up to 2.5 μM did not affect platelet aggregation *in vitro*, while concentrations of 25 μM and higher decreased collagen-induced platelet aggregation. This study suggested that the anti-aggregatory effect of quercetin seen *in vitro* is due to concentrations that cannot be achieved *in vivo*. On the other hand, consumption of isolated green tea polyphenols (100 mg/d, mainly catechins) for 4 weeks decreased ADP-induced platelet aggregation, while collagen-induced platelet aggregation was not affected (Pietta et al., 1996). Thus, there are indications that both black and green tea given orally may inhibit platelet activity in experimental animals and possibly in man, but more evidence from human trials is needed.

The effects of black tea on several hemostatic proteins were measured in a placebo controlled, crossover study in human subjects. Black tea, 6 mugs per day (300 ml each), was consumed for 4 weeks (Vorster et al., 1996). There was no change in plasma fibrinogen, tissue plasminogen activator (TPA), or Plasminogen Activator Inhibitor-1 (PAI-1) levels or activities after consuming the tea. In another study consumption of six cups per day of either black or green tea had no significant effect on plasma fibrinogen or PAI-1 either (de Maat, M.P.M. and Princen, H.M.G., personal communication). Using a different source of flavonoids (e.g., onions), it was shown that quercetin-3-glucoside (100 mg/d) also had no effect on plasma fibrinogen, PAI-1 activity, plasminogen activity, and factor VII activity (Janssen et al., 1997). Taken together, these results suggest that ingestion of flavonoids has little effect on soluble coagulation or fibrinolysis factors in man.

VIII. OTHER BIOLOGICAL EFFECTS OF TEA RELATED TO CVD

There are some indirect indications that tea flavonoids may reduce blood pressure. *In vitro* experiments showed that flavonoids have a relaxant effect on noradrenaline-induced contraction of rat aortic strips (Duarte et al., 1993). Quercetin was the

most active flavonoid tested, while catechin and epicatechin showed limited activity. Purified catechins decreased the systolic, diastolic, and mean blood pressure in rats with renal hypertension probably by directly improving the renal circulation (Yokozawa et al., 1994). However, in spontaneously hypertensive rats the systolic blood pressure was not altered by EGCG. The association between black tea and blood pressure in humans was assessed by Stensvold et al. (1992) in a cross-sectional epidemiological study. The systolic blood pressure was inversely related to black tea consumption in men and women. However, black tea consumption for 4 weeks had no effect on blood pressure in human subjects (Bingham et al., 1997; Ishikawa et al., 1997). The potential of green tea to reduce blood pressure has not been investigated.

IX. CONCLUSION

The LDL oxidative modification hypothesis of atherosclerosis, first postulated by Steinberg and colleagues (1991), has stimulated scientific interest in the potential of flavonoids from vegetables, wine, and also tea to protect against CHD. Even a modest effect of tea on heart disease risk would be important from a public health point of view, because tea is the second most frequently consumed beverage in the world, and CVD is the most frequent cause of death in Western societies. Some prospective epidemiological studies have demonstrated lower rates of coronary heart disease in drinkers of black tea, but the evidence is not consistent.

Tea flavonoids possess various properties that may interfere with the process of atherogenesis. Tea components demonstrate strong antioxidant activity and inhibit LDL oxidizability *in vitro*. A number of tea components are absorbed (Hollman et al., 1997), but the evidence that tea consumption reduces LDL oxidation *in vivo* by increasing the intrinsic resistance of LDL to oxidation is inconclusive. Furthermore, it is not known whether the capacity of flavonoids to inhibit enzymatic and metal ion-catalyzed oxidative processes *in vitro* is relevant *in vivo*. Hypocholesterolemic activity of tea components has been suggested based on experimental animals and epidemiological findings. However,

this could not be confirmed in short-term trials in humans.

Tea flavonoids possess a number of activities that may inhibit inflammatory processes *in vitro*, but the relevance for the *in vitro* situation is not clear as yet. Recent observations that ingestion of black tea and green tea reduces platelet aggregation *in vivo* indicate that flavonoids from tea play role in thrombotic processes in addition to or rather than in the early stages of atherogenesis. This could explain the observation in some epidemiological studies that tea intake is inversely associated with CHD mortality but not disease incidence. However, experimental data in man are as yet scarce.

The concept that tea intake may protect against cardiovascular disease has rested heavily on cellular and animal experiment that used conditions and/or concentrations unlikely to occur in man. More realistic studies in man have eliminated some hypotheses, such as that of a significant effect of black tea on serum cholesterol levels. However, the epidemiological studies definitively leave open the possibility that tea flavonoids have a beneficial effect on CHD and stroke risk. In view of the widespread consumption of tea, the effect of tea on CVD risk merits further exploration. Such exploration should focus on studies in man or in animal models of proven validity.

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