Stellingen

1. Op basis van de onderzoeksresultaten beschreven in dit proefschrift is het niet wetenschappelijk verantwoord om aanbevelingen te doen voor de consumptie van catechinerijke voedingsmiddelen.

2. Catechines uit enerzijds thee en anderzijds appels, de twee belangrijkste catechinebronnen, zijn verschillend geassocieerd met sterfte aan coronaire hartziekten. Dit wijst erop dat andere componenten dan catechines verantwoordelijk zijn voor de gevonden verbanden.

3. Het feit dat vrouwen een significant hogere inname van catechines hebben dan mannen bevestigt hun status als theeleuten.

4. Het is alleen mogelijk de resultaten van een proefschrift samen te vatten in 150 woorden als deze consistent zijn.

5. Het bestuderen van populaties met een lage wijnconsumptie draagt niet bij aan het oplossen van de "Franse paradox".

6. Het is onverstandig de keuze voor (non-)nutriënten in epidemiologisch voedingsonderzoek te baseren op de beschikbaarheid van voedingsmiddelentabellen. Het feit dat stoffen gemakkelijk chemisch gekwantificeerd kunnen worden zegt niets over hun biologische activiteit.

7. There is no justification for early risers to affect moral superiority.
   Gale C, Martyn C. Larks and owls and health, wealth, and wisdom. *BMJ* 1998;317:1675-1677.

8. Als lekkere dingen gezond lijken te zijn, is de media-aandacht niet te overzien.

Stellingen behorend bij het proefschrift "Dietary catechins and their potentially protective role in cardiovascular diseases and cancer" van Ilja Arts.
Wageningen, 27 april 2001.

Dietary catechins and their potentially protective role in cardiovascular diseases and cancer

Ilja Arts

Promotor:	prof.dr.ir. D Kromhout Hoogleraar Volksgezondheidsonderzoek Wageningen Universiteit
Co-promotoren:	dr.ir. PCH Hollman Senior wetenschappelijk onderzoeker RIKILT, Wageningen
	dr.ir. EJM Feskens Projectleider Determinanten van Hart- en Vaatziekten en Diabetes RIVM, Bilthoven
Commissie:	prof.dr. DR Jacobs Jr (University of Minnesota, USA) prof.dr. A Bast (Universiteit Maastricht) prof.dr.ir. FJ Kok (Wageningen Universiteit) prof.dr.ir. AGJ Voragen (Wageningen Universiteit)

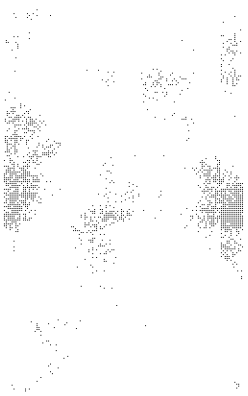
Dietary catechins and their potentially protective role in cardiovascular diseases and cancer

Ilja Catharina Wilhelmina Arts

Proefschrift
ter verkrijging van de graad van doctor
op gezag van de rector magnificus
van Wageningen Universiteit,
prof.dr.ir. L Speelman
in het openbaar te verdedigen
op vrijdag 27 april 2001
des namiddags om half twee in de Aula.

Contents

General introduction



INTRODUCTION

Catechins are compounds that belong to the family of the flavonoids. Six major subclasses of flavonoids, all with a common diphenylpyran skeleton, can be distinguished: catechins, flavonols, flavones, flavanones, anthocyanidins, and isoflavones (Figure 1.1). At present, more than 4000 naturally occurring flavonoids have been identified and this list is still growing.[1] Flavonoids are found in a multitude of plant foods - it is virtually impossible to eat a flavonoid-free diet - and in 1976 their intake from a normal mixed diet in the USA was estimated to be as high as 1 g/day.[2] This, combined with abundant *in vitro* data on their strong antioxidant activity and evidence demonstrating effects on a large variety of mammalian enzyme systems,[3] led researchers to hypothesize that flavonoids could be in part responsible for the widely accepted view that diets high in fruits and vegetables protect against a number of chronic diseases, including cancer[4,5] and cardiovascular diseases.[6]

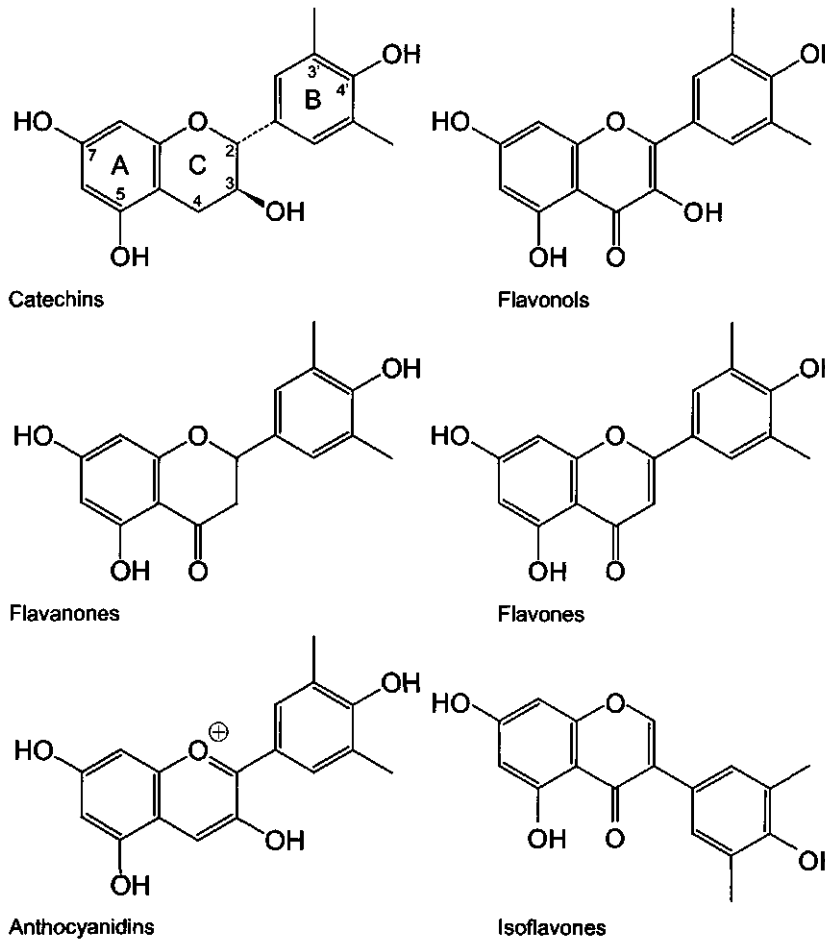


Figure 1.1 Subclasses of flavonoids.

NATURE AND BIOAVAILABILITY OF CATECHINS

The discriminating structural feature of catechins, which they have in common with anthocyanidins only, is the lack of an oxygen group at the 4-position of the heterocyclic C-ring (Figure 1.1). The lack of a double bond at the 2-3 position and the presence of a 3-hydroxyl group create two centers of asymmetry (carbons at position 2 and 3). The predominating catechins are (+)-catechin (2R,3S-3,5,7,3 ,4 -pentahydroxyflavan), (-)-epicatechin (2R,3R-3,5,7,3 ,4 -pentahydroxyflavan), (+)-gallocatechin (GC) (2R,3S-3,5,7,3 ,4 ,5 -hexahydroxyflavan), and (-)-epigallocatechin (EGC) (2R,3R-3,5,7,3 ,4 ,5 -hexahydroxyflavan), and the following gallic acid esters: (-)-epicatechin gallate (ECg), and (-)-epigallocatechin gallate (EGCg) (Figure 1.2).

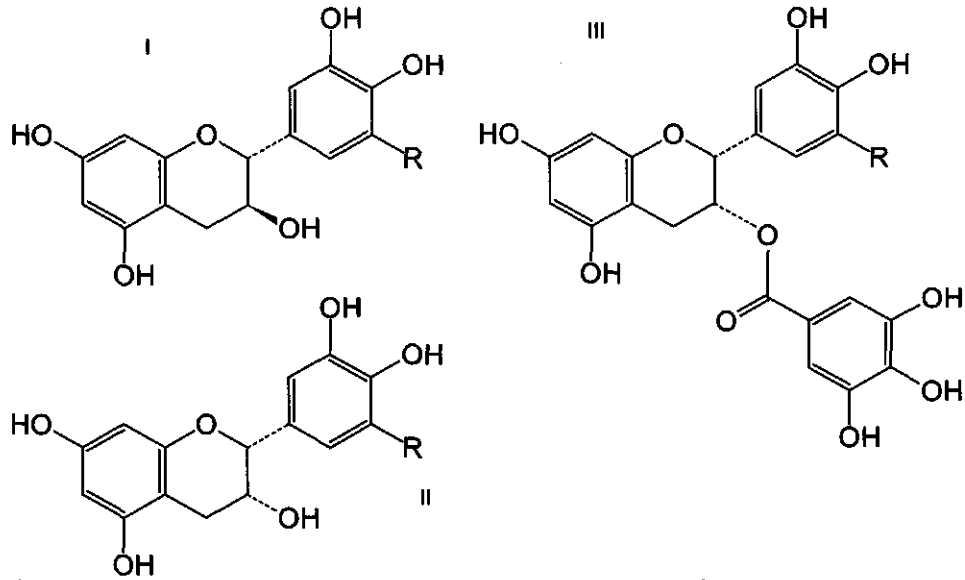


Figure 1.2 Chemical structure of catechins: (I) R = H (+)-catechin, R = OH (+)-gallocatechin (GC); (II) R = H (-)-epicatechin, R = OH (-)-epigallocatechin (EGC); (III) R = H (-)-epicatechin gallate (ECg), R = OH (-)-epigallocatechin gallate (EGCg).

The flavonoid subclass of catechins is sometimes also referred to as flavanols or flavan-3-ols. We prefer the term catechins, because it reduces mix-up with the flavonoid subclass of flavonols. Sometimes, catechins are incorrectly referred to as tannins or proanthocyanidins. The term tannins was historically used for a complex mixture of compounds used to bind proteins in the tanning of animal hides. Proanthocyanidins are a group of complex, often ill-defined compounds that always consist of at least two flavonoid units of which at least one is a catechin monomer.[1] Polymerization of monomeric catechins can occur as a result of autooxidation, but more often it is catalyzed by polyphenoloxidase, an enzyme that is present in most

plant tissues.[7] In intact tissues, polyphenoloxidase is separated from its phenolic substrates. Upon crushing or fermentation, as in the preparation of tea and cacao, polymerization of catechins gives the characteristic brown pigments. Oxidation processes that are induced to produce black tea consequently reduce its catechin content substantially.

Catechins and their microbial degradation products are relatively well absorbed. After oral administration of radioactively labeled (+)-catechin to rodents, monkeys, and men, about 50% of the total administered radioactivity was excreted into urine.[8] After absorption, biotransformation enzymes located mainly in the liver rapidly metabolize catechins. In plasma, catechins appear as products of O-methylation, sulfation and glucuronidation reactions, but low concentrations of free catechins are usually present as well. The conjugate profile depends on the type of catechin.[9,10] Unabsorbed catechins proceed into the colon, where they are degraded by bacteria into valerolactones and phenylpropionic acids.[8,11] These compounds, which are antioxidants themselves,[12] can be absorbed and may thus contribute to biological effects of dietary catechins.

The pharmacokinetic characteristics of (+)-catechin, (-)-epicatechin, EGCg, EGC, and ECg, were determined in volunteers who ingested green tea and black tea extracts,[10,13-17] red wine,[18] and black chocolate.[19] The absorption and excretion of catechins is relatively rapid. Typically, plasma peak values are reached between 0.5 and 4 hours after ingestion, whereas the elimination half-life of catechins varies between 1 and 7 hours.[18,20] There do not appear to be major differences in pharmacokinetic behavior between individual catechins.

CARDIOVASCULAR DISEASES

In spite of the 50% decline in age-specific cardiovascular disease mortality rates over the past decades in many western countries, it is still the leading cause of death among men and women in countries like the Netherlands[21] and the United States.[22] Coronary heart disease (CHD), also referred to as ischaemic heart disease, is the most common cardiovascular cause of death, followed by cerebrovascular disease (ischaemic and haemorraghic stroke). The initial step in atherosclerosis, the pathologic process underlying CHD and ischaemic stroke, is dysfunction of the arterial endothelium, which can result from exposure to agents such as oxidized low-density lipoprotein (oxLDL). The injured endothelium then attracts monocytes and T lymphocytes, which migrate into the subendothelial layer, where monocytes are converted into macrophages. The uptake of oxLDL by the macrophages leads to

foam cell formation. Fatty streaks, and ultimately fibrous plaques, are formed by accumulation of foam cells, T cells and smooth muscle cells. Each of the stages of fatty streak formation is potentially reversible. Rupture of unstable plaques and thrombosis involving platelet aggregation are often involved in the acute onset of a myocardial or cerebral infarction. Growth factors, cytokines, and small molecules such as nitric oxide, are mediators involved in immunity, inflammation, proliferation, and cell recruitment and migration.[23-25] Factors that interfere with any of these steps, could potentially influence the risk of cardiovascular diseases.

Tea, a major catechin source, has been associated with a lower risk of severe atherosclerosis in humans[26] and in experimental animals.[27] Catechins are strong antioxidants *in vitro*[12,28] and the initial hypothesis was therefore that they would lower CHD risk by protecting LDL against oxidation. Indeed, several studies have shown that catechins may reduce *ex vivo* LDL oxidation,[28] by scavenging potentially damaging free radicals and reactive oxygen species, by chelating metal ions,[12] and/or by conserving alpha-tocopherol, a lipid-soluble antioxidant present in LDL.[29-31] Nitric oxide plays a critical role in the regulation of vascular relaxation, which is impaired in humans with atherosclerosis.[32] Catechins have been shown to interact with nitric oxide,[33,34] and may thus influence cardiovascular disease risk through pathways other than oxLDL. Catechins may also be involved in the late stages of cardiovascular diseases by preventing plaque rupture or platelet aggregation.[27,35] In spite of the vast effort targeted at elucidating mechanisms through which catechins could affect cardiovascular disease risk, there is still much uncertainty as to which mechanisms are relevant for the human *in vivo* situation, in particular because most studies do not take into account physiological concentrations and the rapid and extensive metabolism of catechins.

CANCER

Cancer is second only to cardiovascular diseases as a cause of death. For men and women combined, lung, stomach, colorectal, and breast cancer are the most common forms of cancer, even though a large international variation exists in incidence rates of specific tumors.[36,37] Three simplified steps, initiation, promotion, and progression, can define the multistage process of carcinogenesis, the conversion of a normal cell into a malignant state. Initiation is characterized by mutations in DNA through reactions with carcinogens. Most carcinogens need metabolic activation before they can damage DNA. Preventing this activation, enhancing the detoxification of carcinogens, or trapping activated compounds before they reach their target sites are strategies through which tumor initiation can be prevented.

Compounds that can prevent initiation are called blocking agents.[38] A second cancer protective mechanism in the initiation step is DNA repair, which can reverse damage after mutations have occurred. During promotion, the altered DNA is thought to become expressed, and the cells start to proliferate. During tumor progression, when a pre-malignant lesion is converted into a malignant one, further DNA damage, gene expression, and phenotypic cell changes occur. Suppressing agents are compounds that interfere with the tumor promotion and progression steps.[38] Most, if not all cancers can be characterized by six functional changes in cell physiology: self-sufficiency in growth signals, insensitivity to growth-inhibitory signals, evasion of apoptosis, limitless replicative potential, sustained angiogenesis, and tissue invasion and metastasis.[39,40]

Because carcinogenesis is such a complicated process and because cells can take different paths to attaining the physiological changes required to become malignant, there are many possible sites of action of catechins in cancer prevention. There is a large body of evidence, mainly from animal experiments, that suggests that tea can prevent a variety of tumors in different target organs, at most stages of carcinogenesis, but there are very few studies on catechins themselves. Important mechanisms may include inhibition of metabolic activation, induction of phase II detoxifying enzymes, protection of DNA against oxidative damage, induction of apoptosis, inhibition of cell proliferation, and inhibition of angiogenesis.[41,42]

OUTLINE OF THIS THESIS

The aim of the work described in this thesis was to evaluate the associations between dietary catechin intake and risk of coronary heart disease, stroke, and cancer. In order to study associations between nutrient intakes and chronic diseases, data on the nutrient composition of foods are required. At the start of this project, only comprehensive data on two other subclasses of flavonoids, the flavonols and flavones, were available.[43,44] Although some data on catechin contents had been published, most of these were obtained by spectrophotometric detection methods now considered obsolete, and data on a number of commonly consumed foods were missing entirely.[20] In order to create a database with catechin food composition data, we first optimized an analytical method for the determination of six major catechins [(+)-catechin, (+)-gallocatechin (GC), (-)-epicatechin, (-)-epigallocatechin (EGC), (-)-epicatechin gallate (ECg), and (-)-epigallocatechin gallate (EGCg)] in foods (Chapter 2). We then determined the catechin contents of all commonly consumed plant foods (Chapter 3.1) and beverages (Chapter 3.2) in the Netherlands, taking into account seasonal, year-to-year, and varietal differences. With this database, we

estimated the usual intake of catechins in the Netherlands (Chapter 4). Using data from the Zutphen Elderly Study, a prospective cohort study among elderly men in the Netherlands, we then were able to evaluate the relation between catechin intake and risk of CHD and stroke (Chapter 5.1), and epithelial cancer (Chapter 6.1). Because tea was by far the major catechin source in this elderly population, we wanted to repeat our analyses in a population with a low level of tea consumption. The Iowa Women's Health Study in the USA constitutes such a population, and we determined the association between catechins and CHD (Chapter 5.2), and cancer by individual site (Chapter 6.2) in that prospective cohort of postmenopausal women too. This thesis is concluded with a general discussion of our findings (Chapter 7).

REFERENCES

1. Harborne JB. *The Flavonoids: Advances in research since 1986.* London: Chapman & Hall; 1994.
2. Kühnau J. The flavonoids: a class of semi-essential food components: their role in human nutrition. *World Rev Nutr Diet* 1976;24:117-191.
3. Middleton E, Kandaswami C. The impact of plant flavonoids on mammalian biology: implications for immunity, inflammation and cancer. In: Harborne JB, ed. *The Flavonoids: advances in research since 1986.* London: Chapman & Hall;1994:619-652.
4. Steinmetz KA, Potter JD. Vegetables, fruit, and cancer. I. Epidemiology. *Cancer Causes Control* 1991;2:325-357.
5. World Cancer Research Fund. Vegetables and fruits. In: WCRF/AICR. *Food, nutrition and the prevention of cancer: a global perspective.* Washington (DC): World Cancer Research Fund/American Institute for Cancer Research;1997:436-446.
6. Law MR, Morris JK. By how much does fruit and vegetable consumption reduce the risk of ischaemic heart disease? *Eur J Clin Nutr* 1998;52:549-556.
7. Mayer AM, Harel E. Polyphenol oxidases in plants. *Phytochemistry* 1979;18:193-215.
8. Hollman PCH, Tijburg LBM, Yang CS. Bioavailability of flavonoids from tea. *Crit Rev Food Sci Nutr* 1997;37:719-738.
9. Donovan JL, Bell JR, Kasim KS, German JB, Walzem RL, Hansen RJ, Waterhouse AL. Catechin is present as metabolites in human plasma after consumption of red wine. *J Nutr* 1999;129:1662-1668.
10. Lee MJ, Wang ZY, Li H, Chen L, Sun Y, Gobbo S, Balentine DA, Yang CS. Analysis of plasma and urinary tea polyphenols in human subjects. *Cancer Epidemiol Biomarkers Prev* 1995;4:393-399.
11. Li C, Lee MJ, Sheng SQ, Meng XF, Prabhu S, Winnik B, Huang BM, Chung JY, Yan SQ, Ho CT, Yang CS. Structural identification of two metabolites of catechins and their kinetics in human urine and blood after tea ingestion. *Chem Res Toxicol* 2000;13:177-184.
12. Rice-Evans CA, Miller NJ, Paganga G. Structure-antioxidant activity relationships of flavonoids and phenolic acids. *Free Radical Biol Med* 1996;20:933-956.
13. Yang CS, Chen LS, Lee MJ, Balentine D, Kuo MC, Schantz SP. Blood and urine levels of tea catechins after ingestion of different amounts of green tea by human volunteers. *Cancer Epidemiol Biomarkers Prev* 1998;7:351-354.
14. Van het Hof KH, Kivits GAA, Weststrate JA, Tijburg LBM. Bioavailability of catechins from tea: the effect of milk. *Eur J Clin Nutr* 1998;52:356-359.

15. Kivits GAA, Van der Sman FJP, Tijburg LBM. Analysis of catechins from green and black tea in humans: a specific and sensitive colorimetric assay of total catechins in biological fluids. *Int J Food Sci Nutr* 1997;48:387-392.
16. Maiani G, Serafini M, Salucci M, Azzini E, Ferroluzzi A. Application of a new high performance liquid chromatographic method for measuring selected polyphenols in human plasma. *J Chromatogr B* 1997;692:311-317.
17. Nakagawa K, Miyazawa T. Chemiluminescence-high-performance liquid chromatographic determination of tea catechin, (-)-epigallocatechin 3-gallate, at picomole levels in rat and human plasma. *Anal Biochem* 1997;248:41-49.
18. Bell JRC, Donovan JL, Wong R, Waterhouse AL, German JB, Walzem RL, Kasim-Karakas SE. (+)-Catechin in human plasma after ingestion of a single serving of reconstituted red wine. *Am J Clin Nutr* 2000;71:103-108.
19. Richelle M, Tavazzi I, Enslen M, Offord EA. Plasma kinetics in man of epicatechin from black chocolate. *Eur J Clin Nutr* 1999;53:22-26.
20. Hollman PCH, Arts ICW. Flavonols, flavones and flavanols - nature, occurrence and dietary burden. *J Sci Food Agric* 2000;80:1081-1093.
21. Konings-Dalstra JAA, Reitsma JB. *Hart- en vaatziekten in Nederland 1999, cijfers over ziekte en sterfte [in Dutch].* Den Haag: Nederlandse Hartstichting; 1999.
22. Gheorghiade M, Bonow RO. Coronary artery disease. In: Humes HD, ed. *Kelley's textbook of internal medicine.* Philadelphia: Lippincott Williams & Wilkins;2000:428-448.
23. Ross R. Mechanisms of disease - Atherosclerosis - An inflammatory disease. *N Engl J Med* 1999;340:115-126.
24. Steinberg D. Lewis A. Conner Memorial Lecture. Oxidative modification of LDL and atherogenesis. *Circulation* 1997;95:1062-1071.
25. Fuster V, Badimon L, Badimon JJ, Chesebro JH. The pathogenesis of coronary artery disease and the acute coronary syndromes (1). *N Engl J Med* 1992;326:242-250.
26. Geleijnse JM, Launer LJ, Hofman A, Pols HA, Witteman JC. Tea flavonoids may protect against atherosclerosis: the Rotterdam Study. *Arch Intern Med* 1999;159:2170-2174.
27. Tijburg LBM, Mattern T, Folts JD, Weisgerber UM, Katan MB. Tea flavonoids and cardiovascular diseases: A review. *Crit Rev Food Sci Nutr* 1997;37:771-785.
28. Wiseman SA, Balentine DA, Frei B. Antioxidants in tea. *Crit Rev Food Sci Nutr* 1997;37:705-718.
29. Van Acker FAA, Schouten O, Haenen GRMM, Van der Vijgh WJF, Bast A. Flavonoids can replace alpha-tocopherol as an antioxidant. *FEBS Lett* 2000;473:145-148.
30. Zhu QY, Huang Y, Tsang D, Chen ZY. Regeneration of alpha-tocopherol in human low-density lipoprotein by green tea catechin. *J Agric Food Chem* 1999;47:2020-2025.
31. Packer L. Vitamin E and the antioxidant network: protection of human Low Density Lipoprotein from oxidation. In: Ohigashi H, Osawa T, Terao J, Watanabe S, Yoshikawa T, eds. *Food factors for cancer prevention.* Tokyo: Springer-Verlag;1997:452-459.
32. Heinecke JW. Oxidants and antioxidants in the pathogenesis of atherosclerosis: implications for the oxidized low density lipoprotein hypothesis. *Atherosclerosis* 1998;141:1-15.
33. Heijnen CGM, Haenen GRMM, Wiseman SA, Tijburg LBM, Bast A. The interaction of tea flavonoids with the NO-system: discrimination between good and bad NO. *Food Chem* 2000;70:365-370.
34. Liang YC, Huang YT, Tsai SH, Lin-Shiau SY, Chen CF, Lin JK. Suppression of inducible cyclooxygenase and inducible nitric oxide synthase by apigenin and related flavonoids in mouse macrophages. *Carcinogenesis* 1999;20:1945-1952.
35. Kang WS, Lim IH, Yuk DY, Chung KH, Park JB, Yoo HS, Yun YP. Antithrombotic activities of

green tea catechins and (-)-epigallocatechin gallate. *Thromb Res* 1999;96:229-237.

36. Visser O, Coebergh JWW, Schouten LJ, Van Dijck JAAM. *Incidence of cancer in the Netherlands 1996*. Utrecht: Association of Comprehensive Cancer Centres; 2000.
37. Parkin DM, Whelan SL, Ferlay J, Raymond L, Young J. *Cancer incidence in five continents, volume VII, IARC Scientific Publications No. 143*. Lyon: IARC; 1997.
38. Wattenberg LW. An overview of chemoprevention: current status and future prospects. *Proc Soc Exp Biol Med* 1997;216:133-141.
39. Hanahan D, Weinberg RA. The hallmarks of cancer. *Cell* 2000;100:57-70.
40. Halliwell B, Gutteridge JMC. Free radicals, ageing, and disease. In: Halliwell B, Gutteridge JMC, eds. *Free radicals in biology and medicine*. Oxford: Oxford University Press;1989:416-508.
41. Dreosti IE, Wargovich MJ, Yang CS. Inhibition of carcinogenesis by tea: The evidence from experimental studies. *Crit Rev Food Sci Nutr* 1997;37:761-770.
42. Katiyar SK, Mukhtar H. Tea in chemoprevention of cancer: epidemiologic and experimental studies (review). *Int J Oncol* 1996;8:221-238.
43. Hertog MGL, Hollman PCH, Van de Putte B. Content of potentially anticarcinogenic flavonoids of tea infusions, wines, and fruit juices. *J Agric Food Chem* 1993;41:1242-1246.
44. Hertog MGL, Hollman PCH, Katan MB. Content of potentially anticarcinogenic flavonoids of 28 vegetables and 9 fruits commonly consumed in the Netherlands. *J Agric Food Chem* 1992;40:2379-2383.

A quantitative analytical method for the determination of catechins in foods

Catechins are found in many foods of plant origin. We optimized the quantification of catechins in three model foods: apples, black grapes and canned kidney beans. Catechins [(+)-catechin, (-)-epicatechin, (-)-epigallocatechin, (-)-epicatechin gallate, (-)-epigallocatechin gallate] were separated with gradient reversed phase HPLC and quantified by UV (270 nm) or fluorescence (280-310 nm excitation-emission) detection in series. Type (ethanol, methanol or acetone) and concentration (40-100% in water) of extraction solvent influenced catechin yield, whereas extraction time (10-60 min) did not. Adequate extraction was attained with 60-100% methanol for apple and grapes, and 40-80% methanol for beans. Recovery (>94%), within-run repeatability (1-5%), between-run reproducibility (3-9%) and detection limits (0.1-3.9 mg/kg fresh apple or 0.01-0.29 µg/mL extract) were satisfactory. With this method 40 solid food samples a day can be analyzed, without the need for sample clean-up.

Arts ICW, Hollman PCH. Optimization of a quantitative method for the determination of catechins in fruits and legumes. *J Agric Food Chem 1998;46:5156-5162.*

INTRODUCTION

Catechins, or flavanols, are polyphenolic compounds of the flavonoid type, which are present in tea,[1] wine,[2,3] fruits[4,5] and legumes.[6] The five major catechins are (+)-catechin, (-)-epicatechin, (-)-epigallocatechin (EGC), (-)-epicatechin gallate (ECg) and (-)-epigallocatechin gallate (EGCg) (Figure 2.1). Catechins are potentially beneficial to human health: they are strong antioxidants, anticarcinogens, anti-inflammatory agents and inhibitors of platelet aggregation in *in vivo* or *in vitro* studies.[7-9] There is some epidemiological evidence that the consumption of tea, a rich source of catechins, could reduce the risk for certain cancers,[7,10] and for coronary heart disease mortality and stroke.[11] However, not all studies have shown a protective effect of tea consumption. Epidemiological studies that take into account not only tea, but all catechin containing foods could possibly clarify the relation between catechin intake and disease. However, reliable quantitative data on the catechin content of foods are lacking, particularly for EGC, EGCg and ECg, which are the major catechins in tea. EGCg has been suggested as an important compound in the prevention of cancer.[12]

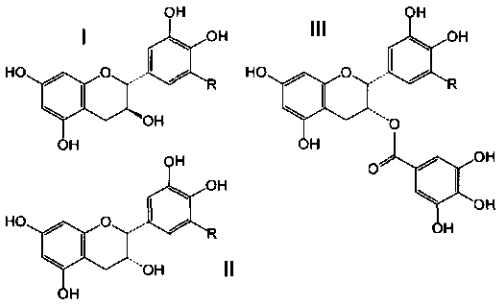


Figure 2.1 Chemical structure of catechins: (I) R=H (+)-catechin, R=OH (+)-gallocatechin (GC); (II) R=H (-)-epicatechin, R=OH (-)-epigallocatechin (EGC); (III) R=H (-)-epicatechin gallate (ECg), R=OH (-)-epigallocatechin gallate (EGCg).

A number of protocols for the quantitative determination of catechins are available. Only, these are mostly developed for liquids such as tea infusions,[1,13-15] wine[3,16] or fruit juices.[17,18] Analytical methods for catechins in solid foods generally focus on identification of new derivatives or polymeric catechins (proanthocyanidins) and are not designed for quantification. In addition, they are lengthy and require considerable sample clean-up. Determination of compounds in solid foods requires extraction from the sample matrix prior to injection into the HPLC system. Thus far, extraction methods for catechins have not been systematically examined.

In this paper we present a quantitative method for the determination of (+)-catechin, (-)-epicatechin, EGC, ECg and EGCg in solid foods, which can be applied to analyze a large number of samples efficiently. We studied fluorescence detection because it has better specificity than UV detection, which may reduce sample clean-up and thus analysis time. Fluorescence detection has been used as a selective and sensitive

method to measure (+)-catechin in plasma.[19] Furthermore, we optimized the extraction procedure using three model foods: apple, black grapes and canned beans.

METHODS

Chromatography

The HPLC equipment consisted of a Gilson 234 autoinjector (Gilson, Villiers-le-Bel, France), which injected 10 µL of sample onto an Inertsil ODS-2 column (150 x 4.6 mm, 5 µm; GL Sciences Inc., Tokyo, Japan) protected by an Opti-Guard PR C18 Violet A guard column (Optimize Technologies Inc., Oregon City, OR), both placed in a column oven (Jones Chromatography 7971 Column Heater, Lakewood, CO) set at 30°C. Merck Hitachi L6000A and L6200 pumps (Hitachi Ltd., Tokyo, Japan) were used to create a gradient with a flow rate of 1.0 mL min^{-1}. Choice of column type and gradient had been optimized before for catechins in tea.[1] However, small adaptations had to be made for the analysis of fruits and legumes, to prevent matrix compounds from accumulating on the column. The solvents used for separation were 5% acetonitrile (eluent A) and 25% acetonitrile (eluent B) in phosphate buffer (0.025 M, pH 2.4). The gradient was as follows: 0-5 min: 10% B, 5-20 min: linear gradient from 10 to 80% B, 20-22 min: linear gradient from 80 to 90% B, 22-25 min: isocratic at 90% B, 25-28 min: linear return to 10% B, 28-37 min: isocratic at 10% B to re-equilibrate. The monitoring of the effluent was done by a Merck Hitachi F1050 fluorescence spectrophotometer (280 nm excitation, 310 nm emission wavelengths) and a Kratos (Kratos Analytical Instruments, Ramsey, NJ) spectroflow 783 UV detector (270 nm), which were series connected. Fluorescence spectra of the pure compounds were measured with a Perkin Elmer (Beaconsfield, England) LS 50 B luminescence spectrometer.

Standards

Pure standards of (+)-catechin (purity >98%, catalog number C1251), (-)-epicatechin (crystalline, E1753) and EGC (purity >98%, E3768) were obtained from Sigma (St. Louis, MO). ECg and EGCg (purity of both >95%) were kindly donated by Alan Davies (Unilever Research Colworth Laboratory, UK). Calibration solutions were freshly prepared for each series of analyses in 90% methanol from stock solutions which were kept at 4°C. Stock solutions contained 1 mg of catechins/mL methanol. The stability of the stock solutions was followed spectrophotometrically every other day for one week; no deterioration occurred. Calibration curves were constructed by linear regression of the peak area against concentration of the calibration solution ((+)-catechin and (-)-epicatechin: 2-10 µg/mL; ECg and EGCg: 5-15 µg/mL; EGC: 10-

30 µg/mL). All calibration curves were linear when forced through the origin, with correlation coefficients close to unity: 1.000 for (+)-catechin, (-)-epicatechin and ECg, 0.996 for EGC and 0.999 for EGCg. Limits of detection were determined by injecting 10 µL of a standard mixture. The limit of detection was defined as the amount of catechin which resulted in a peak height three times the standard deviation of the baseline noise. Peaks were identified by comparing their retention time with the retention time of the pure standards. A diode array detector (HP 1040A Upgraded Version, Hewlett Packard, Palo Alto, CA) was used to confirm peak identity and to check peak purity.

Sample preparation

Jonagold apple and canned kidney-beans were purchased at a local supermarket in February 1997 and black grapes were purchased in June 1997. The apples were quartered and the core was removed. The apple segments, including skin, were immediately chopped under liquid nitrogen and stored at -20°C. Freeze-drying was started at the same day. The black grapes were washed and dried carefully, cut in halves, frozen under liquid nitrogen and freeze-dried immediately. The kidney-beans were allowed to leak out, washed with running water, frozen at -80°C for 24 hours and freeze-dried. After freeze-drying, the samples were ground to a powder and stored at -20°C until analyzed.

Extraction: procedure

Approximately 0.5 gram of freeze-dried sample was mixed with 25 mL of 90% v/v methanol/water for apple and grapes, and 25 mL of 70% v/v methanol/water for beans. The extract was shaken in a mechanical shaker (250 rpm) for 60 minutes at room temperature. After extraction, the volume was made up to 50 mL with the same solvent, filtered over a 0.45 µm Acrodisc filter (Gelman Sciences, Ann Arbor, MI) and injected without further processing.

Extraction: optimization

For apple and beans, the following extraction conditions were optimized: type of extraction solvent, concentration of extraction solvent, extraction time and amount of sample. Methanol concentration was the only variable studied for black grapes. Three extraction solvents were tested: ethanol, methanol and acetone, in concentrations that varied from 40-100% solvent in water. Results are expressed as a percentage of the maximum yield obtained with one of these solvents for each component in each product. Samples were extracted in a mechanical shaker (250 rpm) at room temperature for 10 to 60 minutes. The sample amount (0.25, 0.50, 0.75,

1.00 g) in 25 mL extraction solvent was varied in order to check whether the solubility of catechins was a limiting factor.

Recovery and stability

Recovery was calculated by comparing catechin levels in apple with those in apple spiked with a known amount of standard compound. Standards were added immediately following the addition of extraction solvent, at 50% and 100% of the original level of the apple. Whenever the compound was not present in apple, a well detectable amount of the standard was added. The stability of standard solutions was studied because sample extracts could stay in the autoinjector at room temperature for up to 24 hours during routine analyses. The peak areas of the first standard mixture in a run were compared with the peak areas of the last standard mixture in the same run. Data from five runs were pooled and a two-sided paired t-test was used for statistical analysis.

To test the effect of freeze-drying, we compared the catechin content determined in fresh apple with that in freeze-dried apple. Four apples were quartered; two pieces of each apple were freeze-dried as described under Sample preparation. The remaining two pieces were ground in a Waring blendor (model 8011G, 1 liter, Waring Commercial, New Hartford, CT) under methanol for 2 minutes and subsequently placed in an ultrasonic bath at room temperature for five minutes. Antecedent testing had shown that for freeze-dried samples five minutes ultrasonification gave yields similar to 60 minutes mechanical shaking. The water content of the fresh apple extract totaled approximately 18%. Prior to injection, the extract was filtered over a 0.45 µm Acrodisc filter. There were no indications that freeze-drying notably affected the catechin content of apple: compared with fresh apple, we observed a 0.3% lower (+)-catechin level and a 2.4% higher (-)-epicatechin level in freeze-dried apple.

Repeatability and reproducibility

Within-run repeatability of the method was assessed in apple and black grapes by analyzing 10 samples of each food on one day. Five duplicate analyses were carried out on separate days within a period of three months to determine between-run reproducibility.

RESULTS AND DISCUSSION

Detection and chromatography

Fluorescence detection at 280 nm excitation and 310 nm emission wavelength is a

specific and sensitive method to determine (+)-catechin and (-)-epicatechin in freeze-dried food samples. Detection limits of (+)-catechin and (-)-epicatechin improved more than 10-fold compared to UV detection (Table 2.1), and peak separation and specificity were better when fluorescence detection was used (Figure 2.2). Comparable detection limits have been reported for the fluorescence detection of (+)-catechin in plasma (0.02 µg/mL limit of quantitation, no definition provided)[19] and for electrochemical detection of (+)-catechin (0.002 µg/mL) and (-)-epicatechin (0.005 µg/mL).[20] Lower detection limits have been reported using GC-MS[21] or HPLC after derivatization of catechins with 4-dimethylaminocinnamaldehyde (DMACA).[22] However, the purpose of this study was not to maximize the sensitivity of the method. We intended to develop a method which can efficiently analyze a variety of food samples with a broad range in levels of catechins. Trace quantities of catechins in a food are irrelevant because of their small contribution to dietary intake. As we will show later in this paper, catechin levels may range from 4 up to 226 mg/kg fresh weight.

Table 2.1 Detection limits of catechins

	Fluorescence		UV	
	standard (µg/mL)	apple (mg/kg fresh weight)[a]	standard (µg/mL)	apple (mg/kg fresh weight)[a]
(+)-catechin	0.008	0.1	0.13	1.8
(-)-epicatechin	0.007	0.1	0.09	1.2
EGC	-	-	0.29	3.9
EGCg	-	-	0.03	0.4
ECg	-	-	0.03	0.4

[a] Detection limits in apple are calculated from the standard data as follows: [(value in µg/mL)/sample weight] x 50 mL x (fraction dry weight); sample weight is 0.5 g, fraction dry weight is 0.136.

EGC, EGCg and ECg showed little native fluorescence (Figure 2.3). Attempts to improve the fluorescence of EGC, EGCg and ECg, by changing the eluent composition or by complexation of the catechins with borate, were not successful (data not shown). Therefore, we monitored (+)-catechin and (-)-epicatechin with fluorescence detection and EGC, EGCg and ECg with UV detection at 270 nm, the wavelength which corresponds to an absorption maximum for EGC, the compound with the lowest UV absorbance. The detectors were connected in series so that analysis time was not affected. Fluorescence detection was the preferred method for (+)-catechin and (-)-epicatechin because it increased specificity: these two compounds elute in an area of the chromatogram where many unknown peaks elute (Figure 2.2). Monitoring (+)-catechin and (-)-epicatechin with UV would necessitate

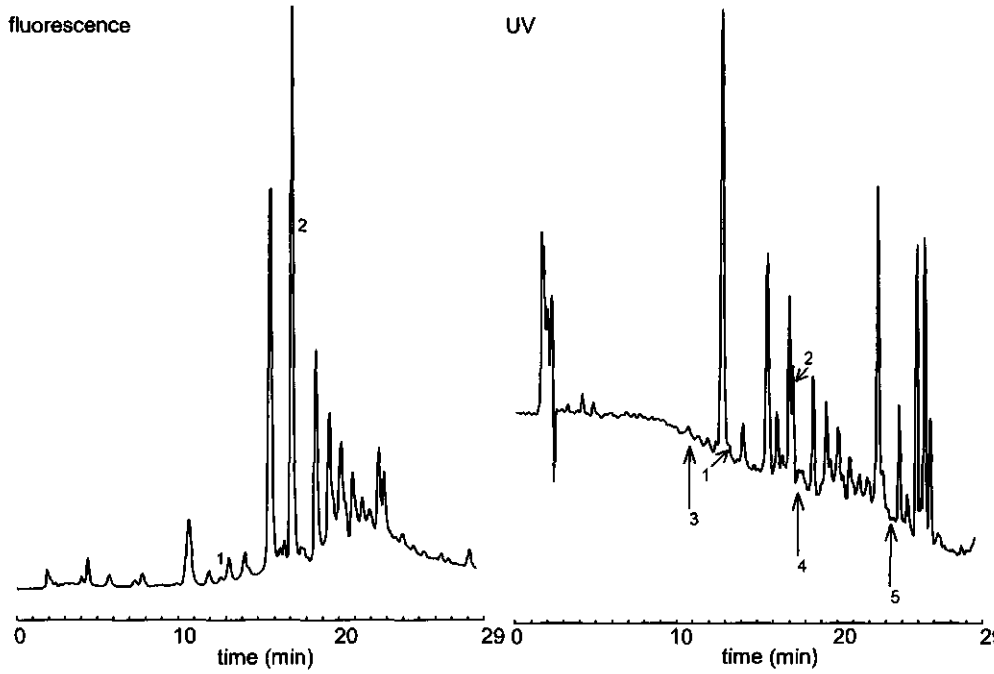


Figure 2.2 Typical chromatograms of an apple extract with UV (270 nm) and fluorescence (280 nm excitation, 310 nm emission) detection (acetonitrile-phosphate buffer gradient, pH 2.4, flow-rate 1.0 mL min^{-1}). Peaks: 1. (+)-catechin; 2. (-)-epicatechin; 3. EGC; 4. EGCg; 5. ECg. Compounds 3-5 are not present in apple. Their peak positions, based on retention times of the pure standards are indicated in the chromatogram.

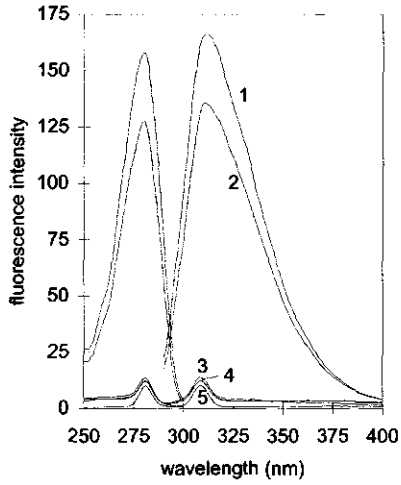


Figure 2.3 Fluorescence excitation (curves on the left side) and emission (curves on the right side) spectra of 1 µg/mL (+)-catechin (1), (-)-epicatechin (2), EGC (3), ECg and EGCg (4) in eluent, and of eluent only (5).

sample clean-up. UV limits of detection of EGCg and ECg (both 0.03 µg/mL) were only approximately four times higher than those for (+)-catechin and (-)-epicatechin with fluorescence detection. EGC had the highest limit of detection: 0.3 µg/mL (Table 2.1). Lower detection limits have been reported in studies with a coulochem electrode array detection system (EGC: 1.5 ng/mL, EGCg: 0.5 ng/mL)[23] and with chemiluminescence detection (EGCg: 0.9 ng/mL).[24] These systems are as yet, however, not suitable for routine analyses.

Extraction

Both type and concentration of the extraction solvent (Figure 2.4 and 2.5) affected yield of catechins. Adequate extraction was achieved with 60 to 100% methanol for apples and grapes, and with 40 to 80% methanol for beans. The extraction solvents chosen were 90% methanol for apples and grapes and 70% methanol for beans. Chromatogram peak shape was adequate when methanol or acetone was used, but extraction with ethanol caused severe peak tailing. Therefore, the ethanol data are not shown in the figures. Extraction with methanol or acetone gave similar maximum catechin yields. Since methanol is more agreeable to work with than acetone, we continued our experiments with methanol as an extraction solvent. When the percentage of methanol in the extraction solvent was reduced to 40%, catechin yield in apple and grapes decreased to approximately 70% of the maximum value. In contrast, beans showed no yield reduction with decreasing methanol concentrations, but the yield reduced to 50% of the maximum value when 100% methanol was used.

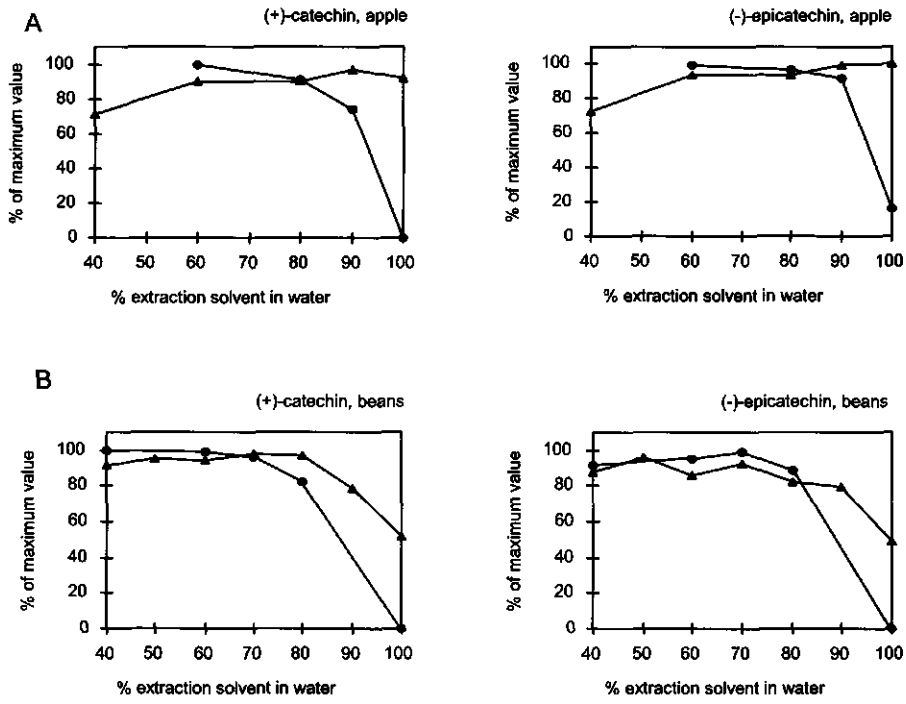


Figure 2.4 (+)-catechin and (-)-epicatechin yield in apple (A) and beans (B), expressed as a percentage of the maximum yield, using 40-100% (v/v) methanol (▲) or acetone (●) in water as an extraction solvent (each data point represents the average of duplicate analyses).

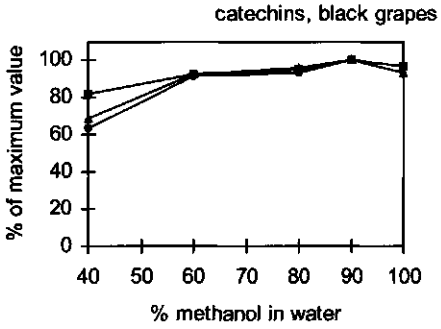
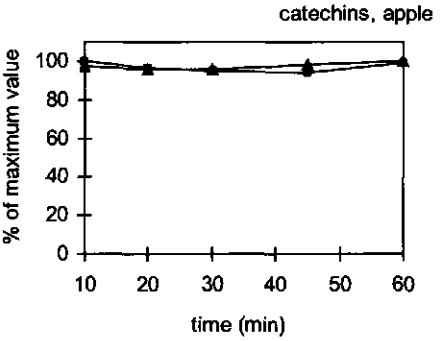


Figure 2.5 (+)-catechin (■), (-)-epicatechin (▲) and (-)-epicatechin gallate (●) yield in black grapes, expressed as a percentage of the maximum yield, using 40-100% (v/v) methanol in water as an extraction solvent (each data point represents the average of duplicate analyses).



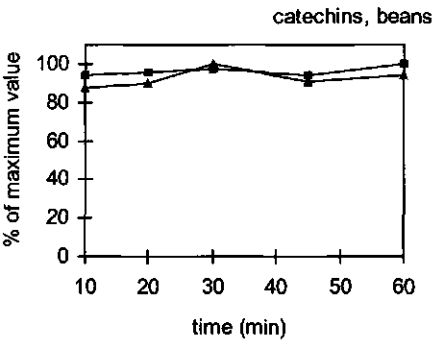


Figure 2.6 Influence of extraction time on (+)-catechin (■) and (-)-epicatechin (▲) yield in apples and beans, expressed as a percentage of the maximum yield (each data point represents the average of duplicate analyses). Apples were extracted in 90% methanol, beans in 70% methanol.

This difference in extraction behavior between canned beans on the one hand and grapes and apple on the other hand, may be explained by the action of polyphenoloxidase. Polyphenoloxidase is an enzyme which is widely distributed in plants and which catalyses the oxidation and polymerization of catechins to brown pigments when cells are ruptured.[25] Methanol reduces the polyphenoloxidase activity.[26] It is therefore possible that extraction with low methanol solvents does not completely inactivate polyphenoloxidase in fresh fruits, which results in reduced catechin yields. We observed browning in the low methanol apple and grape extracts, which supports this hypothesis. Canned beans have undergone a heat treatment, which denatures all polyphenoloxidase. Prolonging the extraction time from 10 up to 60 minutes (Figure 2.6) or increasing the amount of sample from 0.25 up to 1.00 g (data not shown) did not affect extraction efficiency.

When optimum extraction conditions were applied, Jonagold apples contained 68.0 mg (-)-epicatechin/kg fresh edible weight and 3.8 mg (+)-catechin/kg fresh edible weight. Risch and Herrmann[5] reported values in the same order of magnitude for Jonagold apples: 29 mg (-)-epicatechin and 2 mg (+)-catechin per kg fresh weight.

Growing conditions, maturity, and storage period of the fruits in the latter study may be somewhat different from those in our study, and may therefore account for the differences in catechin contents. Maturity stage appears to be the most important contributor to differences within varieties.[27-29] We took particular care to process mature fruits only. In addition, differences in analytical methods may have contributed to some extent. Risch and Herrmann (1988) did not provide data on the recovery of their method. In black grapes we detected ECg (84.4 mg/kg fresh weight) in addition to high levels of (+)-catechin (225.5 mg/kg fresh weight) and (-)-epicatechin (190.3 mg/kg fresh weight). The same compounds were detected in qualitative studies in grape seeds[30] and skins.[31] In spite of the vast amount of literature on catechins in wines, no quantitative data on whole black grapes were found. Canned kidney-beans contain mostly (+)-catechin (16.5 mg/kg fresh weight) and some (-)-epicatechin (7.2 mg/kg fresh weight). None of the other compounds were detected in beans. Hanefeld and Herrmann[6] reported the presence of (+)-catechin and (-)-epicatechin in several varieties of beans, but did not quantify them. Other studies are less reliable because simple colorimetric methods were used to determine the total polyphenol content of beans.[32,33] These data clearly cannot be compared with ours.

Recovery and stability

Recovery of catechins added prior to the extraction procedure approximated 100% for all compounds, ranging from 92% for (+)-catechin to 105% for EGCg (Table 2.2). These data are in accordance with recoveries of (+)-catechin and (-)-epicatechin reported elsewhere in apple must[17,18] and wine,[3,16] and of (-)-epicatechin in cocoa beans (92%).[34] To our knowledge, there are no recovery studies available for EGC, ECg and EGCg in food samples. Lee et al.[23] studied EGC, (-)-epicatechin and EGCg in human plasma and urine. Recoveries from plasma (EGC: 88%; (-)-epicatechin:

Table 2.2 Recovery of catechins added to apple, and coefficients of variation (CV) of the analysis of catechins from apple and black grapes

	Recovery (%)[a]		CV (%)			
			apple		black grapes	
	50% addition	100% addition	within-run[c]	between-run[d]	within-run[c]	between-run[d]
(+)-catechin	96	92	2.5	9.0	2.6	3.4
(-)-epicatechin	100	94	1.3	4.4	3.4	8.2
ECg [b]	98	98	-	-	5.4	5.0
EGCg [b]	96	105	-	-	-	-
EGC [b]	-	101	-	-	-	-

[a] Average of duplicate analyses. [b] Recoveries were calculated by adding a well detectable amount, EGC was added at only one level. [c] n=10. [d] n=5 duplicate analyses.

85%; EGCg: 82%) were lower than those from urine (92, 95 and 87%, respectively). Another study reported a similar recovery for EGCg (84%) from rat plasma.[24] Plasma requires considerable clean-up before analysis, which may have caused the reduced recovery compared to our method in food samples.

The last standard mixture in a run tended to have slightly lower peak areas than the first mixture for (-)-epicatechin (3.1% decrease, P=0.01), (+)-catechin (3.7% decrease, P=0.06), EGC (8.1% decrease, P=0.06) and EGCg (5.2% decrease, P=0.08), but not for ECg (0.1% increase, P=0.96). On average, there were 29 samples in-between the two standards (approximately 18 hours). It is unlikely that the small decreases in standard areas have seriously affected the quantitative analysis of our samples, also because standard peak areas were averaged before calculating the catechin content of food samples.

Repeatability and reproducibility

Within-run repeatability of the method was excellent. Coefficients of variation ranged from 1.3% for the determination of (-)-epicatechin in apple to 5.4% for ECg in grapes (Table 2.2). Comparable coefficients of variation are reported by other authors in wine[3,16] and plasma.[19] Lee and coworkers[23] found a slightly higher intraday variation for (-)-epicatechin in plasma (8.2%) and urine (7.8%). Between-run reproducibility varied between 3.4% for (+)-catechin in black grapes and 9.0% for (+)-catechin in apple (Table 2.2). Literature values for the between-run coefficients of variation in plasma are 2.1-5.2% for (+)-catechin[19] and 10.4% for (-)-epicatechin.[23] The lower (+)-catechin content of apple compared to grapes, causes the relatively large coefficient of variation in apple.

In this paper, we describe a simple method for the determination of (+)-catechin, (-)-epicatechin, (-)-epigallocatechin, (-)-epicatechin gallate and (-)-epigallocatechin gallate in fruits and legumes, without the need for sample clean-up. The relatively short HPLC run of 37 minutes, and the extremely simple extraction method, make it possible to run circa 40 samples a day. Recovery, repeatability and reproducibility are comparable to or better than currently available methods. Sensitivity is adequate, and allows the determination of a wide range of catechin levels in one run.

ACKNOWLEDGMENTS

The authors would like to thank Betty v.d. Struijs-v.d. Putte, John van Trijp and Dini Venema for their expert assistance. This work was carried out with financial support from the Commission of the European Communities, Agriculture and Fisheries (FAIR) specific RTD programme, CT95 0653, the Product Board for Horticulture, the Greenery, and the Ministry of Agriculture, Nature Management and

Fisheries.

REFERENCES

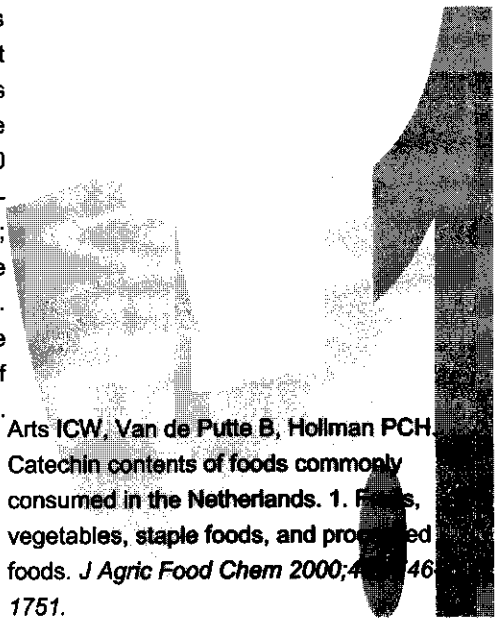
1. Khokhar S, Venema D, Hollman PCH, Dekker M, Jongen W. A RP-HPLC method for the determination of tea catechins. *Cancer Lett* 1997;114:171-172.
2. Soleas GJ, Dam J, Carey M, Goldberg DM. Toward the fingerprinting of wines: cultivar-related patterns of polyphenolic constituents in Ontario wines. *J Agric Food Chem* 1997;45:3871-3880.
3. Goldberg DM, Tsang E, Karumanchiri A, Diamandis EP, Soleas G, Ng E. Method to assay the concentrations of phenolic constituents of biological interest in wines. *Anal Chem* 1996;68:1688-1694.
4. Macheix JJ, Fleuriet A, Billot J. *Fruit phenolics*. Boca Raton (FL): CRC Press; 1990.
5. Risch B, Herrmann K. Die Gehalte an Hydroxyzimtsäure-Verbindungen und Catechinen in Kern- und Steinobst (Contents of hydroxycinnamic acid derivatives and catechins in pome and stone fruit). *Z Lebensm Unters Forsch* 1988;186:225-230.
6. Hanefeld M, Herrmann K. Über das Vorkommen von Proanthocyanidinen, Leukoanthocyanidinen und Catechinen im Gemüse (On the occurrence of proanthocyanidins, leucoanthocyanidins and catechins in vegetables). *Z Lebensm Unters Forsch* 1976;161:243-248.
7. Kohlmeier L, Weterings KGC, Steck S, Kok FJ. Tea and cancer prevention: an evaluation of the epidemiologic literature. *Nutr Cancer* 1997;27:1-13.
8. Cook NC, Samman S. Flavonoids: chemistry, metabolism, cardioprotective effects, and dietary sources. *J Nutr Biochem* 1996;7:66-76.
9. Katiyar SK, Mukhtar H. Tea in chemoprevention of cancer: epidemiologic and experimental studies (review). *Int J Oncol* 1996;8:221-238.
10. Blot WJ, Chow WH, McLaughlin JK. Tea and cancer: a review of the epidemiological evidence. *Eur J Cancer Prev* 1996;5:425-438.
11. Tijburg LBM, Mattern T, Folts JD, Weisgerber UM, Katan MB. Tea flavonoids and cardiovascular diseases: A review. *Crit Rev Food Sci Nutr* 1997;37:771-785.
12. Jankun J, Selman SH, Swiercz R, Skrzypczak Jankun E. Why drinking green tea could prevent cancer. *Nature* 1997;387:561-561.
13. Kuhr S, Engelhardt UH. Determination of flavanols, theogallin, gallic acid and caffeine in tea using HPLC. *Z Lebensm Unters Forsch* 1991;192:526-529.
14. Bailey RG, Nursten HE, McDowell I. Comparative study of the reversed-phase high-performance liquid chromatography of black tea liquors with special reference to the thearubigins. *J Chromatogr* 1991;542:115-128.
15. Goto T, Yoshida Y, Kiso M, Nagashima H. Simultaneous analysis of individual catechins and caffeine in green tea. *J Chromatogr A* 1996;749:295-299.
16. Ricardo da Silva JM, Rosec JP, Bourzeix M, Heredia N. Separation and quantitative determination of grape and wine procyanidins by high performance reversed phase liquid chromatography. *J Sci Food Agric* 1990;53:85-92.
17. Suárez B, Picinelli A, Mangas JJ. Solid-phase extraction and high-performance liquid chromatographic determination of polyphenols in apple musts and ciders. *J Chromatogr A* 1996;727:203-209.
18. Suárez Vallés B, Santamaría Victorero J, Mangas Alonso JJ, Blanco Gomis D. High-performance liquid chromatography of the neutral phenolic compounds of low molecular weight in apple juice. *J Agric Food Chem* 1994;42:2732-2736.
19. Ho Y, Lee YL, Hsu KY. Determination of (+)-catechin in plasma by high-performance liquid

chromatography using fluorescence detection. *J Chromatogr B* 1995;665:383-389.

20. Kermasha S, Goetghebeur M, Dumont J, Couture R. Analyses of phenolic and furfural compounds in concentrated and non-concentrated apple juices. *Food Res Int* 1995;28:245-252.
21. Luthria DL, Jones AD, Donovan JL, Waterhouse AL. GC-MS determination of catechin and epicatechin levels in human plasma. *J High Resolut Chromatogr* 1997;20:621-623.
22. Treutter D. Chemical reaction detection of catechins and proanthocyanidins with 4-dimethylaminocinnamaldehyde. *J Chromatogr* 1989;467:185-193.
23. Lee MJ, Wang ZY, Li H, Chen L, Sun Y, Gobbo S, Balentine DA, Yang CS. Analysis of plasma and urinary tea polyphenols in human subjects. *Cancer Epidemiol Biomarkers Prev* 1995;4:393-399.
24. Nakagawa K, Miyazawa T. Chemiluminescence-high-performance liquid chromatographic determination of tea catechin, (-)-epigallocatechin 3-gallate, at picomole levels in rat and human plasma. *Anal Biochem* 1997;248:41-49.
25. Whitaker JR, Lee CY. Recent advances in chemistry of enzymatic browning. An overview. *ACS Symp Ser* 1995;600:2-7.
26. Tome D, Nicolas J, Drapron R. Influence of water activity on the reaction catalyzed by polyphenoloxidase (E.C.1.14.18.1.) from mushrooms in organic liquid media. *Lebensm Wiss Technol* 1978;11:38-41.
27. Burda S, Oleszek W, Lee CY. Phenolic compounds and their changes in apples during maturation and cold storage. *J Agric Food Chem* 1990;38:945-948.
28. Amiot MJ, Tacchini M, Aubert S, Nicolas J. Phenolic composition and browning susceptibility of various apple cultivars at maturity. *J Food Sci* 1992;57:958-962.
29. McRae KB, Lidster PD, DeMarco AC, Dick AJ. Comparison of the polyphenol profiles of apple fruit cultivars by correspondence analysis. *J Sci Food Agric* 1990;50:329-342.
30. Santos-Buelga C, Francia-Aricha EM, Escribano-Bailón MT. Comparative flavan-3-ol composition of seeds from different grape varieties. *Food Chem* 1995;53:197-201.
31. Escribano-Bailón MT, Guerra MT, Rivas-Gonzalo JC, Santos-Buelga C. Proanthocyanidins in skins from different grape varieties. *Z Lebensm Unters Forsch* 1995;200:221-224.
32. Deshpande SS, Cheryan M. Determination of phenolic compounds of dry beans using vanillin, redox and precipitation assays. *J Food Sci* 1987;52:332-334.
33. Laurena AC, Revilleza MJR, Mendoza EMT. Polyphenols, phytate, cyanogenic glycosides, and trypsin inhibitor activity of several Philippine indigenous food legumes. *J Food Comp Anal* 1994;7:194-202.
34. Kim H, Keeney PG. Method of analysis for (-)-epicatechin in cocoa beans by high performance liquid chromatography. *J Food Sci* 1983;48:548-551.

Catechin contents of foods: fruits, vegetables, staple foods, and processed foods

To enable the epidemiological evaluation of catechins, data on their contents in foods are required. HPLC with UV and fluorescence detection was used to determine the levels of (+)-catechin, (-)-epicatechin, (+)-gallocatechin (GC), (-)-epigallocatechin (EGC), (-)-epicatechin gallate (ECg), and (-)-epigallocatechin gallate (EGCg) in 24 types of fruits, 27 types of vegetables and legumes, some staple foods, and processed foods commonly consumed in the Netherlands. Most fruits, chocolate, and some legumes contained catechins. Levels varied to a large extent: from 4.5 mg/kg in kiwi fruit to 610 mg/kg in black chocolate. (+)-Catechin and (-)-epicatechin were the predominant catechins; GC, EGC, and ECg were detected in some foods, but none of the foods contained EGCg. The data reported here provide a base for the epidemiological evaluation of the effect of catechins on the risk for chronic diseases.



Arts ICW, Van de Putte B, Hollman PCH. Catechin contents of foods commonly consumed in the Netherlands. 1. F[illegible]s, vegetables, staple foods, and pro[illegible]ed foods. *J Agric Food Chem 2000;4[illegible]46-1751.*

INTRODUCTION

Observational studies show that fruits and vegetables protect against cancer and cardiovascular diseases.[1-3] An important question is which compounds might be responsible for this protective effect. Flavonoids, secondary plant metabolites with antioxidant activity, are potentially protective compounds. Effects of flavonoids on the cancer process, the immune system and haemostasis have been reported in cell systems and animals.[4] The average total intake of flavonoids in the USA was estimated to be 1 g/day. Catechins (Figure 3.1.1), one of the six classes of flavonoids, contributed one fifth of the total estimated intake.[5] Catechins are the principal components of tea; they make up 3-10% of black tea solids, and 30-42% of green tea solids.[6] Epidemiological studies have shown that tea may reduce the risk for certain cancers,[7,8] coronary heart disease, and stroke.[9] However, the protective effect of tea is not undisputed. Catechins are also present in fruits and vegetables, and this may partially account for the ambiguity in the epidemiological data on tea. Still, quantitative data on catechin contents of foods are as yet, largely absent, incomplete or unreliable.

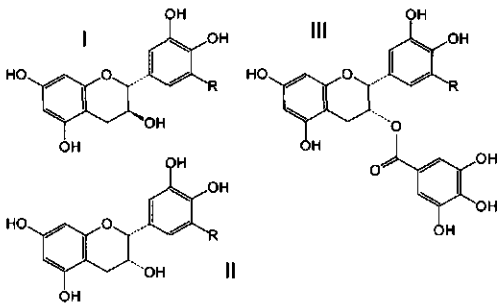


Figure 3.1.1 Chemical structure of catechins: (I) R=H (+)-catechin, R=OH (+)-gallocatechin (GC); (II) R=H (-)-epicatechin, R=OH (-)-epigallocatechin (EGC); (III) R=H (-)-epicatechin gallate (ECg), R=OH (-)-epigallocatechin gallate (EGCg).

Herrmann and coworkers started in the 1970s with the analyses of catechins in fruits using thin-layer chromatography methods with spectrophotometric measurements.[10-13] These methods have been used often since. Their major drawback is that they respond not only to catechins, but also to other compounds, resulting in an over-estimation of the catechin content.[14,15] Over the last ten years, analytical methods have evolved and nowadays selective and sensitive HPLC methods are available. However, reported data on catechin contents of foods are incomplete, and mostly limited to (+)-catechin and (-)-epicatechin. Other catechins may be important to human health as well. For example (-)-epigallocatechin gallate (Figure 3.1.1) has been suggested as an important compound in the prevention of cancer.[16]

Epidemiological research requires reliable and representative data on catechins in

foods. This means that samples from different sale outlets, seasons and years should be included to make sure that natural variation is taken into account. The goal of the present study was to provide these data for commonly consumed foods. In this paper we present data on the catechin [(+)-catechin, (-)-epicatechin, (+)-gallocatechin (GC), (-)-epigallocatechin (EGC), (-)-epicatechin gallate (ECg), and (-)-epigallocatechin gallate (EGCg)] contents of 24 types of fruits, 27 types of vegetables and legumes, some staple foods, and a number of processed foods.

METHODS

Sample Collection and Preparation

Fruits and vegetables were selected using data from the Dutch National Food Consumption Survey 1992, and literature data regarding catechin containing foods. Auction supply data were used to select the common varieties of apples and pears. The Dutch National Food Consumption Survey 1992 consists of a nation-wide 2-day dietary record among 6218 males and females aged 1-92 years. Fruits consumed in quantities in excess of 1 g/person/day, and vegetables consumed in excess of 3 g/person/day were selected. These limits were chosen because products eaten in small quantities, but with high levels of catechins, may make substantial contributions to the intake. We sampled 24 types of fruits, 27 types of vegetables and legumes, some staple foods: rice, macaroni, and wholemeal bread, and a number of processed foods: chocolate, jam, raisins, and currants. Seven varieties of apples and 4 varieties of pears were included, since these fruits are popular in The Netherlands, and supposedly contain relatively high levels of catechins. Rice and macaroni were analyzed after preparation according to the instructions on the packing. Canned and jarred products were sampled if they contributed substantially to the total consumption of a certain food in The Netherlands.

Fresh fruits and vegetables were sampled four times: in August 1997, December 1997, April 1998, and August 1998. We started in April 1997 with apples and pears only, to test the sampling and processing procedures. Fresh products were bought on the same day at three locations: an outlet of a nation-wide supermarket chain (Albert Heijn), a local grocery and an open-air street market. A minimum of 1 kg (or 0.5 kg for very small foods such as berries), or at least three units was sampled at each location. After removing the non-edible parts, these samples were combined per product to a composite in proportions reflecting sales at the three locations. On average, the supermarket accounted for 64%, the grocery for 18%, and the street market for 18% of the sample. Three major brands of each canned or jarred product were bought in the supermarket in April 1998. The contents were allowed to leak out

and the brands were mixed in equal proportions. All composites were either chopped under liquid nitrogen or cut into smaller pieces and frozen in liquid nitrogen, and stored at -20°C until freeze-drying was started within 2 weeks. After freeze-drying, the samples were ground to a powder and stored at -20°C.

Analytical Methods

Six major catechins [(+)-catechin, (-)-epicatechin, GC, EGC, ECg, and EGCg] (Figure 3.1.1) were determined using a method described previously in detail.[17] In short, catechins were extracted from freeze-dried foods with aqueous methanol at room temperature for 1 hour in a mechanical shaker. The concentration of methanol was either 70 or 90%, depending on the previously determined optimal concentration for the product. Sample extracts were injected into a gradient reversed phase HPLC-UV-fluorescence system, operated at 30°C. (+)-Catechin and (-)-epicatechin were measured by means of fluorescence (280 nm excitation, 310 nm emission wavelengths), the other catechins were measured by means of UV (270 nm).

Chocolate was not freeze-dried before extraction, but ground to a fine powder using a handmill. Extraction at room temperature was inefficient, probably due to the high content of hard fat in this product. Reflux extraction (90°C) of 0.5 g of homogenized chocolate in 25 mL 90% methanol in water for 30 min under a continuous nitrogen flow improved the catechin yield by ~40%. Longer extraction (up to 90 min) did not further enhance yield.

In addition to the acetonitrile/phosphate buffer gradient described in the paper by Arts and Hollman,[17] a second gradient using methanol/phosphate buffer was developed. The solvents used for separation were 5% methanol (eluent A) and 40% methanol (eluent B) in phosphate buffer (0.025 M, pH 2.4). The gradient was as follows: 0-30 min: linear gradient from 5 to 90% B, 30-37 min: isocratic at 90% B, 37-40 min: linear return to 5% B, 40-50 min: isocratic at 5% B to re-equilibrate. This gradient was used to obtain baseline peak separation for some samples, and it was used for additional peak identification.

Analytical Quality Control

Because of the large variety of products analyzed and the extended series of analysis, quality control and confirmation of the identity of the peaks was considered an important issue. Control samples consisting of either apple or black grape were included at the beginning and end of each series of analysis. The within and between-run reproducibility of the analysis of these samples was reported

previously[17] and considered satisfactory (<10%). The catechin content of the control sample was recorded after each series of analysis and had to be within the confidence limits (mean ± 2 standard deviations). The recovery of catechins was determined by spiking apple with a known amount of standard compound; recoveries ranged from 92-105%.[17] Limits of detection are shown in Table 3.1.1. All samples were analyzed in duplicate.

Table 3.1.1 Detection limits of catechins

	Fluorescence		UV	
	standard (µg/mL)	apple[a] (mg/kg of fresh wt)	standard (µg/mL)	apple[a] (mg/kg of fresh wt)
(+)-catechin	0.008	0.1	0.13	1.8
(-)-epicatechin	0.007	0.1	0.09	1.2
EGC	-	-	0.29	3.9
EGCg	-	-	0.03	0.4
ECg	-	-	0.03	0.4

[a] Detection limits in apple are calculated from the standard data as follows: [(value in µg/mL)/sample weight] x 50 mL x (fraction dry weight); sample weight is 0.5 g, fraction dry weight is 0.136.

The peaks of interest were identified by two methods. Firstly, all samples were separated with both the acetonitrile and the methanol gradient described under Analytical Methods. The retention times of the peaks were compared to the retention times of pure standards. Peak identity was confirmed when peak retention times were identical to those of the pure standards in both mobile phases. Secondly, all samples were analyzed with diode array detection (HP 1040A upgraded version, Hewlett-Packard, Palo Alto, CA), and the peak spectra were compared to spectra of the pure compounds. Peak purity was also determined by diode array detection. For all samples peak purity was satisfactory with at least one of the mobile phases; catechin contents were calculated based on the chromatogram with the best peak purity.

Catechins in anthocyanin rich fruits, such as red currants, cherries and blackberries were difficult to quantify by UV detection, because a large 'hump' appeared in the chromatogram area where EGCg and ECg normally elute. The determination of (+)-catechin and (-)-epicatechin was not hindered because anthocyanins do not fluoresce at the chosen wavelengths. We developed a method to remove the anthocyanins from the sample, without substantial loss of catechins. 26 mL of a water/ethyl acetate (4:96 v/v) solution was added to 1 g of freeze dried sample. The extract was placed in a mechanical shaker for 60 minutes (250 rpm) at room temperature. After extraction, 2 mL of the clear supernatant was evaporated to

dryness in a heating module (Pierce, Rockford, USA) at 50°C. The residue was redissolved in 90% aqueous methanol, passed through a 0.45 µm Acrodisc filter, and injected. Recoveries were determined by adding known amounts of catechins immediately after adding ethyl acetate. Recoveries ranged from 90% for (-)-epicatechin to 114% for EGC. All anthocyanin rich fruits were analyzed using this extraction method, but none of them contained EGCg or ECg. Thus, all data presented in the Results section of this paper are based on simple aqueous methanol extraction.

RESULTS AND DISCUSSION

Catechins were found particularly in fruits, but black chocolate contained the highest levels of catechins. Vegetables and legumes were poor dietary sources of catechins: only rhubarb, broad beans and marrowfat peas contained catechins (Table 3.1.2). The studied staple foods did not contain catechins. Foods with an average total catechin content >100 mg/kg fresh edible weight were: dark chocolate (459.8-610.0 mg/kg), milk chocolate (153.0-163.2 mg/kg), raw (493.7 mg/kg) and home cooked (206.3 mg/kg) broad beans, black grapes (203.9 mg/kg), blackberries (187.4 mg/kg), cherries (117.1 mg/kg), apricots (110.1 mg/kg), and some apple varieties (71.1-115.4 mg/kg fresh edible weight). (-)-Epicatechin is the quantitatively most important catechin. EGCg was not detected in any of the foods, while GC, EGC, and ECg were limited to a few products.

Literature data on catechin contents of foods determined with appropriate methods (HPLC separation, proper peak identification, analysis of edible parts only, and data reported on a fresh weight basis) are summarized in Table 3.1.3. The remaining data reported in the literature are either qualitative only, quantitative but based on less specific separation (Thin-Layer Chromatography, TLC) or detection methods (e.g. vanillin-HCl or Folin-Ciocalteu), or reported on a dry weight basis. Although the quality of these data is considered inferior, we will compare them to our data whenever no other data are available. Data obtained with vanillin-HCl or Folin-Ciocalteu reagents without chromatographic separation, where results are expressed as 'total catechin equivalents', will not be used.

Apples and pears

Our data for apple correspond well with most of the data reported in the literature (Table 3.1.3). Varietal differences were relatively small (Table 3.1.2). Escarpa and González[18] found exceptionally high levels of (+)-catechin in four varieties of apple (among which Golden Delicious and Granny Smith). The (-)-epicatechin levels

reported by them are in the range usually found, and their analytical method seems adequate. It is not clear what may have caused the difference between their data and other reported data. Possibly, outlying extreme samples were included in the limited number of samples taken: values for six individual apples are given, instead of a composite of several apples. In our study, the reported catechin content of each apple variety is based on the analysis of 12 samples of 1 kg purchased at 3 locations in 4 periods. Two previous studies on the catechin contents of various pear cultivars reported data similar to ours (Table 3.1.3). Both (+)-catechin and (-)-epicatechin are present in most pear cultivars, but (+)-catechin levels are sometimes very low. In general, pears contain lower catechin levels than apples.

Grapes

Our data for black grapes are considerably higher than those reported by Oszmianski and Lee[19] for red grapes; they did not report on ECg (Table 3.1.3). Removal of the seeds, which was done in their study, but not in ours, may have contributed to the difference. However, they themselves report that their data on red grapes are relatively low, even compared to white grapes grown in the same area. Qualitative data confirm the presence of (+)-catechin, (-)-epicatechin, and ECg in black and white grape seeds and skins.[20-22] However, ECg was not present in the skins of all varieties that were tested.[21] Similarly, white grapes contained ECg in only one of our four sampling periods, thus pointing to a varietal effect.

Other fruits

Risch and Herrmann[23] detected (-)-epicatechin in peach, while we did not (Table 3.1.3). Since their levels for (+)-catechin are substantially higher as well, this may have been due to varietal differences. (-)-Epicatechin was, for example, also absent from Desert Gold peaches, a variety which Risch and Herrmann did not study.[24] The variety of the peaches we sampled, has not been recorded. Van Gorsel,[24] reported the presence of (+)-catechin and (-)-epicatechin in nectarines, while we found only (+)-catechin. Although van Gorsel used appropriate separation and detection methods, peak identity was confirmed only by comparing retention times with those of known standards. This can easily lead to the erroneous reporting of a compound.

We found low levels of (-)-epicatechin in kiwi fruit. Dawes and Keen[25] reported similar levels in experimentally prepared kiwi fruit juice using HPLC methods, but they also detected trace amounts of (+)-catechin. Again, the reported difference in types of catechins present may be due to varietal differences. We detected (+)-catechin and (-)-epicatechin in raspberry, which confirmed qualitative data by Rommel and

Table 3.1.2 (+)-Catechin, (-)-epicatechin, GC, EGC and ECg (mg/kg fresh edible weight) in raw and processed foods[a]

Product	Scientific or brand name	Catechin content (mg/kg fresh edible weight)[b]			
		(+)-catechin	(-)-epicatechin	others	total catechins
Apple with skin	*Malus pumila Mill.*				
"cox's orange pippin"		12.8 ± 1.67	96.2 ± 18.37	ND[c]	109.0
"elstar"		12.4 ± 2.11	81.7 ± 12.41	ND	94.0
"golden delicious"		5.3 ± 0.70	74.2 ± 7.44	ND	79.5
"goudreinette"		12.2 ± 0.89	103.2 ± 17.72	ND	115.4
"granny smith"		15.6 ± 4.33	74.8 ± 15.82	ND	90.3
"jonagold"		4.0 ± 0.29	67.1 ± 7.26	ND	71.1
"jonagored"		4.2 ±1.63	72.3 ± 22.33	ND	76.5
Apple without skin	*Malus pumila Mill.*				
"cox's orange pippin"		12.8	66.5	ND	79.3
"elstar"		11.0 ± 3.29	66.4 ± 3.86	ND	77.4
"golden delicious"		4.4 ± 0.26	50.7 ± 8.54	ND	55.1
"goudreinette"		9.6 ± 0.66	86.1 ± 14.81	ND	95.6
"granny smith"		16.5 ± 5.19	65.3 ± 15.24	ND	81.1
"jonagold"		2.8 ± 0.57	48.3 ± 6.84	ND	51.2
"jonagored"		3.0 ± 1.56	51.6 ± 17.17	ND	54.6
Apple-sauce		6.9	54.1	ND	61.0
Apricot	*Prunus armeniaca L.*	49.5 ± 43.68	60.6 ± 78.49	ND	110.1
Avocado	*Persea americana Mill.*	ND	5.6 ± 2.91	ND	5.6
Blackberry	*Rubus sp.*	6.6 ± 0.58	180.8 ± 21.39	ND	187.4
Blueberry	*Vaccinium corymbosum L.*	ND	11.1 ± 1.00	ND	11.1
Broad bean, raw	*Vicia faba L.*	128.3 ± 160.60	225.1 ± 184.78	EGC: 140.3 ± 127.90	493.7
- prepared		81.6 ± 36.44	78.2 ± 40.93	EGC: 46.5 ± 23.17	206.3
- canned		ND	ND	ND	ND
Cherry, sweet	*Prunus avium L.*	21.7 ± 9.18	95.3 ± 24.84	ND	117.1
- canned		ND	43.1	ND	43.1
Cranberry	*Vaccinium oxycoccus L.*	ND	42.0	ND	42.0
Currant, black	*Ribes nigrum L.*	7.0	4.7	ND	11.7
Currant, white	*Ribes pallidum*	3.0	ND	7.0	10.0
Currant, red	*Ribes rubrum-hybriden*	12.2 ± 4.35	ND	GC: 12.2 ± 10.85	24.4
Gooseberry	*Ribes uva-crispa L.*	16.7 ± 3.63	ND	GC: 4.4 ± 6.27	21.2
Grape, black	*Vitis vinifera L.*	89.4 ± 91.80	86.4 ± 71.20	ECg: 28.1 ± 37.93	203.9
- white		24.7 ± 10.59	10.2 ± 5.18	ECg: 4.3 ± 8.54	39.2
Kidney-bean, canned	*Phaseolus vulgaris L.*	16.6	3.5	ND	20.1
Kiwi fruit	*Actinidia chinensis Planch*	ND	4.5 ± 1.05	ND	4.5
Mango	*Mangifera indica L.*	17.2 ± 15.72	ND	ND	17.2
Marrowfat pea, canned	*Pisum sativum L.*	ND	ND	GC: 43.3 EGC: 56.4	99.7
Nectarine	*Prunus persica Batsch*	27.5 ± 2.42	ND	ND	27.5
Peach	*Prunus persica Batsch*	23.3 ± 5.66	ND	ND	23.3
- canned		18.7	ND	ND	18.7
Pear with skin	*Pyrus communis L.*				
"conference"		1.1 ± 0.36	29.5 ± 1.69	ND	30.6
"doyenne du comice"		0.6 ± 0.44	34.8 ± 16.34	ND	35.4
"guyot"		1.9	37.0	ND	38.9
"cooking-pear"[d]		9.6 ± 4.77	75.4 ± 32.42	ND	85.0
- canned		1.8	2.6	ND	4.4
Pear without skin	*Pyrus communis L.*				
"conference"		0.4 ± 0.18	8.2 ± 2.72	ND	8.5
"doyenne du comice"		0.1	14.4	ND	14.6
"cooking-pear"[d]		3.6 ± 0.18	29.6 ± 3.25	ND	33.3
- prepared		3.3	21.2	ND	24.5

Table 3.1.2 Continued

Product	Scientific or brand name	Catechin content (mg/kg fresh edible weight)[b]			
		(+)-catechin	(-)-epicatechin	others	total catechins
Plum	*Prunus domestica L.*	33.5 ± 9.13	28.4 ± 31.89	ND	61.9
Raspberry	*Rubus idaeus L.*	9.7 ± 2.57	82.6 ± 13.06	ND	92.3
Rhubarb	*Rheum rhabarbarum L.*	21.7 ± 11.39	5.1 ± 3.30	ECg: 6.0 ± 3.71	32.8
- prepared		14.8 ± 10.61	3.8 ± 1.31	ECg: 4.9 ± 6.06	23.5
Strawberry	*Fragaria ananassa Duch.*	44.7 ± 13.80	ND	ND	44.7
Chocolate, black	Albert Heijn	132.4	327.4	ND	459.8
	Verkade	107.5	502.5	ND	610.0
Chocolate, milk	Albert Heijn	38.3	124.9	ND	163.2
	Verkade	26.9	126.1	ND	153.0
Chocolate candy bar	Mars	21.7	62.5	ND	84.2
Currant		ND	ND	ND	ND
Jam, apricot	Albert Heijn	4.7	5.0	ND	9.7
Jam, cherry	Albert Heijn	1.6	9.0	ND	10.6
Jam, forest fruit	Albert Heijn	0.7	15.7	ND	16.4
Jam, strawberry	Albert Heijn	9.0	ND	ND	9.0
Raisins		29.7	7.1	ND	36.8

[a] Data include seasonal and regional variation. EGCg was not detected in any food; no catechins were detected in: banana (*Musa sapientum L.*), broccoli (*Brassica oleracea L. cv. botrytis L.*), Brussels sprout (*Brassica oleracea L. cv. gemmifera DC.*), carrot (*Daucus carota L.*), cauliflower (*Brassica oleracea L. cv. botrytis L.*), chicory (*Cichorium intybus L.*), chinese cabbage (*Brassica oleracea L. cv. conica DC.*), cucumber (*Cucumis sativus L.*), endive (*Cichorium endivia L.*), french bean (*Phaseolus vulgaris L.*), green olive (*Olea europaea L.*), green pea (canned) (*Pisum sativum L.*), leek (*Allium porrum L.*), lettuce (*Lactuca sativa L. cv. capitata L.*), macaroni, maize (*Zea mays L. cv. saccharata*), melon (*Cucumis melo L.*), mushroom (*Agaricus campester Fr.*), onion (*Allium cepa L.*), orange (*Citrus sinensis L.*), pineapple (*Ananas comosus L. Merr.*), potato (*Solanum tuberosum L.*), red beet (*Beta vulgaris L. cv. rubra L.*), red cabbage (*Brassica oleracea L. cv. rubra DC.*), rice (*Oryza sativa L.*), slicing bean (*Phaseolus vulgaris L.*), sweet red pepper (*Capsicum annuum L.*), sugar peas (*Pisum sativum L. cv. saccharatum*), tomato (*Lycopersicum esculentum Mill.*), white bean in tomato sauce (*Phaseolus vulgaris L.*), wholemeal bread. [b] Annual mean ± standard deviation, based on duplicate analyses for each season; the food was bought in only one season if no standard deviation is given. [c] ND: not detected (see Table 3.1.1 for limits of detection). [d] Different varieties.

Wrolstad.[26] To our knowledge, no more studies have been published which employed HPLC with online detection to determine catechins in fruits. However, with colorimetric detection after TLC separation, Herrmann and coworkers[12,13,27] reported the presence of catechins in strawberries, black, white, and red currants, gooseberries, blueberries, and rhubarb. Quantitatively, our data are in the same order of magnitude. Herrmann and coworkers, however, occasionally report the presence of a certain catechin, for example (-)-epicatechin in strawberry, which we did not detect. This is most likely due to their unspecific methods and lack of peak identity confirmation, although of course differences in variety may have contributed as well. Our data on strawberries confirm those reported by Van Gorsel and coworkers[24] who did not detect (-)-epicatechin in juice made from strawberries either. Finally, our data confirm an early report on the presence of low levels of (-)-epicatechin in avocado.[28]

Table 3.1.3 Reported data on catechins in foods, determined by HPLC and reported on a fresh weight basis

Product	Content (mg/kg fresh edible weight)			Remarks	Reference
	(+)-catechin	(-)-epicatechin	other		
Apple	trace-17	2-101	-[a]	16 varieties	23
Apple without skin	-	10-140	-	3 varieties	29
Apple without skin	0.2-55	14-81	-	5 varieties	30
Apple without skin	28-182	19-111	-	4 varieties	18
Apricot	26-57	67-171	-	3 varieties	23
Peach	50-129	3-15	-	4 varieties	23
Pear	trace-10	5-59	-	10 varieties	23
Pear	ND[b]-5	6-87	-	7 varieties	31
Plum	5-36	0-16	-	8 varieties	23
Red grapes	<10	17.5-21.4	-	2 varieties, seeds removed	19
Sweet cherry	5-12	14-49	-	5 varieties	23

[a] -: not determined. [b] ND: not detected.

Legumes

In faba beans (Vicia faba L.), which belong to the same family as broad beans, (-)-epicatechin and EGC were detected.[32] We found (+)-catechin in addition to these compounds in broad beans, but it is not clear whether Helsper and coworkers determined (+)-catechin. All other available data on catechins in legumes are expressed as 'total catechin equivalents', and are therefore unreliable estimates of catechin contents. A general trend of increasing 'total catechin equivalent' content with increasing darkness of the legumes within one family can be observed.[33,34] We found that within the Phaseolus family the total catechin content of the dark kidney-bean was higher than the catechin content of the green beans.

Chocolate

We found very high levels of (+)-catechin (107.5-132.4 mg/kg) and (-)-epicatechin (327.4-502.5 mg/kg) in black chocolate (54% cacao), and lower but still substantial levels in milk chocolate (34% cacao). Previous research has shown the presence of (+)-catechin and (-)-epicatechin in cacao liquor (a major ingredient of chocolate),[35] and in fresh and fermented cacao beans.[36,37] Fresh beans contained up to 43,270 mg (-)-epicatechin kg^{-1} defatted sample.[37] On fermentation and roasting of cacao beans, the characteristic red-brown color of chocolate is formed, probably as a result of polymerization of catechins.[36] Fermented cacao beans therefore contain ~90% lower catechin levels than fresh beans.[36,37] It now turns out that chocolate still contains significant amounts of (+)-catechin and (-)-epicatechin.

Seasonal variation and maturity

The seasonal variation was considerable for some products (e.g. black grapes, broad beans), and surprisingly small for others (e.g. apples). Factors that may have contributed to the seasonal variation are varietal differences, maturity of the product, and storage period. Part of the seasonal variation is undoubtedly due to differential availability of varieties over the year. Except for apples and pears, we did not sample specific fruit varieties, but purchased those that were available in the shops. Because all purchases were done at three locations, the analyzed samples are often mixtures of several varieties. Specific varieties were purchased only for apples and pears, and for these fruits the seasonal variation was relatively small. Part of the variation may have been caused by differences in maturity. For apples and pears, it has been shown that during growth there is a rapid decrease in catechin levels.[29,31] Data from the 70s using TLC had shown similar rapid decreases preceding maturity for cherries[38] and strawberries,[13] but much less so for plums,[38] and black currants.[12] However, no unripe fruits are generally for sale for consumers, and fluctuations are reportedly low during maturation in apples and pears.[29,31] Information on changes in catechins during maturation for other fruits than apples and pears is not available, and may differ substantially. Although we took care to purchase ripe fruits only, small differences may have remained. Storage of apples, harvested at commercial maturity, under normal cold-storage conditions for over 6 months changed their (+)-catechin and (-)-epicatechin content only very marginal.[29,39]

Home preparation

Processing resulted in a decrease of the catechin content of foods, although the magnitude of the decrease differed considerably. Rhubarb, broad beans and cooking pears were analyzed both raw and prepared according to standard recipes. Total catechins in the prepared products were respectively 28%, 58%, and 26% lower than in the raw products. In general, it can be concluded that processing is not an important issue for catechins, since they are present mainly in fruits, which are usually consumed raw. In contrast to flavonols in apple, which are almost completely found in the peel,[18,29] only a small part of the catechins were present in apple peel. Removing the apple peel resulted on average in a 23% decrease of the total catechin content. For pears this percentage was higher (45%).

In summary, most fruits, some legumes, and chocolate contain catechins, while vegetables and staple foods do not. Amounts vary from low (4.5 mg/kg in kiwi fruit) to very high (610 mg/kg in black chocolate). Preparation of foods causes a decrease in catechin content, but since most catechin-containing foods are consumed raw, this is

not likely to be an important determinant of catechin intake. (+)-Catechin and (-)-epicatechin are the most common catechins in foods. EGCg was not found, and GC, EGC, and ECg only occasionally. The data reported here provide a base for the epidemiological evaluation of the effect of catechins on the risk for chronic diseases.

ACKNOWLEDGMENTS

We thank Dr. C. Santos-Buelga (University of Salamanca, Spain) for providing GC, EGC, EGCg, and ECg. This work was carried out with financial support from the Commission of the European Communities, Agriculture and Fisheries (FAIR) specific RTD programme, CT95 0653, the Product Board for Horticulture, the Greenery, and the Ministry of Agriculture, Nature Management and Fisheries.

REFERENCES

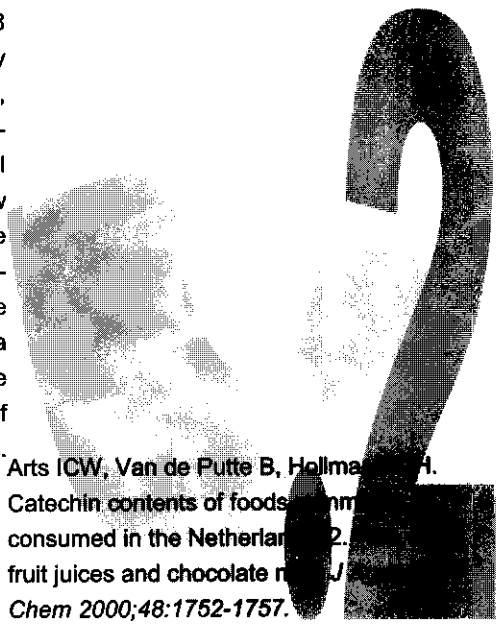
1. Law MR, Morris JK. By how much does fruit and vegetable consumption reduce the risk of ischaemic heart disease? *Eur J Clin Nutr* 1998;52:549-556.
2. Ness AR, Powles JW. Fruit and vegetables, and cardiovascular disease: a review. *Int J Epidemiol* 1997;26:1-13.
3. Steinmetz KA, Potter JD. Vegetables, fruit, and cancer prevention: a review. *J Am Diet Assoc* 1996;96:1027-1039.
4. Middleton E, Kandaswami C. The impact of plant flavonoids on mammalian biology: implications for immunity, inflammation and cancer. In: Harborne JB, ed. *The Flavonoids: advances in research since 1986.* London: Chapman & Hall;1994:619-652.
5. Kühnau J. The flavonoids: a class of semi-essential food components: their role in human nutrition. *World Rev Nutr Diet* 1976;24:117-191.
6. Balentine DA, Wiseman SA, Bouwens LCM. The chemistry of tea flavonoids. *Crit Rev Food Sci Nutr* 1997;37:693-704.
7. Kohlmeier L, Weterings KGC, Steck S, Kok FJ. Tea and cancer prevention: an evaluation of the epidemiologic literature. *Nutr Cancer* 1997;27:1-13.
8. Blot WJ, Chow WH, McLaughlin JK. Tea and cancer: a review of the epidemiological evidence. *Eur J Cancer Prev* 1996;5:425-438.
9. Tijburg LBM, Mattern T, Folts JD, Weisgerber UM, Katan MB. Tea flavonoids and cardiovascular diseases: A review. *Crit Rev Food Sci Nutr* 1997;37:771-785.
10. Berger WG, Herrmann K. Catechine und deren Abbauprodukte in Weintrauben, Trestern, Essigweinen und daraus hergestellten Essigen. I. Qualitativer Nachweis und quantitative Bestimmung der Catechine (Catechins and their degradation products in grapes, husks, vinegar wine, and vinegar. I. Qualitative identification and quantitative determination of catechins). *Z Lebensm Unters Forsch* 1971;146:266-274.
11. Mosel HD, Herrmann K. The phenolics of fruits. III. The contents of catechins and hydroxycinnamic acids in pome and stone fruits. *Z Lebensm Unters Forsch* 1974;154:6-11.
12. Stöhr H, Herrmann K. Die phenolischen Inhaltsstoffe des Obstes. VI. Die phenolischen Inhaltsstoffe der Johannisbeeren, Stachelbeeren und Kulturheidelbeeren. Veranderungen der Phenolsauren und Catechine wahrend Wachstum und Reife von Schwarzen Johannisbeeren (The phenolics of fruits. VI. The phenolics of currants, gooseberries and blueberries. Changes in phenolic acids and catechins during development of black currants). *Z Lebensm Unters Forsch*

1975;159:31-37.
13. Stöhr H, Herrmann K. Die phenolischen Inhaltsstoffe des Obstes. V. Die phenolischen Inhaltsstoffe der Erdbeeren und deren Veranderungen wahrend Wachstum und Reife der Fruchte (The phenolics of fruits. V. The phenolics of strawberries and their changes during development and ripeness of the fruits). *Z Lebensm Unters Forsch* 1975;158:341-348.
14. Solich P, Opletal L, Sovova M. Comparison of different methods for the spectrophotometric determination of (-)-epicatechin. *Pharmazie* 1996;51:954-956.
15. Sarkar SK, Howarth RE. Specificity of the vanillin test for flavanols. *J Agric Food Chem* 1976;24:317-320.
16. Jankun J, Selman SH, Swiercz R, Skrzypczak Jankun E. Why drinking green tea could prevent cancer. *Nature* 1997;387:561-561.
17. Arts ICW, Hollman PCH. Optimization of a quantitative method for the determination of catechins in fruits and legumes. *J Agric Food Chem* 1998;46:5156-5162.
18. Escarpa A, Gonzalez MC. High-performance liquid chromatography with diode-array detection for the determination of phenolic compounds in peel and pulp from different apple varieties. *J Chromatogr A* 1998;823:331-337.
19. Oszmianski J, Lee CY. Isolation and HPLC determination of phenolic compounds in red grapes. *Am J Enol Vitic* 1990;41:204-206.
20. Santos-Buelga C, Francia-Aricha EM, Escribano-Bailón MT. Comparative flavan-3-ol composition of seeds from different grape varieties. *Food Chem* 1995;53:197-201.
21. Escribano-Bailón MT, Guerra MT, Rivas-Gonzalo JC, Santos-Buelga C. Proanthocyanidins in skins from different grape varieties. *Z Lebensm Unters Forsch* 1995;200:221-224.
22. Oszmianski J, Sapis JC. Fractionation and identification of some low molecular weight grape seed phenolics. *J Agric Food Chem* 1989;37:1293-1297.
23. Risch B, Herrmann K. Die Gehalte an Hydroxyzimtsäure-Verbindungen und Catechinen in Kern- und Steinobst (Contents of hydroxycinnamic acid derivatives and catechins in pome and stone fruit). *Z Lebensm Unters Forsch* 1988;186:225-230.
24. Van Gorsel H, Li C, Kerbel EL, Smits M, Kader AA. Compositional characterization of prune juice. *J Agric Food Chem* 1992;40:784-789.
25. Dawes HM, Keene JB. Phenolic composition of kiwifruit juice. *J Agric Food Chem* 1999;47:2398-2403.
26. Rommel A, Wrolstad RE. Influence of acid and base hydrolysis on the phenolic composition of red raspberry juice. *J Agric Food Chem* 1993;41:1237-1241.
27. Hanefeld M, Herrmann K. Über das Vorkommen von Proanthocyanidinen, Leukoanthocyanidinen und Catechinen im Gemüse (On the occurrence of proanthocyanidins, leucoanthocyanidins and catechins in vegetables). *Z Lebensm Unters Forsch* 1976;161:243-248.
28. Ramírez-Martínez JR, Luh BS. Phenolic compounds in frozen avocados. *J Sci Food Agric* 1973;24:219-225.
29. Burda S, Oleszek W, Lee CY. Phenolic compounds and their changes in apples during maturation and cold storage. *J Agric Food Chem* 1990;38:945-948.
30. Perez-Ilzarbe J, Hernandez T, Estrella I. Phenolic compounds in apples: varietal differences. *Z Lebensm Unters Forsch* 1991;192:551-554.
31. Amiot MJ, Tacchini M, Aubert SY, Oleszek W. Influence of cultivar, maturity stage, and storage conditions on phenolic composition and enzymatic browning of pear fruits. *J Agric Food Chem* 1995;43:1132-1137.
32. Helsper JPFG, Kolodziej H, Hoogendijk JM, Van Norel A. Characterization and trypsin inhibitor activity of proanthocyanidins from vicia-faba. *Phytochemistry* 1993;34:1255-1260.

33. Moneam NMA. Effects of presoaking on faba bean enzyme inhibitors and polyphenols after cooking. *J Agric Food Chem* 1990;38:1479-1482.
34. Deshpande SS, Cheryan M. Determination of phenolic compounds of dry beans using vanillin, redox and precipitation assays. *J Food Sci* 1987;52:332-334.
35. Sanbongi C, Osakabe N, Natsume M, Takizawa T, Gomi S, Osawa T. Antioxidative polyphenols isolated from Theobroma cacao. *J Agric Food Chem* 1998;46:454-457.
36. Porter LJ, Ma Z, Chan BG. Cacao procyanidins: major flavanoids and identification of some minor metabolites. *Phytochemistry* 1991;30:1657-1663.
37. Kim H, Keeney PG. (-)-Epicatechin content in fermented and unfermented cocoa beans. *J Food Sci* 1984;49:1090-1092.
38. Stöhr H, Mosel HD, Herrmann K. The phenolics of fruits. VII. The phenolics of cherries and plums and the changes in catechins and hydroxycinnamic acid derivatives during the development of fruits. *Z Lebensm Unters Forsch* 1975;159:85-91.
39. Mosel HD, Herrmann K. Changes in catechins and hydroxycinnamic acid derivatives during development of apples and pears. *J Sci Food Agric* 1974;25:251-256.

Catechin contents of beverages: tea, wine, fruit juices and chocolate milk

To enable the epidemiological evaluation of catechins, data on their contents in beverages are required. HPLC with UV and fluorescence detection was used to determine the levels of (+)-catechin, (-)-epicatechin, (+)-gallocatechin (GC), (-)-epigallocatechin (EGC), (-)-epicatechin gallate (ECg), and (-)-epigallocatechin gallate (EGCg) in 8 types of black tea, 18 types of red and white wine, apple juice, grape juice, ice-tea, beer, chocolate milk and coffee. Tea infusions contained high levels of catechins (102-418 mg catechins/L), and tea was the only beverage which contained GC, EGC, ECg, and EGCg in addition to (+)-catechin and (-)-epicatechin. Catechin concentrations were still substantial in red wine (27-96 mg/L), but low to negligible amounts were found in white wine, commercially available fruit juices, ice-tea, and chocolate milk. Catechins were absent from beer and coffee. The data reported here provide a base for the epidemiological evaluation of the effect of catechins on the risk for chronic diseases.



Arts ICW, Van de Putte B, Hollma[illegible]H. Catechin contents of foods [illegible]nm consumed in the Netherlan[illegible]2. fruit juices and chocolate m[illegible]J *Chem 2000;48:1752-1757.*

INTRODUCTION

A number of epidemiological studies have demonstrated a protective effect of tea against the development of certain cancers,[1,2] coronary heart disease, and stroke.[3] Catechins, or flavanols, one of the six classes of flavonoids, are the principal components of tea and may be responsible for its alleged protective effect. *In vitro* and *in vivo* experiments have shown that catechins are potentially beneficial to human health: they are strong antioxidants, anticarcinogens, anti-inflammatory agents, and inhibitors of platelet aggregation.[2,4,5] Not all epidemiological studies, however, have found a protective effect of tea against chronic diseases.[6-8] One of the reasons for this inconsistency may be that tea is not the only catechin containing food. Including all sources of catechins in epidemiological studies may clarify the association between catechins, tea as one of its major sources, and chronic diseases. We already reported on the catechin contents of a comprehensive set of fruits, vegetables, and certain staple and processed foods.[9]

In spite of the fact that tea has been studied extensively for its biological actions, there are surprisingly few data on the catechin content of tea infusions. Previous studies typically determined the catechin contents of fresh tea leaves on a dry weight basis after exhaustive extraction with organic solvents. This type of data does not take into account infusion rates into ordinary hot water, and is not suitable for use in epidemiological studies. The goal of the present study was to determine the catechin contents of commonly consumed beverages. We determined the following catechins, (+)-catechin, (-)-epicatechin, (+)-gallocatechin (GC), (-)-epigallocatechin (EGC), (-)-epicatechin gallate (ECg), and (-)-epigallocatechin gallate (EGCg) (Figure 3.2.1) in 8 types of black tea, 18 types of red and white wine, apple juice, grape juice, ice-tea, beer, chocolate milk and coffee.

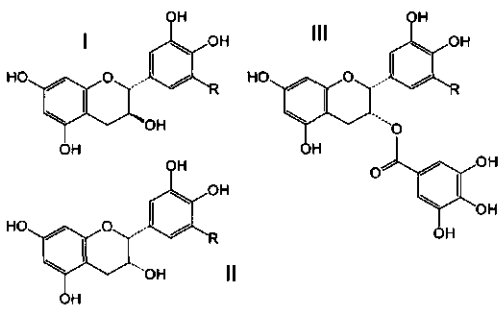


Figure 3.2.1 Chemical structure of catechins: (I) R=H (+)-catechin, R=OH (+)-gallocatechin (GC); (II) R=H (-)-epicatechin, R=OH (-)-epigallocatechin (EGC); (III) R=H (-)-epicatechin gallate (ECg), R=OH (-)-epigallocatechin gallate (EGCg).

METHODS

Sample collection and preparation: tea

The three most frequently consumed blends of black tea from two major brands, and

two fruit flavored black teas were purchased (consumption data were obtained from the Coffee and Tea Information Bureau), and prepared according to Dutch custom. A 2-g tea bag was placed in 200 mL of boiled tap water for 5 min. Before removing the bag it was stirred through the brew a few times, after which the brew was allowed to cool. For the analysis of GC and EGC the pH of the brew was lowered by adding 50 µL 1M citric acid in methanol to 8 mL tea, and the volume was made up to 10 mL with methanol (final pH = 3). Lowering the pH stabilized these compounds in the HPLC autosampler for at least 24 hours at room temperature. For the analysis of all other catechins the brew was diluted 10 times with methanol; stabilization was not necessary. The effect of the brewing method on catechin yield of Ceylon tea was studied by varying the infusion time (2, 5, and 10 min) and the amount of tea used for brewing (2, 2.5, 3, or 4 g).

Sample collection and preparation: wine

Twelve red and six white wines commonly consumed in The Netherlands were purchased at an outlet of a nation-wide supermarket-chain that holds the major share of Dutch wine sales (Albert Heijn). Types of wine were selected based on information from the Dutch Commodity Board of Wine. Two types of French red wine (Bordeaux and Cotes-du-Rhone) were chosen. Of each, three brands (one low-priced, one medium-priced, and one higher-priced) were purchased. In addition, two Spanish, two South African, and two Italian red wines were selected. Three white Bordeaux wines, and three German white Mosel-Saar-Ruwer wines in different price-categories were purchased. 5 mL of white wine was diluted with 5 mL 90% methanol in water. Red wines were diluted 10 times by adding 18 mL 90% methanol in water to 2 mL of wine.

Sample collection and preparation: other beverages

Two major brands (Riedel, and Albert Heijn's own brand) of apple juice and black grape juice, and one brand (Albert Heijn's own brand) of white grape juice were analyzed. Although orange juice is the most frequently consumed fruit juice in the Netherlands, it was not included because we did not detect catechins in oranges.[9] One brand of ice-tea (Liptonice lemon without carbondioxide) was purchased, and one brand of lager beer (Heineken). The beer was degassed in an ultrasonic bath at room temperature before injection. Two brands of commonly consumed semi-skimmed chocolate milk (Albert Heijn's own brand and Nutricia), and a commonly consumed brand of coffee (Roodmerk, DE) were bought. The coffee was prepared according to Dutch custom in a coffee maker with a paper filter; 275 mL of boiling water was dripped on 14 g of finely ground coffee.

Analytical methods

Six major catechins [(+)-catechin, (-)-epicatechin, GC, EGC, ECg, and EGCg] (Figure 3.2.1) were determined according to a method described previously in detail for solid foods.[10] Modifications were made to take the liquid sample matrix into account. After filtration over a 0.45 µm Acrodisc filter, the beverages were injected directly, without extraction or sample cleanup, onto a HPLC-UV-fluorescence system, operated at 30°C. Prior to filtration, tea and wine were diluted as described. Chocolate milk required some cleanup because of its protein and fat content: 15 mL of chocolate milk was extracted for 1 hour in a mechanical shaker with 5 mL 1M citric acid in methanol, and subsequently centrifuged for 5 minutes at 2000 g; the supernatant was injected. Quantification of (+)-catechin and (-)-epicatechin was done with fluorescence detection (280 nm excitation, 310 nm emission wavelengths), while the other compounds were measured with UV (270 nm). In addition to an acetonitrile/phosphate buffer gradient,[10] a second gradient using methanol/phosphate buffer was applied.[9]

Analytical quality control

A control sample of black tea infusion was included at the beginning and end of each series of analysis. For this control sample, the contents of 25 tea bags (2 g each) from different batches of one tea blend (Ceylon, Pickwick DE) were mixed, passed through a set of sieves retaining the >0.4 and <0.8 mm fraction, and stored in an air tight container. The control tea infusion was prepared fresh as follows: 200 mL of tap water were boiled, 2 g of the control tea was added and allowed to infuse for 5 min. After infusion, the tea was passed through a household tea sieve, diluted similar to the other tea samples, passed though a 0.45 µm Acrodisc filter, and injected. The catechin content of the control sample was recorded after each series of analysis and had to be within the confidence limits (mean ± 2 standard deviations). All samples were analyzed in duplicate. Peak identification was done by comparing retention times, and UV-spectra obtained by diode array detection to those of pure standards. See the accompanying paper for details.[9]

RESULTS AND DISCUSSION

Tea

Tea was the only beverage that contained EGCg (Table 3.2.1). Together with ECg it was in fact the most abundant compound present in black tea. (+)-Catechin and GC represented only a small part of the catechin content of our black teas, while (-)-epicatechin and EGC were low in English melange and Earl Grey, but much higher in

Ceylon tea. The total catechin content of Ceylon tea was very high (up to 418 mg/L) and approximated the composition of green tea,[11] but also that of a mixture of Orange Pekoe and Pekoe cut.[12] The English melange and Earl Grey infusions analyzed in our study resembled black teas from India and the UK, except for ECg and EGCg which were higher in our tea brews.[11] Because of the limited number of reports on catechin contents of tea infusions, we recalculated literature data on tea extracted with water, but with catechin contents expressed on a tea leaf dry weight basis, to enable comparisons to our data (Table 3.2.1). The large variation in contents of individual catechins in black tea brews from varying origins is apparent. Tea blend, manufacturing practices, and methods of beverage preparation influence the composition of a tea brew.[13] Regarding beverage preparation, it is apparent from our data that the quantity of tea used, had more impact on the catechin concentration of the brew than the infusion time (Figure 3.2.2). In the range we tested (2-4 g/200 mL), catechin concentrations increased fairly linearly with the amount of tea used. On the other hand, maximum catechin concentrations were nearly reached after 5 minutes of infusion, and did not increase substantially after that period. Clearly, if the composition of tea brews varies to the extent seen here, it may be difficult to interpret results from epidemiological studies on the health effects of tea consumption in an unambiguous way.

Table 3.2.1 Catechin content (mg/L) of black tea infusions[a]

Tea	Catechin content (mg/L)[b]							Ref.
	(+)-catechin	(-)-epicatechin	GC	EGC	ECg	EGCg	total catechins	
English melange (Albert Heijn)	5.2	22.1	8.9	12.2	71.4	56.0	175.7	
English melange (Pickwick DE)	3.5	12.7	5.6	6.4	46.6	27.2	101.9	
Ceylon (Albert Heijn)	8.7	62.4	26.1	92.7	100.0	128.4	418.4	
Ceylon (Pickwick DE)	8.8	47.7	27.8	88.4	79.1	100.4	352.1	
Earl Grey (Albert Heijn)	6.0	13.5	6.1	2.9	70.5	51.3	150.3	
Earl Grey (Pickwick DE)	6.2	15.1	7.0	6.5	60.6	43.7	139.1	
Lemon melange (Pickwick DE)	5.8	21.3	10.5	12.6	64.6	50.9	165.6	
Forest fruit melange (Pickwick DE)	4.7	17.8	8.9	8.7	59.1	42.7	141.9	
Literature data on black tea								
Orange Pekoe/Pekoe cut (USA)	-[c]	40	-	60	110	120	330	12
Ceylon (Netherlands)	-	79	-	91	85	229	484	11
Yule (India)	-	31	-	ND[d]	8	18	57	11
PG-tips (UK)	-	40	-	ND	10	26	76	11
Black tea (Taiwan)[e]	-	9	-	116	7	32	164	14
Black tea[e]	-	22	-	59	75	191	347	15
Black tea (China)[e]	120	54	-	52	349	40	615	16

[a] One tea bag (2 g) in 200 mL of boiling tap water during 5 min. [b] Average of duplicate analyses. [c] -: not determined. [d] ND: not detected. [e] Data have been recalculated from tea leaf dry weight to mg/L and corrected for brewing method to resemble 1 g tea in 100 mL water assuming linearity of infusion.

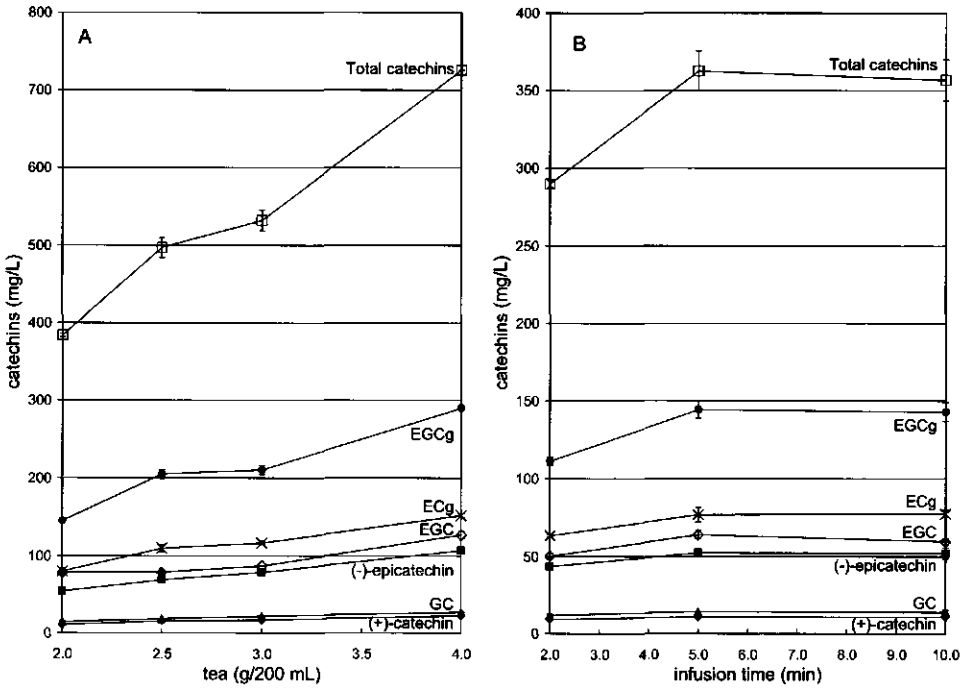


Figure 3.2.2 Catechin yield (mg/L) with variations in brewing method: (A) amount of tea used for brewing, (B) infusion time; (□) total catechins, (◆) (+)-catechin, (▲) (+)-gallocatechin (GC), (■) (-)-epicatechin, (◊) (-)-epigallocatechin (EGC), (x) (-)-epicatechin gallate (ECg), (●) (-)-epigallocatechin gallate (EGCg). Values are means of duplicate analyses ± the standard deviation of the duplicates.

Wine

Wine contained only (+)-catechin and (-)-epicatechin (Table 3.2.2); levels in red wine (16.3-53.4 mg (+)-catechin/L, 9.2-42.1 mg (-)-epicatechin/L) were much higher than those in white wine (1.5-6.1 mg (+)-catechin/L, 0.5-1.3 mg (-)-epicatechin/L). French red wines appeared to have higher levels of catechins than other red wines, which is consistent with reported data on the catechin content of 836 red wines from a large number of wine-making regions of the world.[17] Goldberg and coworkers noted the large differences in catechin levels between wines made from different grape cultivars, and the exceptionally high catechin content of wine made from Pinot Noir grapes (up to 287 mg/L). Pinot Noir wine was not included in our study, since it is not frequently consumed in the Netherlands. Most of the wine from Burgundy, France, is made from this grape. Comparison of our data to those reported in the literature is difficult, since grape cultivar has such a pronounced impact on the catechin concentration of wine. However, in general results are in the same order of magnitude as ours.[17-20] Next to grape cultivar, which is the most important

Table 3.2.2 Catechin content (mg/L) of red and white wine, fruit juices, beer, chocolate milk and coffee

Product	Catechin content (mg/L)[a]		
	(+)-catechin	(-)-epicatechin	total catechins
Red wine			
Bordeaux (Appellation Controlee, Rineau, 1997)	53.4	42.1	95.5
Bordeaux Rouge (Appellation Controlee, Euroshopper, table wine)	47.7	41.5	89.2
Bordeaux (Appellation Controlee, Christian Moueix, 1993)	48.2	29.9	78.0
Cotes-du-Rhone (Appelation Controlee, Les Vendanges, 1997)	47.7	18.8	66.5
Cotes-du-Rhone (Appelation Controlee, Cuvee des Amoureuses, 1996)	44.5	19.0	63.6
Cotes-du-Rhone (Appelation Controlee, Reserve de l'Arche, 1996)	43.3	19.5	62.8
Rioja (Siglo, 1996)	22.5	9.2	31.7
Valdepenas (Albert Heijn's own brand, table wine)	29.0	13.2	42.1
Pinotage/Merlot (Stellenbosch Welmoed Winery, 1997)	25.2	18.9	44.1
Droe Rooi (Suid-Afrika, Albert Heijn's own brand, table wine)	16.3	11.0	27.3
Chianti Classico (Castellani, 1995)	23.2	16.2	39.4
Rosso del Salento (Albert Heijn's own brand, table wine)	21.6	16.4	38.1
White wine			
Bordeaux Sauvignon (Appellation Controlee, Chateau de Nivelle, 1997)	2.1	0.7	2.8
Bordeaux (Appellation Controlee, Rineau, 1997)	4.7	1.0	5.7
Bordeaux Sauvignon (Appellation Controlee, Euroshopper, table wine)	4.8	1.2	6.0
Mosel-Saar-Ruwer (Zeller Schwarze Katz, 1997)	3.6	1.3	4.8
Mosel-Saar-Ruwer (Qualitatswein, 1995)	1.5	0.5	2.0
Mosel-Saar-Ruwer (Riesling Spatlese Qualitatswein mit Pradikat, 1996)	6.1	0.8	6.8
Other beverages			
Apple juice (Riedel)	ND[b]	ND	ND
Apple juice (Albert Heijn)	ND	ND	ND
Black grape juice (Albert Heijn)	8.0	ND	8.0
Black grape juice (Riedel)	9.1	ND	9.1
White grape juice (Albert Heijn)	2.0	ND	2.0
Ice-tea (Liptonice lemon without carbon dioxide)	ND	0.8	0.8
Lager beer (Heineken)	ND	ND	ND
Chocolate milk (semi-skimmed, Nutricia)	16.1	5.0	21.1
Chocolate milk (semi-skimmed, Albert Heijn)	1.2	0.6	1.8
Coffee (Roodmerk, DE)	ND	ND	ND

[a] Average of duplicate analyses; GC, EGC, ECg, EGCg: not detected in any of the samples. [b] ND: not detected.

determinant of catechin content of wine, climatic conditions appear to play an important role. Within cultivars, the highest catechin levels were found in wines grown under damp cool conditions, whereas dry sunny climates yielded lower catechin concentrations.[17] Even within France, an association was observed between the average temperature in a region, and the catechin content of the wine produced there.[18] These observations are consistent with our results: we found higher catechin levels in French wines than in Spanish and Italian wines. In our data, however, we cannot distinguish between cultivar and climate effects, because we did not analyze wine from the same grape cultivar grown under different climatic conditions. Moreover, winemaking methods differ between regions as well, and may explain some of the observed climatic differences.

Other beverages

Despite the fact that both consumer apples[9,21] and homemade juice from apples used for commercial apple juice manufacture[22] contain relatively high levels of (+)-catechin and (-)-epicatechin, no catechins were detected in commercial apple juices (Table 3.2.2). Previous studies likewise reported only very low levels of catechins in commercial apple juice (up to 2.3 mg (+)-catechin and up to 0.7 mg (-)-epicatechin/L juice), or catechins were not detected.[23-26] Spanos and coworkers[26] showed that the complex process of commercial apple juice preparation results in a stepwise decrease in catechins. In particular crushing and pressing, storage of the concentrated juice at room temperature, and decolorization by treatment with activated carbon are steps that lead to complete catechin loss. The same applies to grape juice: relatively high levels in homemade juice,[27] whereas commercial juice, depending on the manufacturing method used, does not contain any catechins.[28] In our study we found low levels of catechins in both white and black grape juice (Table 3.2.2).

In commercially available ice-tea we detected very low levels of (-)-epicatechin (0.8 mg/L), whereas none of the other catechins normally present in tea were found. Ice-tea is made from instant tea, and production methods of instant tea powder cause dimerization and polymerization of catechin monomers. Moreover, the amount of instant tea in commercially available ice-tea is presumably low, and further degradation may have occurred during storage. The quantities of catechins in a number of instant tea powders ranged considerably, from 0.36-7.92% of dry weight.[29] These data suggest that somewhat higher catechin levels may be found in other brands of ice-tea. We did not investigate other brands than the most frequently consumed Dutch brand, because ice-tea consumption is not very high in the Netherlands.

Low levels of (+)-catechin (~5 mg/L) and (-)-epicatechin (~1 mg/L) have been reported in lager beers.[30-32] We did not detect catechins in Dutch lager beer. Polyvinylpolypyrrolidone (PVPP) is an adsorbent for phenolics, which is used by breweries to prolong the stability of beers against haze formation. PVPP was shown to reduce the catechin content of lager beer.[30,33] One might hypothesize that differences in the efficiency with which breweries clarify their beer explain the lack of catechins in the beer we analyzed.

Chocolate milk contained low levels of (+)-catechin and (-)-epicatechin. There was a large difference in catechin contents between the two brands tested. High levels of

(+)-catechin and (-)-epicatechin have been reported previously in chocolate,[34] cacao liquor,[35] and cacao beans.[36,37] The cacao content of chocolate milk is low, which results as a matter-of-course in low catechin levels. The difference in catechin content between the two brands tested may be due to different cacao contents of the beverage, but the use of cacao varieties with different initial catechin levels may be an alternative partial explanation.

In summary, tea and red wine contain substantial quantities of catechins, while low to negligible amounts were found in white wine, commercially available fruit juices, ice-tea, and chocolate milk. Catechins were absent from beer and coffee. Tea is the only beverage that contains all six catechins studied; only (+)-catechin and (-)-epicatechin were present in other beverages. Catechin contents may vary substantially between types, brands, and blends, particularly in tea. The data reported here, together with our data on solid foods,[9] provide a base for the epidemiological evaluation of the effect of catechins on the risk for chronic diseases.

ACKNOWLEDGMENTS

We thank Dr. C. Santos-Buelga (University of Salamanca, Spain) for providing GC, EGC, EGCg, and ECg. This work was carried out with financial support from the Commission of the European Communities, Agriculture and Fisheries (FAIR) specific RTD programme, CT95 0653, the Product Board for Horticulture, the Greenery, and the Ministry of Agriculture, Nature Management and Fisheries.

REFERENCES

1. Blot WJ, Chow WH, McLaughlin JK. Tea and cancer: a review of the epidemiological evidence. *Eur J Cancer Prev* 1996;5:425-438.
2. Kohlmeier L, Weterings KGC, Steck S, Kok FJ. Tea and cancer prevention: an evaluation of the epidemiologic literature. *Nutr Cancer* 1997;27:1-13.
3. Tijburg LBM, Mattern T, Folts JD, Weisgerber UM, Katan MB. Tea flavonoids and cardiovascular diseases: A review. *Crit Rev Food Sci Nutr* 1997;37:771-785.
4. Cook NC, Samman S. Flavonoids: chemistry, metabolism, cardioprotective effects, and dietary sources. *J Nutr Biochem* 1996;7:66-76.
5. Katiyar SK, Mukhtar H. Tea in chemoprevention of cancer: epidemiologic and experimental studies (review). *Int J Oncol* 1996;8:221-238.
6. Klatsky AL, Armstrong MA, Friedman GD. Coffee, tea, and mortality. *Ann Epidemiol* 1993;3:375-381.
7. Hertog MGL, Sweetnam PM, Fehily AM, Elwood PC, Kromhout D. Antioxidant flavonols and ischemic heart disease in a Welsh population of men: the Caerphilly Study. *Am J Clin Nutr* 1997;65:1489-1494.
8. Woodward M, Tunstall-Pedoe H. Coffee and tea consumption in the Scottish Heart Health Study follow up: conflicting relations with coronary risk factors, coronary disease, and all cause mortality. *J Epidemiol Community Health* 1999;53:481-487.

9. Arts ICW, Van de Putte B, Hollman PCH. Catechin contents of foods commonly consumed in The Netherlands. 1. Fruits, vegetables, staple foods, and processed foods. *J Agric Food Chem* 2000;48:1746-1751.
10. Arts ICW, Hollman PCH. Optimization of a quantitative method for the determination of catechins in fruits and legumes. *J Agric Food Chem* 1998;46:5156-5162.
11. Khokhar S, Venema D, Hollman PCH, Dekker M, Jongen W. A RP-HPLC method for the determination of tea catechins. *Cancer Lett* 1997;114:171-172.
12. Bronner WE, Beecher GR. Method for determining the content of catechins in tea infusions by high-performance liquid chromatography. *J Chromatogr A* 1998;805:137-142.
13. Graham HN. Green tea composition, consumption, and polyphenol chemistry. *Prev Med* 1992;21:334-350.
14. Yen GC, Chen HY. Relationship between antimutagenic activity and major components of various teas. *Mutagenesis* 1996;11:37-41.
15. Kuhr S, Engelhardt UH. Determination of flavanols, theogallin, gallic acid and caffeine in tea using HPLC. *Z Lebensm Unters Forsch* 1991;192:526-529.
16. Shao W, Powell C, Clifford MN. The analysis by HPLC of green, black and puer teas produced in Yunnan. *J Sci Food Agric* 1995;69:535-540.
17. Goldberg DM, Karumanchiri A, Tsang E, Soleas GJ. Catechin and epicatechin concentrations of red wines: Regional and cultivar-related differences. *Am J Enol Vitic* 1998;49:23-34.
18. Etiévant P, Schlich P, Bertrand A, Symonds P, Bouvier JC. Varietal and geographic classification of French red wines in terms of pigments and flavonoid compounds. *J Sci Food Agric* 1988;42:39-54.
19. Archier P, Coen S, Roggero JP. Phenolic contents of single variety wines. *Sci Aliments* 1992;12:453-466.
20. Soleas GJ, Dam J, Carey M, Goldberg DM. Toward the fingerprinting of wines: cultivar-related patterns of polyphenolic constituents in Ontario wines. *J Agric Food Chem* 1997;45:3871-3880.
21. Risch B, Herrmann K. Die Gehalte an Hydroxyzimtsäure-Verbindungen und Catechinen in Kern- und Steinobst (Contents of hydroxycinnamic acid derivatives and catechins in pome and stone fruit). *Z Lebensm Unters Forsch* 1988;186:225-230.
22. Suárez Vallés B, Santamaría Victorero J, Mangas Alonso JJ, Blanco Gomis D. High-performance liquid chromatography of the neutral phenolic compounds of low molecular weight in apple juice. *J Agric Food Chem* 1994;42:2732-2736.
23. Bengoechea ML, Sancho AI, Bartolomé B, Estrella I, Gómez-Cordovés C, Hernández MT. Phenolic composition of industrially manufactured purees and concentrates from peach and apple fruits. *J Agric Food Chem* 1997;45:4071-4075.
24. Kermasha S, Goetghebeur M, Dumont J, Couture R. Analyses of phenolic and furfural compounds in concentrated and non-concentrated apple juices. *Food Res Int* 1995;28:245-252.
25. Fernández de Simón B, Pérez-Ilzarbe J, Hernández T, Gómez-Cordovés C, Estrella I. Importance of phenolic compounds for the characterization of fruit juices. *J Agric Food Chem* 1992;40:1531-1535.
26. Spanos GA, Wrolstad RE, Heatherbell DA. Influence of processing and storage on the phenolic composition of apple juice. *J Agric Food Chem* 1990;38:1572-1579.
27. Lee CY, Jaworski A. Phenolic compounds in white grapes grown in New York. *Am J Enol Vitic* 1987;38:277-281.
28. Spanos GA, Wrolstad RE. Influence of processing and storage on the phenolic composition of Thompson Seedless grape juice. *J Agric Food Chem* 1990;38:1565-1571.
29. Constable A, Varga N, Richoz J, Stadler RH. Antimutagenicity and catechin content of soluble

instant teas. *Mutagenesis* 1996;11:189-194.

30. Madigan D, McMurrough I, Smyth MR. Determination of proanthocyanidins and catechins in beer and barley by high-performance liquid chromatography with dual-electrode electrochemical detection. *Analyst* 1994;119:863-868.
31. Achilli G, Cellerino GP, Gamache PH, Melzi d'Eril GV. Identification and determination of phenolic constituents in natural beverages and plant extracts by means of a coulometric electrode array system. *J Chromatogr* 1993;632:111-117.
32. Kaneda H, Kano Y, Osawa T, Kawakishi S, Kamimura M. Effect of free radicals on haze formation in beer. *J Agric Food Chem* 1990;38:1909-1912.
33. McMurrough I, Madigan D, Smyth MR. Adsorption by polyvinylpolypyrrolidone of catechins and proanthocyanidins from beer. *J Agric Food Chem* 1995;43:2687-2691.
34. Arts ICW, Hollman PCH, Kromhout D. Chocolate as a source of tea flavonoids. *Lancet* 1999;354:488.
35. Sanbongi C, Osakabe N, Natsume M, Takizawa T, Gomi S, Osawa T. Antioxidative polyphenols isolated from Theobroma cacao. *J Agric Food Chem* 1998;46:454-457.
36. Porter LJ, Ma Z, Chan BG. Cacao procyanidins: major flavanoids and identification of some minor metabolites. *Phytochemistry* 1991;30:1657-1663.
37. Kim H, Keeney PG. (-)-Epicatechin content in fermented and unfermented cocoa beans. *J Food Sci* 1984;49:1090-1092.

Catechin intake and associated dietary and lifestyle factors in a representative sample of Dutch men and women

In this study we estimated the intake of catechins in the Dutch population and assessed the relation between catechin intake and other dietary factors. Data were used from a nation-wide dietary survey carried out in 1998 among a representative sample of 6,200 Dutch men and women aged 1-97 years. Dietary data were collected using a two-day dietary record method. The average daily catechin intake was 50 mg (standard deviation: 56 mg/day). Catechin intake increased with age, and the intake was higher in women (60 mg/day) than in men (40 mg/day). Tea was the main catechin source in all age groups, whereas chocolate was second in children, and apples and pears were second in adults and elderly. Catechin intake was lower in smokers than in non-smokers, and increased with socio-economic status. A high intake was associated with a high intake of fiber (r=0.20), vitamin C (r=0.17) and beta-carotene (r=0.10). Catechins are quantitatively important bioactive components of the daily diet, which should be taken into account when studying the relation between diet and chronic diseases. Catechin intake is only moderately associated with the intake of other nutrients, but much stronger with certain health behaviours such as smoking.

Arts ICW, Hollman PCH, Feskens EJM, Bueno de Mesquita HB, Kromhout, D. Catechin intake and associated dietary and lifestyle factors in a representative sample of Dutch men and women. *Eur J Clin Nutr 2001;55:76-81.*

INTRODUCTION

Catechins, also referred to as flavanols, are one of the six subclasses of flavonoids. Flavonoids are non-nutritive secondary plant metabolites, which are common components of the human diet. Currently more than 4000 different flavonoids have been identified.[1] *In vitro* and *in vivo* animal experiments have shown that catechins have antimutagenic and anticarcinogenic properties, and that they may play a role in the prevention of cardiovascular diseases. Proposed mechanisms for these potential human health promoting properties include inhibition of the metabolic activation of procarcinogens to DNA-reactive species, induction of enzyme systems involved in detoxification, and protection against DNA damaging free radicals through their antioxidant activity.[2,3] Catechins may help prevent LDL from oxidative damage through their free radical quenching and metal chelating abilities,[2,4] or play a role in the inflammatory and thrombotic processes involved in atherosclerosis.[5,6]

Catechins are the major components of tea; they constitute about 30% of the dry weight of green tea, and 9% of the dry weight of black tea.[7] Epidemiological studies on health effects of tea have suggested that a high tea intake may protect against certain cancers and coronary heart disease, but results are inconsistent.[8-11] On the other hand, there is firm evidence from experimental studies in laboratory animals that tea may prevent chronic diseases.[12,13] One possible explanation for this apparent inconsistency is that tea is not the only catechin containing food in the human diet. In particular in countries were tea intake is low, other foods may be more important sources of catechins than tea.

Recently, we reported data on catechin contents of a comprehensive set of foods and beverages.[14,15] These data enable us to estimate the intake of these compounds in a population-based sample of men and women in The Netherlands, and to study dietary and lifestyle factors that are associated with a high intake of catechins.

METHODS

Food Consumption Survey

The Dutch National Food Consumption Survey 1998 was carried out among a sample of households representative for the Dutch population. A household was defined as one or more persons living together in one house, eating together a home prepared hot meal for at least 4 days a week. Based on this definition, institutionalized persons were excluded. Also, subjects who did not master the Dutch language sufficiently, and children younger than 1 year of age were excluded. 6,250

persons (2,885 men and 3,365 women) aged 1-97 years coming from 2,564 households participated in the study. The response rate was 68.5%. Trained dieticians collected dietary data between April 1997 and March 1998 using a two-day dietary record method. No data were collected on holidays; otherwise, record-days were distributed equally over the seven days of the week and over the year. Other measurements included age, height, weight, socio-economic status, and life-style variables such as smoking. As indicators of socio-economic status, (former) occupation and attained educational level of the head of the household were used.[16,17]

Food analysis

Six major catechins [(+)-catechin, (-)-epicatechin, (+)-gallocatechin (GC), (-)-epigallocatechin (EGC), (-)-epicatechin gallate (ECg), and (-)-epigallocatechin gallate (EGCg)] were determined in a comprehensive set of plant foods by Reversed-Phase High Performance Liquid Chromatography with online ultraviolet and fluorescence detection.[14,15,18] Analyzed foods included 24 types of fruits, 27 types of vegetables and legumes, some staple foods, and a number of processed foods such as chocolate, 8 types of black tea, 18 types of red and white wine, fruit juices, ice-tea, beer, chocolate milk and coffee. All perishable foods were purchased at three outlets: a nation-wide supermarket chain, an open-air street market, and a grocery. To take into account seasonal and year-to-year variability, fruits and vegetables were purchased in August and December 1997 and in April and August 1998, if available in that period.

Catechin intake calculation

The catechin intake for each individual was calculated by multiplying the consumption of each food by its catechin content. Catechin contents were estimated either by direct chemical analysis of the food concerned (37%), calculated using standard recipes if one or more ingredients contained catechins (57%), or derived from similar foods (6%). Data on catechin contents of several varieties of apples and pears were combined into single values according to auction supply data. Similarly, data on catechin contents of tea blends, and white and red wines were combined into single values according to supply data provided by, respectively, the Coffee and Tea Information Bureau, and the Dutch Commodity Board of Wine. Standard recipes were used to calculate the catechin contents of foods that contained one or more chemically analyzed ingredients, except for chocolate containing foods, where data were derived from the Conversion Model Primary Agricultural Products.[19] Because seasonal variation was relatively low,[14] year average catechin values were used.

Since catechins are predominantly present in foods that are usually consumed raw, e.g. fruit, catechin loss due to home preparation is not an important issue.

Statistical analysis

Statistical analyses were performed using the SAS statistical package (SAS, release 6.12, SAS Institute, North Carolina, USA). Pregnant women (n=50) were excluded from the analyses, because they tended to have a deviant dietary pattern. Wilcoxon rank sum tests were used to compare mean catechin intakes between groups. Partial rank-order correlation coefficients were calculated between total catechin intake and intake of alcohol, saturated and polyunsaturated fatty acids, fiber, vitamin C, vitamin E, and beta-carotene, after adjusting for total energy intake. Mean intakes stratified by smoking status, and socio-economic status were standardized for age and sex using analysis of covariance. Children (<19 years of age) were excluded from the smoking status analysis.

RESULTS

The mean intake of catechins in the total population was 50 mg/day (standard deviation: 56 mg/day), and ranged from 0 (284 subjects, 4.6%) to 958 mg/day on the two particular days on which subjects were surveyed. The distribution of catechin intake in the adult population (19 years and older), was strongly skewed towards higher values (Figure 4.1). Only 123 subjects (2% of the total population) had an average intake on the two survey days that was higher than 200 mg/day.

Of the individual catechins, ECg contributed most to the total catechin intake (33%), followed by EGCg (24%) and (-)-epicatechin (23%). GC (5%), EGC (8%), and (+)-catechin (8%) were minor contributors. Women had a significantly higher intake of all catechins than men: the total intake was 60 mg/day in women versus 40 mg/day in men (P-value: 0.0001) (Table 4.1). This gender difference persisted when we classified subjects by age group, and standardized catechin intake on total energy intake (Figure 4.2). After the age of 16, women had a significantly higher catechin intake per MJ energy intake than men. Furthermore, total catechin intake increased with age, even after subjects had attained adulthood (Figure 4.2).

The most important source of catechins in children, adults and elderly people was tea (Table 4.2). Tea consumption increased with age: both the number of consumers, the mean intake, and the contribution to the total catechin intake showed a steady increase, up to a contribution of 87% in elderly subjects. Chocolate was an important source of catechins in children only: among the 1-18 year olds, 83% consumed

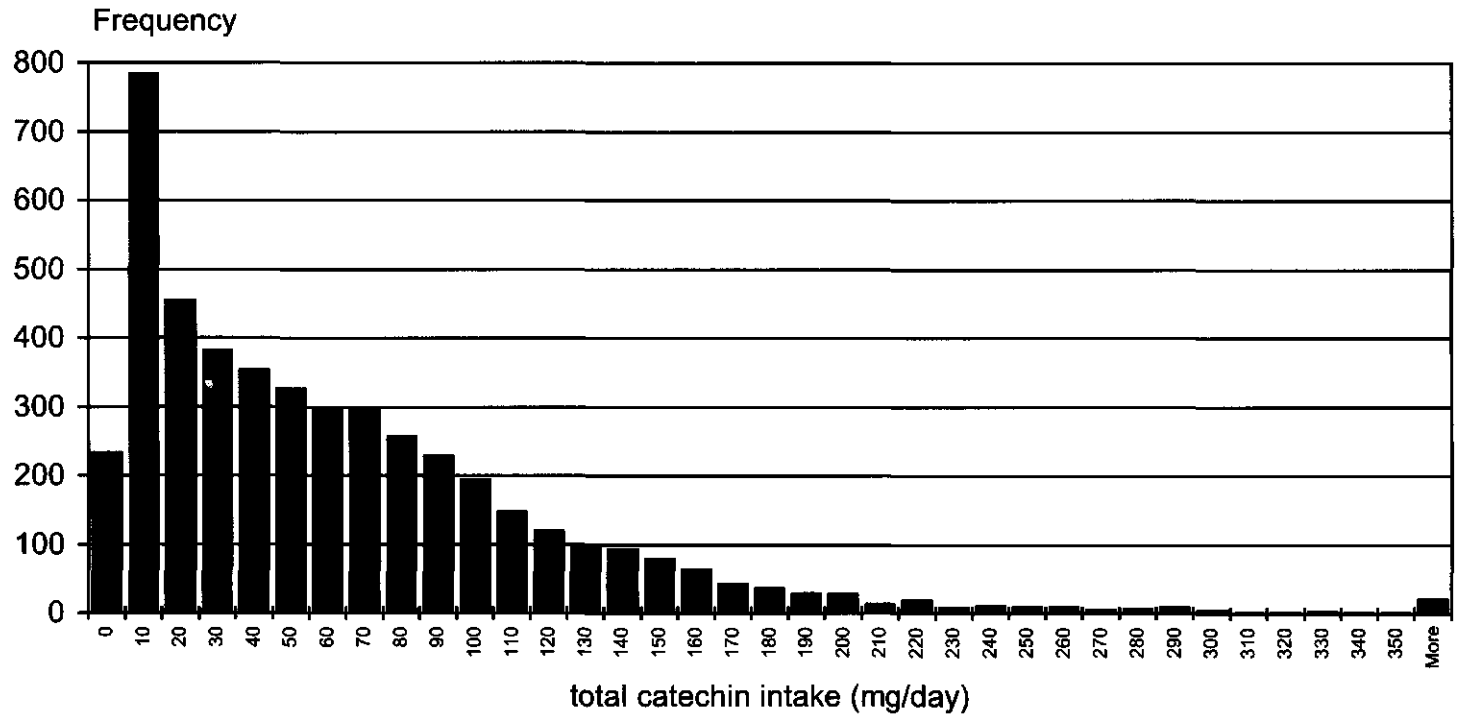


Figure 4.1 Distribution of the total catechin intake of 4,661 adults (19 years and older), the Dutch National Food Consumption Survey 1998.

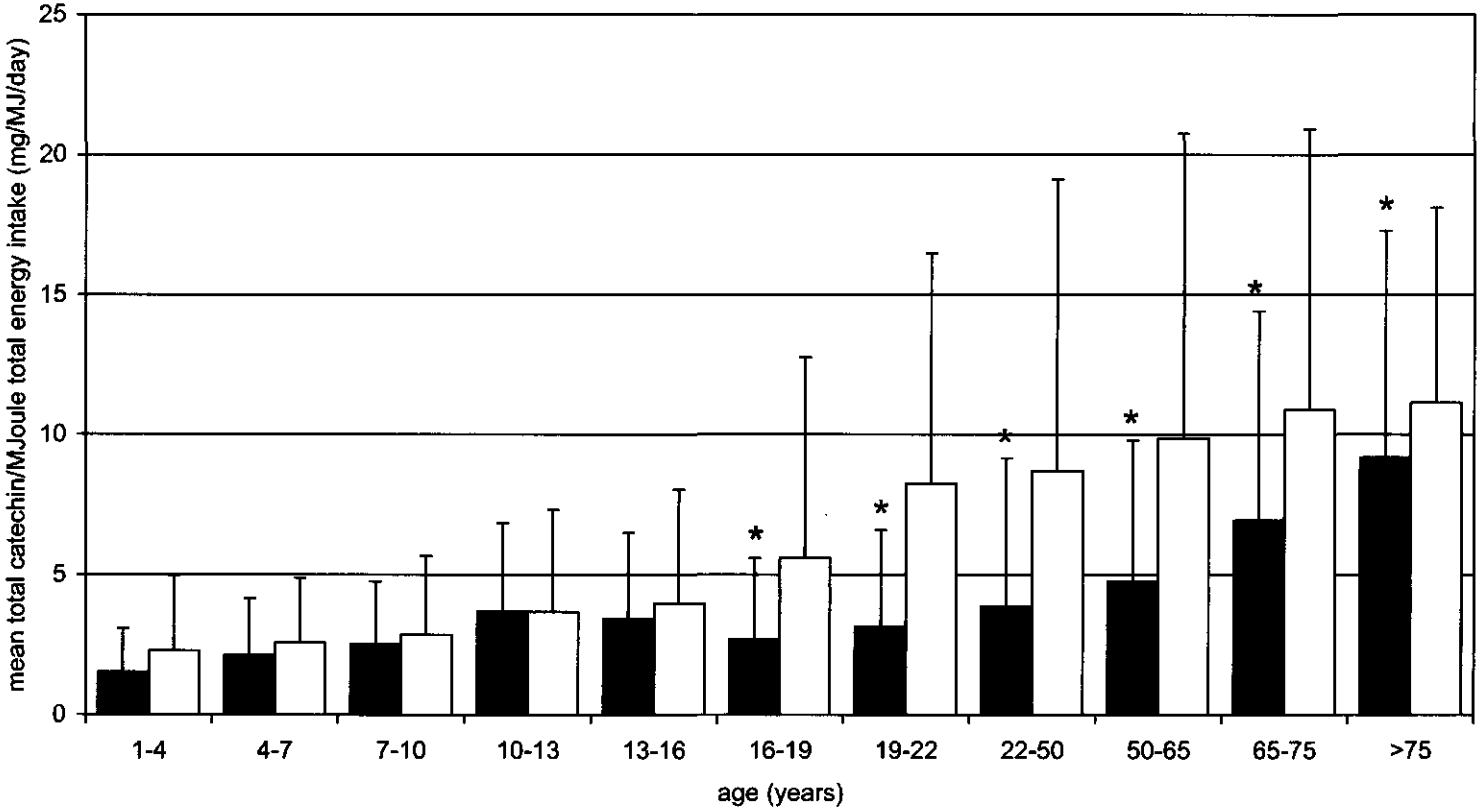


*Figure 4.2 Mean total catechin intake per MJoule total energy intake, by age group and sex (■ men; □ women) among 6,200 subjects, the Dutch National Food Consumption Survey 1998. Error bars indicate standard deviations. *: P<0.05 for difference between men and women.*

chocolate on at least one of the two survey days (20% of total catechin intake). Chocolate and apples/pears were equally important sources of catechins in adults (6%), while apple and pear were more important in elderly people. Wine, other fruits such as cherries, strawberries and peaches, and legumes such as kidney beans contributed only marginally to the total catechin intake of this population, and were generally consumed by only a small part of the population. Within consumers, however, they may be important sources.

Table 4.1 Mean intakes of total catechins, (+)-catechin, (-)-epicatechin, (+)-gallocatechin (GC), (-)-epigallocatechin (EGC), (-)-epicatechin gallate (ECg), and (-)-epigallocatechin gallate (EGCg) among 6,200 men and women, the Dutch National Food Consumption Survey 1998

	mean catechin intake (mg/day) ± standard deviation	
	Men	Women
N	2885	3315
total catechins	39.5 ± 46.4[a]	59.8 ± 62.5
(+)-catechin	3.5 ± 3.6[a]	4.4 ± 4.1
(-)-epicatechin	10.2 ± 9.0[a]	12.8 ± 10.1
GC	1.8 ± 2.6[a]	3.0 ± 3.6
EGC	2.8 ± 4.1[a]	4.6 ± 5.6
ECg	12.1 ± 17.8[a]	20.1 ± 24.4
EGCg	9.0 ± 13.2[a]	14.9 ± 18.1

[a] P = 0.0001 for difference between men and women.

Table 4.2 Contribution of various food groups to the mean catechin intake in the total population by age group

Source	Children (1-18 yrs) (N=1539)			Adults (19-64 yrs) (N=3954)			Elderly (>65 yrs) (N=707)		
	% users	mean intake (mg/day) ± SD[a]	% contribution	% users	mean intake (mg/day) ± SD	% contribution	% users	mean intake (mg/day) ± SD	% contribution
Tea	47	16.6 ± 25.6	65.2	67	46.3 ± 58.8	83.3	84	65.6 ± 62.3	87.3
Chocolate	83	5.1 ± 5.8	20.1	65	3.1 ± 4.8	5.7	61	2.0 ± 3.4	2.6
Apple/pear	50	3.1 ± 4.5	12.0	44	3.3 ± 5.3	5.9	58	4.0 ± 5.0	5.3
Other fruits	15	0.4 ± 1.5	1.5	20	0.8 ± 3.0	1.4	29	1.6 ± 4.6	2.1
Wine	2	0.0 ± 0.3	0.1	20	1.6 ± 5.0	2.8	20	1.1 ± 3.7	1.5
Legumes	3	0.1 ± 0.6	0.2	4	0.2 ± 1.4	0.3	5	0.5 ± 3.6	0.7
Other foods	37	0.2 ± 0.6	0.9	45	0.4 ± 0.9	0.7	56	0.4 ± 1.0	0.6
All foods	97	25.5 ± 27.2	100	95	55.6 ± 60.2	100	96	75.1± 63.4	100

[a] SD: standard deviation

After adjusting for total energy intake, we observed weak positive associations between intake of catechins and intake of fiber, vitamin C and beta-carotene among

children, adults, and elderly (Table 4.3). The strongest associations were observed for fiber (r=0.21 for children). Catechin intake was not associated with intake of vitamin E, saturated fatty acids, and polyunsaturated fatty acids, and inversely associated with intake of alcohol in the elderly. The mean catechin intake standardized for age and sex, was significantly lower among smokers compared to non-smokers, and increased with socio-economic status (Table 4.4).

Table 4.3 Partial rank-order correlation coefficients between total catechin intake and other dietary factors stratified by age group and adjusted for total energy intake

	Children (1-18 yrs) (N=1539)	Adults (19-64 yrs) (N=3954)	Elderly (>65 yrs) (N=707)
alcohol	-0.01	-0.08[a]	-0.15[a]
saturated fatty acids	0.05	-0.03	0.02
polyunsaturated fatty acids	-0.02	-0.02	0.01
fiber	0.21[a]	0.20[a]	0.13[a]
vitamin C	0.07[a]	0.17[a]	0.11[a]
vitamin E	0.01	0.05[a]	0.07
beta-carotene	0.06[a]	0.10[a]	0.11[a]

[a] $P < 0.05$.

Table 4.4 Mean total catechin intake according to smoking status (adults only) and socio-economic status, standardized for age and sex

	N	mean catechin intake (mg/day)[a]
Smoking status		
Yes	1431	43.7[a]
No	3226	65.2
Socio-economic status		
A (high)	794	59.8[b]
B1	1630	54.0
B2	1298	53.0
C	2230	44.5
D (low)	242	33.5

[a] $P < 0.05$ for difference between smokers and non-smokers. [b] P-value for a linear trend: 0.0001.

Discussion

In a representative sample of 6,200 Dutch men and women aged 1-97 years, the intake of six major catechins was on average 50 mg/day. Kühnau[20] first estimated the dietary intake of catechins using data on the average American diet which were based on food disappearance values (Food Consumption Statistics 1955-1971, OECD, Paris, 1973). The estimated catechin intake in the US was 220 mg/day expressed as quercitrin (molecular weight = 464), which equals 140 mg catechins per day. This is roughly three times our estimate for the Dutch population. Although such

an intake is possible (in our study 7% of the population had an intake of at least 140 mg/day), Kühnau most likely overestimated the average catechin intake in the US, because he used food disappearance data, which are known to overestimate the true food intake. In addition he only had available catechin data obtained with methods now considered obsolete. The most advanced technique used at that time was thin-layer chromatography with spectrophotometric measurement, which is likely to overestimate the catechin content of a food. Most likely the American daily catechin intake is lower than the intake in The Netherlands because the consumption of tea, a major catechin source, is relatively low in the US.[21,22] More recently a paper was published on the catechin intake in the Danish population.[23] An estimated average daily intake of 20-50 mg was reported using data from the Danish Household Consumption Survey and literature data on catechin contents of foods. The calculated range was wide because of lack of detail in the survey data and wide ranges of reported catechin contents of foods in the literature.

In our study a 2-day dietary record method was used to measure food intake. This is a reliable method to estimate population intakes. At the individual level, however, the 2 particular days included in the study probably do not represent the habitual dietary pattern. We determined catechin contents of more than 100 plant foods and beverages commonly consumed in The Netherlands using state-of-the-art High Performance Liquid Chromatography methods, taking into account seasonal and year-to-year variation.[14,15] This comprehensive database represents catechin contents of foods as consumed. It is unlikely that we have missed important catechin sources in the Dutch diet because of the extensive sampling scheme.[14,15]

The major sources of catechins were tea, chocolate, and apples/pears. Absolute consumption levels of tea were much higher than those of chocolate, which resulted in a lower contribution of chocolate to the total catechin intake, despite the fact that catechin concentrations are higher in chocolate than in tea.[14,15] The major catechins in our study were ECg, EGCg and (-)-epicatechin. This partly justifies the relatively large research effort directed at EGCg in *in vitro* and animal experimental studies. However, it also warrants more attention for ECg and (-)-epicatechin, and comparative studies would be extremely useful.

Catechin intake was higher among adult women than men. There were more females that consumed tea (75%, versus 58% of adult males), other fruits than apples/pears (23%, versus 16% of adult males), and wine (24%, versus 15%). Additionally, adult female tea consumers drank on average more tea than adult male tea consumers. In elderly, gender differences were similar, but less pronounced. Catechin intake

increased with age among both sexes. Again, the observed differences were largely due to variations in tea intake. On the basis of these cross-sectional data, it is not possible to conclude that with increasing age, people increase their catechin intake: we cannot distinguish between age and cohort effects. The increasing catechin intake with age may be due to an increase in tea consumption with aging, but it may also be due to decreasing popularity of tea among youths nowadays. If the latter would be true, the adults of today would have a lower catechin intake by the time they become elderly than the current elderly.

In epidemiological studies, it is important to consider lifestyles and health behaviors associated with the exposure of interest to take into account potential confounders or effect modifiers. Several studies have reported that catechin-rich beverages such as wine and tea are associated with a healthy dietary pattern.[24,25] In our study, subjects with a high catechin intake tended to have a high intake of vitamin C, beta-carotene and fiber, consumed less alcohol, and were more often non-smokers. There was a clear increase in catechin intake with increasing socio-economic status. When studying health effects of catechins using epidemiological data, these dietary and lifestyle factors should be taken into account.

ACKNOWLEDGMENTS

The authors would like to thank Betty van de Putte for excellent technical assistance, and Jacob van Klaveren and Margo van Dooren-Flipsen at RIKILT for use of data from the Conversion Model Primary Agricultural Products. This work was carried out with financial support from the Commission of the European Communities, Agriculture and Fisheries (FAIR) specific RTD programme, CT95 0653.

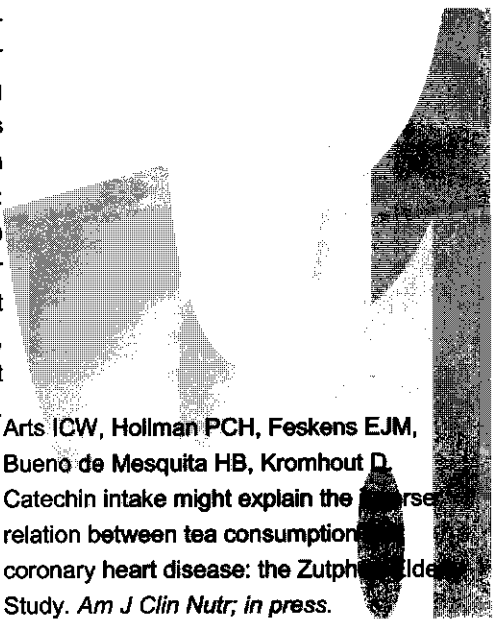
REFERENCES

1. Harborne JB. *The Flavonoids: Advances in research since 1986.* London: Chapman & Hall; 1994.
2. Rice-Evans CA, Miller NJ, Paganga G. Structure-antioxidant activity relationships of flavonoids and phenolic acids. *Free Radical Biol Med* 1996;20:933-956.
3. Middleton E, Kandaswami C. The impact of plant flavonoids on mammalian biology: implications for immunity, inflammation and cancer. In: Harborne JB, ed. *The Flavonoids: advances in research since 1986.* London: Chapman & Hall;1994:619-652.
4. Wiseman SA, Balentine DA, Frei B. Antioxidants in tea. *Crit Rev Food Sci Nutr* 1997;37:705-718.
5. Middleton E. Effect of plant flavonoids on immune and inflammatory cell function. *Adv Exp Med Biol* 1998;439:175-182.
6. Wollny T, Aiello L, Di Tommaso D, Bellavia V, Rotilio D, Donati MB, De Gaetano G, Iacoviello L. Modulation of haemostatic function and prevention of experimental thrombosis by red wine in rats: a role for increased nitric oxide production. *Br J Pharmacol* 1999;127:747-755.
7. Harbowy ME, Balentine DA. Tea chemistry. *Crit Rev Plant Sciences* 1997;16:415-480.
8. Bushman JL. Green tea and cancer in humans: a review of the literature. *Nutr Cancer* 1998;31:151-159.

9. Kohlmeier L, Weterings KGC, Steck S, Kok FJ. Tea and cancer prevention: an evaluation of the epidemiologic literature. *Nutr Cancer* 1997;27:1-13.
10. Tijburg LBM, Mattern T, Folts JD, Weisgerber UM, Katan MB. Tea flavonoids and cardiovascular diseases: A review. *Crit Rev Food Sci Nutr* 1997;37:771-785.
11. Blot WJ, Chow WH, McLaughlin JK. Tea and cancer: a review of the epidemiological evidence. *Eur J Cancer Prev* 1996;5:425-438.
12. Katiyar SK, Mukhtar H. Tea in chemoprevention of cancer: epidemiologic and experimental studies (review). *Int J Oncol* 1996;8:221-238.
13. Dreosti IE, Wargovich MJ, Yang CS. Inhibition of carcinogenesis by tea: The evidence from experimental studies. *Crit Rev Food Sci Nutr* 1997;37:761-770.
14. Arts ICW, Van de Putte B, Hollman PCH. Catechin contents of foods commonly consumed in The Netherlands. 1. Fruits, vegetables, staple foods, and processed foods. *J Agric Food Chem* 2000;48:1746-1751.
15. Arts ICW, Van de Putte B, Hollman PCH. Catechin contents of foods commonly consumed in The Netherlands. 2. Tea, wine, fruit juices, and chocolate milk. *J Agric Food Chem* 2000;48:1752-1757.
16. Hulshof KFAM, Van Staveren WA. The Dutch National Food Consumption Survey: design, methods and first results. *Food Policy* 1991;16:257-260.
17. Voedingscentrum. *Zo eet Nederland; Resultaten van de Voedselconsumptiepeiling 1997-1998 [in Dutch].* Den Haag: Voedingscentrum; 1998.
18. Arts ICW, Hollman PCH. Optimization of a quantitative method for the determination of catechins in fruits and legumes. *J Agric Food Chem* 1998;46:5156-5162.
19. Van Dooren-Flipsen MMH, Boeijen I, Van Klaveren JD, Van Donkersgoed G. Conversie van consumeerbare voedingsmiddelen naar primaire agrarische producten. Berekening inname residuen en contaminanten [in Dutch]. *Voeding* 1996;57:6-9.
20. Kühnau J. The flavonoids: a class of semi-essential food components: their role in human nutrition. *World Rev Nutr Diet* 1976;24:117-191.
21. Zheng W, Doyle TJ, Kushi LH, Sellers TA, Hong CP, Folsom AR. Tea consumption and cancer incidence in a prospective cohort study of postmenopausal women. *Am J Epidemiol* 1996;144:175-182.
22. Rimm EB, Katan MB, Ascherio A, Stampfer MJ, Willett WC. Relation between intake of flavonoids and risk for coronary heart disease in male health professionals. *Ann Intern Med* 1996;125:384-389.
23. Dragsted LO, Strube M, Leth T. Dietary levels of plant phenols and other non-nutritive components: could they prevent cancer? *Eur J Cancer Prev* 1997;6:522-528.
24. Tjonneland A, Gronbaek M, Stripp C, Overvad K. Wine intake and diet in a random sample of 48763 Danish men and women. *Am J Clin Nutr* 1999;69:49-54.
25. Tsubono Y, Takahashi T, Iwase Y, Iitoi Y, Akabane M, Tsugane S. Dietary differences with green tea intake among middle-aged japanese men and women. *Prev Med* 1997;26:704-710.

Dietary catechins, coronary heart disease and stroke: the Zutphen Elderly Study

We evaluated the effect of a high catechin intake on coronary heart disease (CHD) and stroke incidence and mortality using data from the Zutphen Elderly Study, a prospective cohort study among 806 men aged 65-84 years at baseline in 1985. The average catechin intake at baseline was 72 mg/day (standard deviation: 47.8 mg/day), mainly from black tea, apples, and chocolate. A total of 90 deaths from CHD were documented. Catechin intake was inversely associated with CHD mortality, the multivariate adjusted risk ratio in the highest tertile of intake was 0.49 (95% confidence interval (CI): 0.27-0.88; P for trend: 0.017). After multivariate adjustment, catechin intake was not associated with myocardial infarction incidence (risk ratio in highest tertile of intake: 0.70; 95% CI: 0.39-1.26; P for trend: 0.232). After adjustment for tea consumption and flavonol intake, a 7.5 mg increase in catechin intake from other sources than tea, was associated with a tendency for a 20% reduction in CHD mortality risk (P-value: 0.114). There was no association between catechin intake and stroke incidence or mortality. These results suggest that catechins, whether from tea or other sources, may reduce risk of CHD mortality, but not stroke.



Arts ICW, Hollman PCH, Feskens EJM, Bueno de Mesquita HB, Kromhout D. Catechin intake might explain the inverse relation between tea consumption and coronary heart disease: the Zutphen Elderly Study. *Am J Clin Nutr; in press.*

INTRODUCTION

Epidemiological studies suggest that tea may reduce the risk of cardiovascular and cerebrovascular diseases.[1-5] However, results published up to now are not consistent. Studies from both the United Kingdom (with a high intake of black tea) and the USA (with a relatively low intake) found no effect of tea consumption on coronary heart disease risk,[6-8] or even a slightly increased risk.[9] Tea is a rich source of flavonoids, the compounds which are held responsible for its alleged protective effect. At present, more than 4000 different flavonoids have been identified.[10] They occur ubiquitously in plant foods, and can be categorized into six major subclasses: catechins, flavonols, flavones, flavanones, anthocyanidins, and isoflavonoids. To date, only comprehensive food composition data on the subclasses of flavonols and flavones are available.[11,12] In a number of prospective studies, flavonols and flavones have been related to a reduced risk of coronary death,[2,13-15] and stroke.[3]

Catechins are the major components of tea; they constitute about 30% of the dry weight of green tea, and 9% of the dry weight of black tea.[16] Several mechanisms by which catechins could prevent cardiovascular diseases have been suggested; they have been reviewed by Middleton.[17] Catechins may prevent LDL from oxidative damage either through their free radical quenching and metal chelating abilities,[18,19] or by recycling other antioxidants such as vitamin E.[20,21] Catechins also have been shown to interfere with several stages of the inflammatory process involved in atherosclerosis,[22,23] and there are indications that catechins may influence hemostatic parameters and reduce thrombosis.[24] However, the relevance of these proposed mechanisms to the *in vivo* situation remains to be established.

Up to now, an epidemiological evaluation of catechins was impossible, because reliable data on the catechin content of foods were lacking. We have developed a method to determine catechins in foods[25] and we have determined six major catechins: (+)-catechin, (+)-gallocatechin, (-)-epicatechin, (-)-epigallocatechin, (-)-epicatechin gallate, and (-)-epigallocatechin gallate in foods and beverages commonly consumed in the Netherlands.[26,27] Using these data we evaluated for the first time the effect of catechin intake on the risk of fatal and incident coronary heart disease and stroke in a cohort of elderly men.

METHODS

Study population

The Zutphen Elderly Study is a prospective cohort study on risk factors for chronic

diseases in elderly men. It is an extension of the Zutphen Study, the Dutch contribution to the Seven Countries Study. In 1985, 555 men of the original Zutphen cohort recruited in 1960 were still alive, and were invited to participate in the Zutphen Elderly Study. A random sample of all other men of the same age also living in Zutphen but not belonging to the original cohort, was invited additionally. This resulted in a total target population of 1266 men aged 65-84. Of these 1266 men, 939 men (74%) participated in the study, of which 876 men completed a dietary questionnaire. Complete information on both diet and other risk factors was available for 806 men.

Data collection

Dietary and medical examinations were conducted between March and June 1985. Medical examinations were carried out by trained physicians and included anthropometry, blood sampling, blood pressure measurement, and detailed questionnaires on smoking behavior and physical activity. The latter was determined with a validated questionnaire designed for retired men.[28] The habitual diet in the month preceding the interview was determined using a cross-check dietary history method adapted to the Dutch situation.[29] Participants were interviewed at home by an experienced dietitian in the presence of the person usually preparing meals. If the respondent followed a prescribed diet, the type of diet was denoted. The food intake data were encoded by the dietitians, and converted into energy and nutrient data using the 1985 release of the Dutch Food Table,[30] updated with 1993 data for beta-carotene and vitamin E, with flavonol and flavone data, and with catechin data. Flavonol and flavone contents of foods were previously determined by Hertog and colleagues.[11,12] Flavones are a minor group of flavonoids compared to the flavonols; the term flavonols will be used here for the sum of both groups. Catechin, or total catechin, is defined as the sum of (+)-catechin, (+)-gallocatechin, (-)-epicatechin, (-)epigallocatechin, (-)-epicatechin gallate, and (-)-epigallocatechin gallate. We determined these six catechins by Reversed-Phase HPLC with online ultraviolet and fluorescence detection.[25] More than 120 commonly consumed plant foods and beverages in the Dutch diet were analyzed.[26,27] Each food was purchased at three outlets: a nation-wide supermarket chain, an open-air street market, and a grocery. To take into account seasonal and year-to-year variability, each food was purchased in August and December 1997 and in April and August 1998, if available in that period. Different brands and varieties of the most important catechin sources were analyzed, and combined into average values taking into account consumption levels.

Case ascertainment

Information on the vital status of the participants until January 1995 was obtained from municipal population registries. Three men were lost to follow-up in 1991. Of these, two had moved abroad, and one had moved with unknown destination. These men were included in the analyses, but censored at 31 December 1990. Causes of death were obtained from the Central Bureau of Statistics; between June 1990 and January 1995 information was also obtained from the participants' general practitioner. Information was verified with either hospital discharge data or written information from the general practitioner. Since it is often difficult to determine the underlying cause of death in elderly people, both the primary and secondary cause of death were included in the analyses. Coding of the causes of death followed the ninth Revision of the International Classification of Diseases.[31] ICD-codes 410-414 refer to coronary heart disease (CHD) and codes 430-438 refer to cerebrovascular disease, further referred to as stroke.

Disease prevalence at baseline and first clinical diagnosis of disease during follow-up were recorded at the examinations in 1985, 1990, 1993, and 1995 using the standardized Rose-questionnaire. Non-responders at follow-up examinations received a short questionnaire on disease history. Data on baseline prevalence of angina pectoris (AP), and on baseline prevalence and first clinical diagnosis of myocardial infarction (MI) and stroke, were verified with hospital discharge data and written information from the general practitioner. Data on first, fatal or non-fatal, events (referred to as incidence) were uniformly coded by three physicians. At baseline, there were 115 men (14%) with prevalent MI, these were excluded in the MI incidence analyses. Prevalent MI or AP at baseline was included as a covariate in the MI mortality analyses. Similarly, the 39 men (5%) with prevalent stroke at baseline were excluded in the stroke incidence analyses, and stroke at baseline was included as a covariate in the stroke mortality analyses.

Statistical analysis

Baseline characteristics of the participants were compared between tertiles of catechin intake using the Chi-square test for categorical variables, one-way analysis of variance for normally distributed variables and the Kruskal-Wallis test for skewed variables. Spearman rank correlation coefficients were calculated between catechin intake and other dietary factors. Correlation coefficients ranged from –0.27 for coffee to 0.23 for fiber. Risk ratios of fatal and non-fatal incidence of MI and stroke, and death due to CHD and stroke were estimated by Cox proportional hazards analysis, using the SAS procedure PHREG (SAS, release 6.12, SAS Institute, North Carolina,

USA). Following age-adjusted analyses, two multivariate models were tested. The first model was adjusted for baseline prevalence of the disease of interest (mortality analyses only), age, smoking status, total energy intake, body mass index (BMI), alcohol intake, and physical activity. The second model was additionally controlled for dietary factors: coffee consumption, fish consumption, vitamin C, vitamin E, beta-carotene, saturated fatty acids, polyunsaturated fatty acids, dietary cholesterol, fiber, and whether or not a prescribed diet was followed. Probability values for a linear trend were derived from tertile medians.

RESULTS

The average daily catechin intake in the 806 elderly men that participated in 1985 was 72 mg (standard deviation: 47.8 mg/day). It ranged from 0 to 355.4 mg/day. Only one subject had a catechin intake of 0. The major source of catechins in this population was tea (87%). Other foods that contributed substantially to the catechin intake were apples (8%), and chocolate (3%). Other fruits and legumes were minor sources, while vegetables did not contribute. For persons in the lowest tertile of catechin intake (<49.1 mg/day), tea was a less important source (71%), while apples (17%) and chocolate (7%), were more important. Of the individual catechins, (-)-epicatechin gallate (34%), (-)-epigallocatechin gallate (26%), and (-)-epicatechin (21%) contributed most to the catechin intake.

At baseline, participants that belonged to the highest tertile of catechin intake were less likely to be current smokers, more likely to have never smoked, and tended to be more physically active (Table 5.1.1). They had a higher total energy, fiber, vitamin C, vitamin E and beta-carotene intake. They ate more fruits and vegetables, but less fish and drank less coffee. Since tea was the most important source of catechins in this population, tea consumption increased in a dose-response manner with catechin intake (correlation coefficient, r_s=0.98).

After 10 years of follow-up (6025 person years) 374 men (46%) had died. Of these, 90 men had coronary heart disease as a primary or secondary cause of death, and 47 men died from stroke. Age-adjusted catechin intake showed a statistically significant inverse association with the risk of death from coronary heart disease (P for trend: 0.007) (Table 5.1.2). The risk ratio in the highest tertile of catechin intake was 0.48 (95% confidence interval (CI): 0.28-0.82). Adjustment for baseline prevalent MI or AP, age, physical activity, total energy intake, BMI, alcohol intake, and smoking status (model 1), and dietary factors (model 2) did not essentially change the relationship. Nor did additional adjustment for serum total or HDL cholesterol, systolic

Table 5.1.1 Baseline characteristics of 806 elderly men by tertile of total catechin intake

	Tertile of total catechin intake			
	low	middle	high	P-value[a]
Range of total catechin intake (mg/day)	0-49.0	49.1-85.8	85.9-355.4	
Number of men	268	269	269	
Number of men with history of MI or AP	54	55	57	
Number of men with history of stroke	20	8	11	
Current smokers (%)	42.2	28.6	19.3	0.001
Never smokers (%)	15.7	16.4	23.4	0.038
Prescribed diet (%)	25.4	24.5	30.1	0.289
	mean (standard deviation)			
Catechin intake (mg/day)	25.3 (15.4)	66.8 (10.7)	124.0 (40.0)	
Flavonol + flavone intake (mg/day)	14.0 (8.7)	24.5 (7.8)	38.9 (13.0)	0.0001
Age in 1985 (yrs)	70.8 (5.0)	71.9 (5.5)	71.3 (5.2)	0.088
Energy intake (MJ/day)	9.3 (2.2)	9.3 (2.1)	9.8 (2.1)	0.010
Alcohol (g/day)	15.2 (18.4)	11.9 (17.0)	12.6 (15.6)	0.189
Physical activity (min/week)	657.6 (665.2)	645.4 (617.4)	735.4 (662.6)	0.052
Serum total cholesterol (mmol/L)	6.18 (1.1)	6.13 (1.2)	6.03 (1.0)	0.285
Serum HDL cholesterol (mmol/L)	1.15 (0.3)	1.11 (0.3)	1.11 (0.3)	0.464
Systolic blood pressure (mmHg)	150.2 (20.8)	151.6 (21.3)	150.6 (21.6)	0.555
Body mass index (kg/m^2)	25.5 (3.5)	25.3 (2.9)	25.6 (3.0)	0.602
Tea (mL/day)	122.9 (102.9)	390.3 (84.9)	768.7 (276.1)	0.0001
Coffee (mL/day)	538.1 (340.2)	403.1 (210.6)	364.8 (229.5)	0.0001
Fish (g/day)	22.1 (24.8)	16.2 (18.8)	17.0 (21.6)	0.002
Fruits (g/day)	177.2 (146.0)	202.4 (139.6)	222.2 (133.2)	0.0001
Vegetables (g/day)	168.8 (73.8)	173.8 (67.0)	185.8 (73.8)	0.011
Vitamin C (mg/day)	87.7 (48.3)	95.9 (42.9)	104.7 (46.6)	0.0001
Vitamin E (mg/day)	8.1 (2.6)	8.3 (2.5)	8.7 (2.6)	0.030
Beta-carotene (mg/day)	1.3 (0.7)	1.4 (0.6)	1.5 (0.6)	0.001
Saturated fatty acids (g/day)	43.7 (15.6)	43.2 (13.8)	44.3 (14.2)	0.686
Polyunsaturated fatty acids (g/day)	15.9 (8.5)	15.9 (7.8)	16.6 (8.1)	0.355
Dietary cholesterol	336.6 (117.9)	335.9 (110.7)	341.4 (127.2)	0.842
Fiber (g/day)	23.7 (7.7)	25.0 (6.5)	27.5 (7.9)	0.0001

[a] Tests any difference among catechin intake tertiles. Chi-square test for categorical variables, one-way analysis of variance for normally distributed variables (total energy intake, serum total cholesterol, body mass index, vitamin E, saturated fatty acids, dietary cholesterol, and fiber), and Kruskal-Wallis test for all other variables.

blood pressure, prevalent hypertension, or prevalent diabetes (data not shown). Baseline prevalence of MI or AP was an important determinant of mortality. Catechin intake was inversely associated with CHD mortality both in subjects free of disease at baseline and in subjects with already prevalent disease; there was no statistically significant interaction between catechin intake and baseline disease (P=0.63). When

Table 5.1.2 Risk ratios of death from coronary heart disease and fatal or non-fatal first myocardial infarction in 806 elderly men according to tertiles of total catechin intake

	Tertile of total catechin intake			P for trend
	low	middle	high	
Range of total catechin intake (mg/day)	0-49.0	49.1-85.8	85.9-355.4	
Coronary heart disease mortality				
Number of men	268	269	269	
Number of person years	1908	2039	2078	
Deaths	38	31	21	
Age adjusted RR (95% CI)[a]	1.00	0.71 (0.44-1.15)	0.48 (0.28-0.82)	0.007
RR adjusted model 1[b]	1.00	0.73 (0.45-1.19)	0.46 (0.26-0.80)	0.006
RR adjusted model 2[c]	1.00	0.76 (0.46-1.26)	0.49 (0.27-0.88)	0.017
Myocardial infarction incidence				
Number of men	230	231	230	
Number of person years	1537	1625	1646	
Cases	36	33	21	
Age adjusted RR (95% CI)[a]	1.00	0.85 (0.53-1.37)	0.54 (0.32-0.93)	0.026
RR adjusted model 1[b]	1.00	0.90 (0.56-1.46)	0.63 (0.36-1.10)	0.103
RR adjusted model 2[c]	1.00	0.96 (0.58-1.59)	0.70 (0.39-1.26)	0.232

[a] RR: risk ratio; CI: confidence interval. [b] Adjusted for prevalent myocardial infarction or angina pectoris at baseline (mortality analyses only), age, physical activity, total energy intake, body mass index, alcohol intake, smoking status. [c] Adjusted for above covariates plus: fish, coffee, saturated fatty acids, polyunsaturated fatty acids, dietary cholesterol, fiber, vitamin C, vitamin E, beta-carotene, prescribed diet.

catechin intake was modeled as a continuous variable, one standard deviation (50 mg) increase in intake was associated with a 25% decrease in risk (95% CI: 0.56-0.99). 50 mg catechins are equivalent to 1 cup of black tea (200 mL) with a small piece of dark chocolate (20 g), or to two large apples. The age-adjusted association of catechin intake with fatal or non-fatal incidence of MI was less strong than that with CHD mortality (Table 5.1.2). After adjustment for potential confounders, the risk ratio of incidence of MI in the highest tertile of catechin intake was 0.70 (95% CI: 0.39-1.26) and no longer statistically significant. Catechin intake was not associated with the risk of death from stroke, nor with the fatal or non-fatal incidence of stroke (Table 5.1.3).

Catechin intake was highly correlated with both tea consumption (r_s=0.98), and with the intake of flavonols (r_s=0.85). Therefore, it was impossible to examine the effect of catechin intake on CHD risk after adjusting for flavonol and tea intake. It was considered important, however, to examine whether flavonols, or some other component of tea could be responsible for the observed protective effect of a high catechin intake on CHD mortality, rather than catechins as such. If catechins are indeed responsible for the observed protective effect of tea, then catechins from other sources than tea would be expected to be inversely associated with CHD risk

Table 5.1.3 Risk ratios of death from stroke and fatal or non-fatal first stroke in 806 elderly men according to tertiles of total catechin intake

	Tertile of total catechin intake			P for trend
	low	middle	high	
Range of total catechin intake (mg/day)	0-49.0	49.1-85.8	85.9-355.4	
Stroke mortality				
Number of men	268	269	269	
Number of person years	1908	2039	2078	
Deaths	17	15	15	
Age adjusted RR (95% CI)[a]	1.00	0.73 (0.37-1.47)	0.74 (0.37-1.48)	0.404
RR adjusted model 1[b]	1.00	0.81 (0.39-1.65)	0.78 (0.37-1.62)	0.508
RR adjusted model 2[c]	1.00	1.02 (0.48-2.16)	0.81 (0.36-1.83)	0.606
Stroke incidence				
Number of men	255	256	256	
Number of person years	1724	1784	1824	
Cases	27	36	25	
Age adjusted RR (95% CI)[a]	1.00	1.24 (0.75-2.04)	0.85 (0.49-1.46)	0.516
RR adjusted model 1[b]	1.00	1.26 (0.76-2.09)	0.83 (0.48-1.46)	0.482
RR adjusted model 2[c]	1.00	1.40 (0.83-2.36)	0.92 (0.51-1.68)	0.749

[a] RR: risk ratio; CI: confidence interval. [b] Adjusted for prevalent stroke at baseline (mortality analyses only), age, physical activity, total energy intake, body mass index, alcohol intake, smoking status. [c] Adjusted for above covariates plus: fish, coffee, saturated fatty acids, polyunsaturated fatty acids, dietary cholesterol, fiber, vitamin C, vitamin E, beta-carotene, prescribed diet.

as well. Catechin intake from sources other than tea was relatively independent of tea intake (r_s=0.11), and of flavonol intake from sources other than tea (r_s=0.44). Also, tea intake was independent of flavonols from sources other than tea (r_s=0.08). These variables and potential confounders were entered into the model simultaneously (Table 5.1.4). Tea intake was borderline statistically significantly associated with a reduced risk for CHD death (P-value: 0.056). For catechins from sources other than tea the risk ratio was 0.80 for a standard deviation (7.5 mg) increase in intake of catechins (P-value: 0.114).

Table 5.1.4 Intake of tea, catechins from other sources than tea, and flavonols and flavones from other sources than tea, and mutually independent risk ratios of coronary heart disease mortality in 806 elderly men

	mean intake (standard deviation) (mg)	mutually independent RR model 2, per SD increase in intake[a]	P-value
Tea (mL)	427.7 (318.7)	0.78	0.056
Catechins not from tea[b] (mg)	9.5 (7.2)	0.80	0.114
Flavonols + flavones not from tea[c] (mg)	10.6 (7.6)	0.93	0.581

[a] RR: risk ratio; SD: standard deviation; adjusted for prevalent myocardial infarction or angina pectoris at baseline, age, physical activity, total energy intake, alcohol intake, smoking status, body mass index, fish, coffee, saturated fatty acids, polyunsaturated fatty acids, dietary cholesterol, fiber, vitamin C, vitamin E, beta-carotene, prescribed diet. [b] (+)-catechin, (+)-gallocatechin, (-)-epicatechin, (-)-epigallocatechin, and (-)-epicatechin gallate. [c] quercetin, kaempferol, myricetin, luteolin, and apigenin.

DISCUSSION

In this population of elderly men, catechin intake was inversely related to coronary heart disease mortality and myocardial infarction incidence. Adjusting for cardiovascular disease risk factors including diet, attenuated the association with myocardial infarction incidence, but not with mortality. We observed a statistically significant 51% lower CHD mortality risk in the highest tertile of catechin intake. The risk of stroke mortality or incidence was not associated with the intake of catechins.

We found that subjects with a high catechin intake are more likely to have never smoked, are less likely to be a current smoker, are more physically active, have a higher intake of total energy, fiber, fruits and vegetables, and a higher intake of vitamin C, E, and beta-carotene. It could therefore be hypothesized that a high catechin intake is merely an indicator of a healthy lifestyle. However, adjustment for these variables, and additional adjustment for pack-years of smoking and socio-economic status did not affect the risk ratios for CHD mortality or MI incidence, nor did an analysis restricted to former and never smokers, adjusted for all risk factors and pack-years of smoking. Nevertheless, we cannot rule out residual confounding as an explanation for the protective effect of catechin intake on CHD mortality.

The use of updated dietary data instead of baseline data only, has been advocated to reduce measurement error due to intraindividual variation.[32] In our study, dietary data were collected at baseline and in 1990. We were therefore able to perform our analyses using updated catechin intake data. The results using updated models did not essentially differ from those using baseline data only. The men in our study are relatively old, and it is possible that they therefore show little change in their dietary habits. Also, our dietary history method yielded information on the habitual diet. A validation study performed 12 months after the initial investigation showed that the reproducibility of the dietary history method was sufficient.[29]

Because of the high correlations between catechin, flavonol and tea intake, it is impossible to clearly discern their effects on CHD risk in this population. Tea consumption was inversely associated with CHD mortality. After adjustment for tea consumption, a 7.5 mg increase in catechin intake from other sources than tea was associated with a tendency for a 20% reduction in risk (P-value: 0.114). The risk reduction of a 7.5 mg increase in flavonol intake was small (7%) and far from significant. These findings suggest that catechins, whether from tea or other sources, may lower risk of CHD mortality, and that, in this population, catechins may be of more importance than flavonols. Including catechins from other sources than tea

might clarify the inconsistencies in reported health effects of tea, particularly in populations where tea drinking is relatively low.[6,7,13,15]

The results of the present study can, however, not explain the lack of effect of tea consumption reported in the United Kingdom by Woodward and Tunstall-Pedoe[8] and the increased risk reported by Hertog and colleagues.[9] Black tea consumption in these populations is twice that of the intake in our study, and it is therefore unlikely that catechins from other sources than tea play a major role. It has been suggested that the addition of milk to tea, a habit which is common in the United Kingdom, but not in the Netherlands, could explain the lack of a protective effect in the UK studies.[9] However, milk proteins do not impede the absorption of catechins from the gut,[33] which makes this explanation unlikely. The catechin content of tea infusions is influenced by brewing method, and type of tea used. These differ notably between countries, and may partially explain the reported differences in effect. Another explanation for the UK findings could be residual confounding. In contrast to most other countries, tea consumption in the United Kingdom is positively associated with a less healthy lifestyle (e.g., smoking and fat intake) and with lower social class.[8,9] Residual confounding by inaccurately measured or unmeasured confounders has been suggested as a likely explanation for the reported increased risk of ischemic heart disease in the Caerphilly Study.[9]

Studies on flavonols have reported a slightly stronger protective effect on CHD mortality than on MI incidence[2] or a trend towards a protective effect on CHD mortality limited to those who had previously had cardiovascular disease.[15] As a consequence, it was suggested that flavonols could possibly influence coronary heart disease through platelet aggregation and thrombosis, rather than through reducing atherosclerosis. In our study, we find a similar result for catechins. However, tea has been found to protect against the development of severe atherosclerosis, assessed by radiographic films of the abdomen, in a population-based follow-up study among more than 6000 men and women.[4] Also, we did not find an effect of catechins on stroke risk, which would be expected if platelet aggregation and thrombosis are involved in the causal pathway. An alternative explanation of our findings may therefore be that causes of death may have been recorded more reliably than non-fatal events, resulting in larger measurement error, and thus weaker associations. We made great effort to confirm morbidity data from questionnaires with hospital discharge data or written information from the general practitioner, but cannot completely disregard this as an explanation. The number of cases in our study is relatively small, and misclassification of a few cases may have attenuated the strength of the association.

To our knowledge, Keli and coworkers,[3] using data from the original Zutphen Study cohort, were the first to report on stroke risk and tea or flavonol intake. They found an inverse association between tea and flavonol intake, and incident stroke. We did not find such an effect for catechins in the Zutphen Elderly Study. The power of picking up an effect of catechin intake on stroke risk was low, because of the small number of cases of both incident and fatal stroke, but this was also the case in Keli's study. Possibly, the older age of the men in our study plays a role in observed differences, but more research is needed to clarify the relation between stroke risk and intake of flavonoids.

The present prospective study is the first on catechin intake and cardiovascular diseases. In our study among elderly men in the Netherlands, catechin intake was inversely associated with coronary heart disease mortality, but not with myocardial infarction incidence or stroke. The results suggest that catechins, rather than flavonols, could explain the inverse relation between tea consumption and coronary heart disease. However, our study has only a limited ability to discern the effects of catechins, flavonols, and tea. More research is needed to verify our results, in particular in populations with a lower intake of tea, in order to determine whether catechins, or other constituents of tea, are indeed protective against coronary heart disease.

ACKNOWLEDGMENTS

This work was carried out with financial support from the Commission of the European Communities, Agriculture and Fisheries (FAIR) specific RTD programme, CT95 0653, and The Netherlands Prevention Foundation.

REFERENCES

1. Stensvold I, Tverdal A, Solvoll K, Foss OP. Tea consumption. relationship to cholesterol, blood pressure, and coronary and total mortality. *Prev Med* 1992;21:546-553.
2. Hertog MGL, Feskens EJ, Hollman PCH, Katan MB, Kromhout D. Dietary antioxidant flavonoids and risk of coronary heart disease: the Zutphen Elderly Study. *Lancet* 1993;342:1007-1011.
3. Keli SO, Hertog MGL, Feskens EJ, Kromhout D. Dietary flavonoids, antioxidant vitamins, and incidence of stroke: the Zutphen study. *Arch Intern Med* 1996;156:637-642.
4. Geleijnse JM, Launer LJ, Hofman A, Pols HA, Witteman JC. Tea flavonoids may protect against atherosclerosis: the Rotterdam Study. *Arch Intern Med* 1999;159:2170-2174.
5. Sesso HD, Gaziano JM, Buring JE, Hennekens CH. Coffee and tea intake and the risk of myocardial infarction. *Am J Epidemiol* 1999;149:162-167.
6. Klatsky AL, Friedman GD, Armstrong MA. Coffee use prior to myocardial infarction restudied: heavier intake may increase the risk. *Am J Epidemiol* 1990;132:479-488.
7. Klatsky AL, Armstrong MA, Friedman GD. Coffee, tea, and mortality. *Ann Epidemiol* 1993;3:375-381.

8. Woodward M, Tunstall-Pedoe H. Coffee and tea consumption in the Scottish Heart Health Study follow up: conflicting relations with coronary risk factors, coronary disease, and all cause mortality. *J Epidemiol Community Health* 1999;53:481-487.
9. Hertog MGL, Sweetnam PM, Fehily AM, Elwood PC, Kromhout D. Antioxidant flavonols and ischemic heart disease in a Welsh population of men: the Caerphilly Study. *Am J Clin Nutr* 1997;65:1489-1494.
10. Harborne JB. *The Flavonoids: Advances in research since 1986.* London: Chapman & Hall; 1994.
11. Hertog MGL, Hollman PCH, Van de Putte B. Content of potentially anticarcinogenic flavonoids of tea infusions, wines, and fruit juices. *J Agric Food Chem* 1993;41:1242-1246.
12. Hertog MGL, Hollman PCH, Katan MB. Content of potentially anticarcinogenic flavonoids of 28 vegetables and 9 fruits commonly consumed in the Netherlands. *J Agric Food Chem* 1992;40:2379-2383.
13. Yochum L, Kushi LH, Meyer K, Folsom AR. Dietary flavonoid intake and risk of cardiovascular disease in postmenopausal women. *Am J Epidemiol* 1999;149:943-949.
14. Knekt P, Järvinen R, Reunanen A, Maatela J. Flavonoid intake and coronary mortality in Finland: A cohort study. *BMJ* 1996;312:478-481.
15. Rimm EB, Katan MB, Ascherio A, Stampfer MJ, Willett WC. Relation between intake of flavonoids and risk for coronary heart disease in male health professionals. *Ann Intern Med* 1996;125:384-389.
16. Harbowy ME, Balentine DA. Tea chemistry. *Crit Rev Plant Sciences* 1997;16:415-480.
17. Middleton E, Kandaswami C. The impact of plant flavonoids on mammalian biology: implications for immunity, inflammation and cancer. In: Harborne JB, ed. *The Flavonoids: advances in research since 1986.* London: Chapman & Hall;1994:619-652.
18. Rice-Evans CA, Miller NJ, Paganga G. Structure-antioxidant activity relationships of flavonoids and phenolic acids. *Free Radical Biol Med* 1996;20:933-956.
19. Wiseman SA, Balentine DA, Frei B. Antioxidants in tea. *Crit Rev Food Sci Nutr* 1997;37:705-718.
20. Zhu QY, Huang Y, Tsang D, Chen ZY. Regeneration of alpha-tocopherol in human low-density lipoprotein by green tea catechin. *J Agric Food Chem* 1999;47:2020-2025.
21. Packer L. Vitamin E and the antioxidant network: protection of human Low Density Lipoprotein from oxidation. In: Ohigashi H, Osawa T, Terao J, Watanabe S, Yoshikawa T, eds. *Food factors for cancer prevention.* Tokyo: Springer-Verlag;1997:452-459.
22. Ross R. Mechanisms of disease - Atherosclerosis - An inflammatory disease. *N Engl J Med* 1999;340:115-126.
23. Middleton E. Effect of plant flavonoids on immune and inflammatory cell function. *Adv Exp Med Biol* 1998;439:175-182.
24. Wollny T, Aiello L, Di Tommaso D, Bellavia V, Rotilio D, Donati MB, De Gaetano G, Iacoviello L. Modulation of haemostatic function and prevention of experimental thrombosis by red wine in rats: a role for increased nitric oxide production. *Br J Pharmacol* 1999;127:747-755.
25. Arts ICW, Hollman PCH. Optimization of a quantitative method for the determination of catechins in fruits and legumes. *J Agric Food Chem* 1998;46:5156-5162.
26. Arts ICW, Van de Putte B, Hollman PCH. Catechin contents of foods commonly consumed in The Netherlands. 1. Fruits, vegetables, staple foods, and processed foods. *J Agric Food Chem* 2000;48:1746-1751.
27. Arts ICW, Van de Putte B, Hollman PCH. Catechin contents of foods commonly consumed in The Netherlands. 2. Tea, wine, fruit juices, and chocolate milk. *J Agric Food Chem* 2000;48:1752-1757.
28. Caspersen CJ, Bloemberg BP, Saris WH, Merritt RK, Kromhout D. The prevalence of selected

physical activities and their relation with coronary heart disease risk factors in elderly men: the Zutphen Study, 1985. *Am J Epidemiol* 1991;133:1078-1092.

29. Bloemberg BP, Kromhout D, Obermann-De Boer GL, Van Kampen-Donker M. The reproducibility of dietary intake data assessed with the cross-check dietary history method. *Am J Epidemiol* 1989;130:1047-1056.
30. Kommissie UCV. *UCV Tabel: Uitgebreide voedingsmiddelentabel 1985 [in Dutch].* Den Haag: Voorlichtingsbureau voor de Voeding (Nutrition Education Bureau); 1985.
31. World Health Organization. *Manual of the international statistical classification of diseases, injuries, and causes of death. Ninth Revision, 1975.* Geneva: WHO; 1977.
32. Hu FB, Stampfer MJ, Rimm E, Ascherio A, Rosner BA, Spiegelman D, Willett WC. Dietary fat and coronary heart disease: a comparison of approaches for adjusting for total energy intake and modeling repeated dietary measurements. *Am J Epidemiol* 1999;149:531-540.
33. Van het Hof KH, Kivits GAA, Weststrate JA, Tijburg LBM. Bioavailability of catechins from tea: the effect of milk. *Eur J Clin Nutr* 1998;52:356-359.

Dietary catechins and coronary heart disease mortality: the Iowa Women's Health Study

The objective of this study was to assess whether the intake of catechins was inversely associated with the risk of coronary heart disease death in a prospective study of postmenopausal women from Iowa. Between 1986 and 1998, 767 of 34,492 participants initially free of cardiovascular disease died from coronary heart disease. There was a strong inverse association between the intake of (+)-catechin and (-)-epicatechin and coronary heart disease death, which was somewhat attenuated after multivariate adjustment (risk ratios from lowest to highest quintile: 1.00, 0.95, 0.97, 0.77, 0.76). This inverse association was most pronounced in women at low risk of coronary heart disease (non-smokers, free of diabetes and cardiovascular disease). A high intake of 'gallates', catechins typical of tea, was not associated with coronary heart disease death. Of the major catechin sources, apples and wine were inversely associated with coronary heart disease death. Our data suggest that preventive effects might be limited to certain types of catechins, or that they are indicators of other dietary components or a healthy lifestyle in general.

Arts ICW, Jacobs Jr DR, Harnack LJ, Gross M, Folsom AR. Dietary catechins in relation to coronary heart disease death among postmenopausal women. *Epidemiology; in press.*

INTRODUCTION

Catechins, one of the six major groups of flavonoids, are bioactive compounds present in a variety of plant foods and beverages. *In vitro* and animal experiments suggest that they might help to prevent chronic diseases in humans.[1] The catechins may reduce cardiovascular disease risk through several mechanisms. Through their antioxidant activity, catechins could reduce LDL oxidation either by quenching free radicals, chelating metals, or recycling other antioxidants such as vitamin E.[2-5] Catechins have also been shown to interfere with several stages of the inflammatory process,[6] and to reduce thrombosis.[7,8] A recent epidemiological cohort study among elderly men in the Netherlands supported the experimental evidence by showing a strong inverse association between the intake of catechins and the risk of coronary heart disease (CHD) mortality.[9] In the Dutch study, tea was the major source of catechins, which made it difficult to determine whether catechins versus other tea constituents were responsible for the observed association. Moreover, tea has been associated with a healthy lifestyle in some countries such as the Netherlands, but not in others such as the UK.[10] Prospective studies on the relation between tea consumption and the risk of cardiovascular disease have provided inconsistent results,[11-14] which were partly attributed to residual confounding by clustering of health-conscious behaviors.

We wanted to test the hypothesis that the intake of catechins is inversely associated with the risk of CHD death, independent of the catechin source. Moreover, levels of oxidative stress, resulting from an increased generation of reactive oxygen species, are known to be elevated in pathological states such as diabetes and rheumatoid arthritis. In the human body, enzymic antioxidant defense systems are operative, and dietary antioxidants may be necessary mostly to supplement these systems in subjects with elevated levels of oxidative stress.[15] Previous epidemiological studies have suggested that the protective effects of flavonoids may be limited to subjects that are at high risk of dying from cardiovascular causes because of prevalent disease[16] or the presence of severe atherosclerosis.[11] Our secondary objective was therefore to assess whether a high intake of catechins was more strongly associated with CHD death in subjects who smoked at baseline, or had prevalent diabetes, or cardiovascular disease, compared to apparently healthy, low risk, subjects.

METHODS

Study population

The Iowa Women's Health Study was approved by the Committee on the Use of

Human Subjects in Research, University of Minnesota. In January 1986, a 16-page survey was sent to a random sample of 99,826 women aged 55-69 years who had a valid Iowa driver's license. 41,836 women (42 percent) returned the questionnaire, and were enrolled in the study. We excluded women from the analyses if they were not postmenopausal at baseline (n = 569), or if they left > 30 items blank on the food frequency questionnaire or had improbably high (5,000 Kcal) or low (< 600 Kcal) energy intakes (n = 3096). Also, women who reported having cardiovascular disease at baseline, defined as having been told by a doctor that they had suffered a heart attack or had angina or other heart disease (n = 4115), were excluded from all analyses, except for analyses concerning women at high risk. This left 34,492 women for analysis; due to missing covariates this number was reduced to 32,857 in the multivariate analysis. We considered women to be at high risk of death from CHD if they reported at baseline that they had been told by a doctor they had diabetes (yes or unsure) or were taking diabetes medication (n = 1991), had suffered a heart attack or had angina or other heart disease (n = 3713), or were smoking at the time of the baseline examination (n = 5177). Numbers excluded are not mutually exclusive.

Data collection

The baseline questionnaire included questions regarding known or suspected CHD risk factors, as well as a 127-item semiquantitative food frequency questionnaire similar to that used in the 1984 survey of the Nurses' Health Study.[17] Although this questionnaire was not validated for its ability to assess catechin intake, Feskanich and co-authors[18] assessed the ability of the questionnaire to determine the intake of foods compared to a 28-day diet record among male health professionals. Correlation coefficients for the main sources of catechins were: 0.77 for tea, 0.70 for apples, 0.45 – 0.83 for chocolate containing foods, and 0.83 for red and 0.78 for white wine. Moreover, no important dietary sources of catechins were omitted. We weighted catechin contents of questionnaire items that referred to more than one food, e.g. 'fresh apples or pears', using US food disappearance data for the year 1986.[19] We used data on catechin contents of Dutch foods and beverages,[20,21] supplemented with analyses of US tea, apples, chocolate, beans, and lentils done at the same laboratory in the Netherlands. Catechin contents of US foods were similar to comparable Dutch foods, except for US apples, which tended to have higher catechin levels than those from the Netherlands.

Case ascertainment

The women were followed annually through the State Health Registry of Iowa, which

collects information on deaths in Iowa. Deaths were also reported in response to follow-up questionnaires mailed in 1987, 1989, 1992, and 1997. For non-responders not found in the Health Registry, the occurrence and causes of death were obtained from the National Death Index. Coding of the underlying cause of death followed the ninth Revision of the International Classification of Diseases.[22] Underlying ICD-codes 410-414 and 429.2 were taken as CHD.

Statistical analysis

We defined total catechin as the sum of six major catechins: (+)-catechin, (+)-gallocatechin, (-)-epicatechin, (-)epigallocatechin, (-)-epicatechin gallate, and (-)-epigallocatechin gallate. We created two subgroups of catechins, the first one reflecting catechins derived mainly or solely from tea (the sum of (+)-gallocatechin, (-)-epigallocatechin, (-)-epicatechin gallate, and (-)-epigallocatechin gallate), further referred to as 'gallates', and the second one reflecting catechins derived mainly from sources other than tea (the sum of (+)-catechin and (-)-epicatechin). We calculated the intake of catechins, 'gallates' and the sum of (+)-catechin and (-)-epicatechin for each woman and stratified by quintiles of intake. Forty three percent of the population did not drink tea, and their catechin intake was therefore completely derived from sources other than tea. We adjusted all baseline means of dietary characteristics for total energy intake using linear regression.

We combined questionnaire food items that contained catechins to form six groups: tea, apples and pears, chocolate, wine, legumes, and fruits other than apples or pears. We combined items by converting each individual's response to an item into milligrams of catechins per day, and taking the sum of these values. Values for all groups except tea were based on more than one food frequency questionnaire item. Apples combined the items "fresh apples or pears" and "applesauce"; chocolate combined "chocolate", "candy bars", "cookies", "brownies" and "cake"; wine combined "red wine" and "white wine"; legumes combined "beans or lentils" and "fava beans"; and other fruits combined "raisins or grapes", "peaches, apricots or plums", "strawberries", "blueberries", "other fruit juices", and several fruits that were not included on the printed questionnaire, but were reported in an "other foods" category as eaten at least once a week.

We calculated the follow-up time for each participant from completion of the baseline questionnaire until the day of death or December 31, 1998, whichever came first. We estimated risk ratios of death from CHD by Cox proportional hazards analysis, using the SAS procedure PHREG (SAS, release 6.12, SAS Institute, North Carolina, USA).

We adjusted the initial analyses for age and total energy intake only. We adjusted the multivariate models also for marital status (yes or no), educational attainment (did not graduate from high school, high school graduate, more than high school education), self reported high blood pressure (yes or no), self reported diabetes (yes/unsure or no), body mass index (quintiles), waist-to-hip ratio (quintiles), physical activity (low, moderate, or high, based on the frequency and intensity of leisure activity), pack-years of smoking (none, 1-19, 20-39, or ≥40), use of estrogen replacement therapy (current, former, never), use of vitamin supplements (yes or no), alcohol intake, intake of whole grains (quintiles), intake of saturated fatty acids, intake of polyunsaturated fatty acids, intake of cholesterol, and intake of dietary vitamin C, vitamin E, folate, and carotene. We did not have information on serum cholesterol or triglyceride levels. Probability values for a trend in risk ratios were derived based on category medians.

RESULTS

The average intake of catechins in this population of 34,492 postmenopausal women was 25.4 mg/day (standard deviation (SD): 32.0). The intake ranged from 0 (24 participants) to 278 mg/day. A high intake of catechins was associated with lifestyle and dietary habits that are known to be associated with a lower risk of CHD (Table 5.2.1). Women in the higher quintiles of catechin intake tended to smoke less, exercise more, use vitamin supplements more often, and have a higher level of education. Their dietary pattern was also more favorable, with a lower energy adjusted intake of alcohol, saturated fatty acids, and cholesterol, and a higher intake of whole grains, and dietary vitamin C, E, folate and carotenoids.

After 13 years of follow-up (422,648 person-years) 767 participants had died from CHD. Table 5.2.2 shows age- and energy-adjusted risk ratios of CHD death by quintiles of catechin intake. The risk ratio for women in the second quintile of catechin intake was lower (risk ratio: 0.73; 95% confidence interval: 0.58-0.90) than that for women in the lowest quintile. After the second quintile, the risk ratios did not decrease much further. After adjusting for lifestyle and dietary potential confounders, the inverse association was attenuated. The risk ratios from the lowest to the highest category of intake were 1.00, 0.80, 0.86, 0.83, and 0.85. Table 5.2.2 also shows the risk ratios of CHD death by quintiles of (+)-catechin plus (-)-epicatechin and by quintiles of 'gallates', both adjusted for age and total energy intake and for each other. There was a striking difference in association for the two subgroups: the 'gallates' were not clearly associated with CHD death, but there was a strong inverse association between the intake of (+)-catechin plus (-)-epicatechin

Table 5.2.1 Baseline characteristics by quintiles of catechin intake for 34,492, cardiovascular disease free, postmenopausal women, 1986. Iowa Women's Health Study

	Catechin intake quintile				
	1	2	3	4	5
Flavonoid intake					
Total catechins (mg/day)[a]	3.6 (1.6)[b]	8.8 (1.3)	14.9 (2.2)	24.8 (5.2)	74.8 (42.3)
(+)-catechin	1.4 (0.7)	2.5 (0.9)	3.6 (1.2)	4.5 (2.2)	6.1 (3.1)
(-)-epicatechin	1.9 (0.9)	5.1 (1.7)	8.9 (3.0)	11.2 (4.2)	18.1 (10.0)
(+)-gallocatechin	0.0 (0.1)	0.1 (0.1)	0.2 (0.2)	0.6 (0.5)	3.5 (2.6)
(-)-epigallocatechin	0.0 (0.1)	0.1 (0.2)	0.2 (0.3)	1.0 (0.8)	5.5 (4.1)
(-)-epicatechin gallate	0.2 (0.4)	0.6 (0.7)	1.1 (1.5)	4.3 (3.5)	23.9 (17.9)
(-)-epigallocatechin gallate	0.1 (0.3)	0.4 (0.5)	0.8 (1.1)	3.2 (2.6)	17.7 (13.3)
Flavonols + flavones (mg/day)	5.3 (3.4)	7.8 (4.2)	10.7 (4.6)	14.5 (6.5)	31.3 (15.5)
Demographics					
Age (y)	61.3 (4.2)	61.5 (4.2)	61.4 (4.1)	61.7 (4.2)	61.6 (4.2)
Education (% > high school)	34.0	38.7	41.5	42.8	43.2
Anthropometry					
Body mass index (kg/m^2)	26.8 (5.1)	27.0 (5.1)	27.0 (5.0)	26.9 (5.0)	26.9 (5.1)
Waist-to-hip ratio	0.843 (0.092)	0.836 (0.084)	0.833 (0.085)	0.834 (0.083)	0.836 (0.084)
Self-reported illness					
Diabetes (%)	5.7	5.5	5.8	5.4	6.5
Hypertension (%)	34.8	34.8	34.1	34.2	34.2
Cardiovascular disease (%)[c]	10.4	10.0	9.3	9.5	9.4
Lifestyle behaviors					
Current smoker (%)	25.1	16.7	12.1	10.4	11.8
Pack-years of smoking	14.2 (20.8)	9.7 (17.7)	7.8 (16.0)	7.0 (15.4)	8.0 (16.6)
Vitamin supplement use (%)	29.8	32.7	34.1	34.9	32.9
Alcohol (% never drink)	52.5	53.9	54.5	53.4	57.1
Physical activity (% engaging in moderate to high activity)	42.6	50.6	56.3	58.7	55.0
Hormone replacement therapy (% ever)	37.7	37.8	38.5	38.2	38.5
Diet[d]					
Energy intake (kcal/day)	1,526 (492.4)	1,715 (541.5)	1,845 (584.6)	1,973 (638.5)	1,946 (649.2)
Alcohol intake (g/day)	5.9	4.0	3.3	2.9	3.2
Saturated fat intake (g/day)	25.6	24.6	23.8	23.2	23.4
Polyunsaturated fat intake (g/day)	12.1	12.1	12.1	12.0	12.0
Cholesterol intake (mg/day)	286	276	273	267	268
Whole grain intake (servings/week)	10.0	11.0	11.7	12.1	11.8
Dietary vitamin C intake (mg/day)	133	149	161	167	163
Dietary vitamin E intake (mg/day)	7.6	8.1	8.5	8.7	8.6
Dietary folate intake (µg/day)	286	301	314	322	336
Dietary carotenoid intake (IU/day)	7,419	8,610	9,482	10,085	9,831

[a] Sum of: (+)-catechin, (-)-epicatechin, (+)-gallocatechin, (-)-epigallocatechin, (-)-epicatechin gallate, and (-)-epigallocatechin gallate. [b] Values are means (standard deviation) or percentages where indicated. [c] Includes women with self-reported cardiovascular disease at baseline, n = 38,205. [d] All diet means, except energy intake, were adjusted for total energy intake.

Table 5.2.2 Risk ratios of coronary heart disease (CHD) death by quintiles of catechin intake for 34,492, cardiovascular disease free, postmenopausal women, 1986-1998. Iowa Women's Health Study

	Catechin intake quintile					P for trend
	1	2	3	4	5	
Total catechins						
Median intake (range) mg/day	3.7 (0-6.3)	8.7 (6.3-11.2)	14.8 (11.2-18.5)	23.5 (18.5-36.8)	52.7 (36.8-277.7)	
CHD deaths	186	143	148	140	150	
Person-years	83,563	84,599	84,885	84,975	84,626	
RR (95% CI)[a]	1.00	0.73 (0.58-0.90)	0.74 (0.60-0.92)	0.66 (0.53-0.83)	0.73 (0.59-0.91)	0.08
RR (95% CI) multivariate[b]	1.00	0.80 (0.63-1.00)	0.86 (0.69-1.09)	0.83 (0.66-1.06)	0.85 (0.67-1.07)	0.50
Sum of (+)-catechin and (-)-epicatechin[c]						
Median intake (range) mg/day	3.2 (0-4.8)	7.0 (4.8-8.8)	10.4 (8.8-12.8)	15.9 (12.8-18.6)	23.1 (18.6-124.8)	
CHD deaths	184	162	147	130	144	
Person-years	83,691	84,518	84,734	84,881	84,823	
RR (95% CI)	1.00	0.86 (0.69-1.07)	0.76 (0.60-0.96)	0.64 (0.50-0.82)	0.65 (0.50-0.85)	0.0002
RR (95% CI) multivariate	1.00	0.95 (0.76-1.20)	0.97 (0.76-1.24)	0.77 (0.60-1.00)	0.76 (0.58-1.03)	0.02
'Gallates'[c,d]						
Median intake (range) mg/day	0.0 (0-0.02)	0.05 (0.03-0.2)	2.1 (0.2-3.7)	12.5 (4.2-22.9)	29.2 (22.9-176.5)	
CHD deaths	174	155	134	148	156	
Person-years	76,119	93,027	81,502	88,512	83,487	
RR (95% CI)	1.00	0.72 (0.58-0.90)	0.71 (0.57-0.90)	0.75 (0.59-0.94)	0.93 (0.73-1.20)	0.21
RR (95% CI) multivariate	1.00	0.78 (0.62-0.98)	0.78 (0.62-0.99)	0.85 (0.67-1.09)	1.00 (0.77-1.29)	0.16

[a] RR: risk ratio; CI: confidence interval; adjusted for age and total energy intake (n = 34,492). [b] Adjusted for age, total energy intake, marital status, educational attainment, high blood pressure, diabetes, body mass index, waist-to-hip ratio, physical activity, pack-years of smoking, use of estrogen replacement therapy, use of vitamin supplements, alcohol intake, intake of whole grains, intake of saturated fatty acids, intake of polyunsaturated fatty acids, intake of cholesterol, and intake of dietary vitamin C, vitamin E, folate, and carotene (n = 32,857). [c] Risk ratios also adjusted for other catechins listed in table. [d] Sum of (+)-gallocatechin, (-)-epigallocatechin, (-)-epicatechin gallate, and (-)-epigallocatechin gallate.

and CHD death (risk ratios from lowest to highest quintile of intake: 1.00, 0.86, 0.76, 0.64, and 0.65). After multivariate adjustment the association was attenuated, but an inverse trend remained.

The major sources of catechins were tea, which provided 59 percent of the total catechin intake in this population, apples and pears (26 percent), chocolate (6 percent), and fruits other than apples or pears (5 percent). Wine and legumes contributed little to the average catechin intake. The total intake of catechins among women drinking tea was 36.9 mg/day compared to 10.0 mg/day for women not drinking tea (Table 5.2.3). Tea drinkers obtained on average 55 percent of their catechin intake from tea; their intake of catechins from sources other than tea was only slightly higher than that of women not drinking tea (Table 5.2.3). The intake of (-)-epigallocatechin gallate and (-)-epigallocatechin was zero in non-tea drinkers because the occurrence of these catechins is limited to tea. (-)-Epigallocatechin is, apart from tea, also present in certain types of legumes,[20] but these particular types (broad bean and marrowfat pea) were not ascertained by the food frequency questionnaire. Tea drinking was associated with a healthy lifestyle and a diet that was relatively high in vitamins and whole grains, and low in saturated fat and alcohol (Table 5.2.3).

In Table 5.2.4 risk ratios of CHD death are presented for tertiles of catechins from various sources, adjusted for the other sources listed in the table. In the age and energy adjusted model, catechins from apples, chocolate and wine were, independent of each other, inversely associated with CHD death. After multivariate adjustment, only apples (risk ratios from lowest to highest tertile of intake: 1.00, 0.95, and 0.78), and wine (risk ratios from lowest to highest tertile of intake: 1.00, 0.73, and 0.77) remained inversely associated with CHD death. However, almost 70 percent of the participants never drank wine, and even among wine drinkers, wine generally contributed only marginally to the total catechin intake. Catechins from tea, with non-tea drinkers as the reference category, were not associated with CHD death, and neither were catechins from legumes, or fruits other than apples or pears. Similar results were obtained when the numbers of servings of the foods themselves were entered into the models, rather than the levels of catechins (data not shown).

Table 5.2.5 shows risk ratios of CHD death for women at high risk and for women at low risk by quintiles of intake of (+)-catechin plus (-)-epicatechin (top panel), and 'gallates' (bottom panel). Contrary to our hypothesis, there was an inverse trend between the intake of (+)-catechin and (-)-epicatechin and CHD death in the women at low risk only. The association showed an inconsistent dose response: only the

Table 5.2.3 Baseline characteristics by tea drinking status for 34,492, cardiovascular disease free, postmenopausal women, 1986. Iowa Women's Health Study

	Non drinkers of tea (n=14,768)	Tea drinkers (n=19,724)
Flavonoid intake		
Total catechins (mg/day)[a]	10.0 (8.5)[b]	36.9 (37.8)
(+)-catechin	3.0 (2.3)	4.1 (2.5)
(-)-epicatechin	6.9 (6.6)	10.7 (7.8)
(+)-gallocatechin	0.01 (0.02)	1.5 (2.1)
(-)-epigallocatechin	0 (0)	2.4 (3.3)
(-)-epicatechin gallate	0.04 (0.1)	10.5 (14.6)
(-)-epigallocatechin gallate	0 (0)	7.8 (10.9)
Catechins from tea (mg/day)	0 (0)	26.3 (36.8)
Catechins not from tea (mg/day)	10.0 (8.5)	10.7 (8.0)
Flavonols + flavones (mg/day)	8.3 (6.5)	18.1 (13.8)
Demographics		
Age (y)	61.5 (4.2)	61.6 (4.2)
Education (% > high school)	36.1	43.0
Anthropometry		
Body mass index (kg/m^2)	26.8 (5.1)	27.0 (5.0)
Waist-to-hip ratio	0.837 (0.088)	0.836 (0.083)
Self-reported illness		
Diabetes (%)	5.8	5.8
Hypertension (%)	34.3	34.5
Cardiovascular disease (%)[c]	10.1	9.4
Lifestyle behaviors		
Current smoker (%)	18.3	12.9
Pack-years of smoking	11.0 (19.0)	8.1 (16.4)
Vitamin supplement use (%)	32.4	33.2
Alcohol (% never drink)	55.5	53.3
Physical activity (% engaging in moderate to high activity)	50.5	54.2
Hormone replacement therapy (% ever)	36.7	39.2
Diet[d]		
Energy intake (kcal/day)	1,736 (604.3)	1,850 (604.4)
Alcohol intake (g/day)	4.3	3.5
Saturated fat intake (g/day)	24.4	23.9
Polyunsaturated fat intake (g/day)	12.0	12.1
Cholesterol intake (mg/day)	275	274
Whole grain intake (servings/week)	11.0	11.6
Dietary vitamin C intake (mg/day)	150	158
Dietary vitamin E intake (mg/day)	8.2	8.4
Dietary folate intake (µg/day)	300	321
Dietary carotenoid intake (IU/day)	8,650	9,411

[a] Sum of: (+)-catechin, (-)-epicatechin, (+)-gallocatechin, (-)-epigallocatechin, (-)-epicatechin gallate, and (-)-epigallocatechin gallate. [b] Values are means (standard deviation) or percentages where indicated. [c] Includes women with self-reported cardiovascular disease at baseline, n = 38,205. [d] All diet means, except energy intake, were adjusted for total energy intake.

Table 5.2.4 Risk ratios of coronary heart disease (CHD) death according to intake of catechins from selected foods for 34,492, cardiovascular disease free, postmenopausal women, 1986-1998. Iowa Women's Health Study

Catechin source	Catechin intake category			P for trend
	1	2	3	
Tea				
Median catechin intake (range) mg/day	0	2.5 (2.5-5.0)	34.6 (14.9-207.9)	
CHD deaths	351	177	239	
Person-years	180,264	111,563	130,821	
RR (95% CI)[a]	1.00	0.86 (0.71-1.03)	0.94 (0.80-1.12)	0.87
RR (95% CI) multivariate[b]	1.00	0.89 (0.73-1.08)	0.99 (0.83-1.18)	0.70
Apples and pears				
Median catechin intake (range) mg/day	1.1 (0-1.7)	6.6 (2.2-6.6)	15.5 (6.7-93.2)	
CHD deaths	228	374	165	
Person-years	104,464	212,400	105,785	
RR (95% CI)	1.00	0.82 (0.70-0.98)	0.67 (0.54-0.83)	0.0001
RR (95% CI) multivariate	1.00	0.95 (0.79-1.14)	0.78 (0.62-0.98)	0.02
Chocolate				
Median catechin intake (range) mg/day	0.2 (0-0.5)	0.9 (0.6-1.2)	2.3 (1.3-49.7)	
CHD deaths	301	238	228	
Person-years	140,414	141,385	140,849	
RR (95% CI)	1.00	0.76 (0.64-0.90)	0.68 (0.56-0.81)	0.0001
RR (95% CI) multivariate	1.00	0.94 (0.78-1.13)	0.88 (0.71-1.08)	0.23
Wine				
Median catechin intake (range) mg/day	0	0.2 (0.04-0.5)	0.7 (0.6-36.5)	
CHD deaths	606	79	82	
Person-years	293,169	61,964	67,515	
RR (95% CI)	1.00	0.65 (0.51-0.82)	0.63 (0.50-0.79)	0.0001
RR (95% CI) multivariate	1.00	0.73 (0.57-0.94)	0.77 (0.59-0.98)	0.01
Legumes				
Median catechin intake (range) mg/day	0	0.2	0.4 (0.4-16.7)	
CHD deaths	290	328	149	
Person-years	162,482	172,388	87,778	
RR (95% CI)	1.00	1.11 (0.94-1.30)	0.95 (0.77-1.16)	0.70
RR (95% CI) multivariate	1.00	1.14 (0.97-1.35)	0.97 (0.78-1.21)	0.93
Other fruits				
Median catechin intake (range) mg/day	0.4 (0-0.5)	0.8 (0.6-1.1)	2.0 (1.2-39.2)	
CHD deaths	258	237	272	
Person-years	133,910	150,405	138,334	
RR (95% CI)	1.00	0.86 (0.72-1.04)	0.98 (0.82-1.18)	0.92
RR (95% CI) multivariate	1.00	0.92 (0.76-1.11)	1.07 (0.88-1.31)	0.30

[a] RR: risk ratio; CI: confidence interval; adjusted for other foods listed in table, age, and total energy intake (n = 34,492). [b] Adjusted for other foods listed in table, age, total energy intake, marital status, educational attainment, high blood pressure, diabetes, body mass index, waist-to-hip ratio, physical activity, pack-years of smoking, use of estrogen replacement therapy, use of vitamin supplements, alcohol intake, intake of whole grains, intake of saturated fatty acids, intake of polyunsaturated fatty acids, intake of cholesterol, and intake of dietary vitamin C, vitamin E, folate, and carotene (n = 32,857).

Table 5.2.5 Risk ratios of coronary heart disease (CHD) death among high and low risk groups by quintiles of catechin intake for 38,205 postmenopausal women, including women with prevalent cardiovascular disease at baseline, 1986-1998

	Quintile of intake					P for trend
	1	2	3	4	5	
Sum of (+)-catechin and (-)-epicatechin						
Low risk group[a]						
CHD deaths	79	85	93	61	96	
Person-years	58,749	66,392	72,564	71,638	72,373	
RR (95% CI)[b]	1.00	0.87 (0.64-1.20)	0.83 (0.60-1.14)	0.54 (0.37-0.77)	0.74 (0.52-1.07)	0.02
RR (95% CI) multivariate[c]	1.00	0.90 (0.65-1.25)	0.88 (0.63-1.23)	0.54 (0.37-0.80)	0.76 (0.52-1.12)	0.03
High risk group [a]						
CHD deaths	196	149	109	129	110	
Person-years	33,014	26,252	20,773	21,749	21,060	
RR (95% CI)	1.00	0.95 (0.77-1.19)	0.88 (0.69-1.13)	0.94 (0.74-1.20)	0.83 (0.62-1.10)	0.22
RR (95% CI) multivariate	1.00	0.94 (0.74-1.18)	0.96 (0.73-1.24)	1.00 (0.77-1.29)	0.85 (0.63-1.15)	0.49
'Gallates' [d]						
Low risk group						
CHD deaths	70	74	85	93	92	
Person-years	55,888	75,542	66,722	72,299	71,265	
RR (95% CI)	1.00	0.74 (0.53-1.03)	0.96 (0.69-1.33)	0.98 (0.71-1.36)	1.07 (0.75-1.53)	0.12
RR (95% CI) multivariate	1.00	0.71 (0.50-1.01)	0.99 (0.70-1.38)	1.03 (0.74-1.44)	1.10 (0.76-1.59)	0.08
High risk group						
CHD deaths	174	161	107	115	136	
Person-years	28,112	27,166	22,544	22,372	22,655	
RR (95% CI)	1.00	0.96 (0.77-1.19)	0.77 (0.60-0.99)	0.86 (0.67-1.10)	1.03 (1.79-1.34)	0.49
RR (95% CI) multivariate	1.00	0.96 (0.77-1.21)	0.78 (0.60-1.01)	0.87 (0.67-1.12)	1.05 (0.80-1.37)	0.47

[a] Low risk: free of baseline cardiovascular disease and diabetes, and not smoking at baseline (n = 27,573). High risk: prevalent cardiovascular disease or diabetes at baseline, current smokers (n = 10,632). [b] RR: risk ratio; CI: confidence interval; adjusted for other catechins listed, age, and total energy intake. [c] Adjusted for other catechins listed, age, total energy intake, marital status, educational attainment, high blood pressure, body mass index, waist-to-hip ratio, physical activity, pack-years of smoking, use of estrogen replacement therapy, use of vitamin supplements, alcohol intake, intake of whole grains, intake of saturated fatty acids, intake of polyunsaturated fatty acids, intake of cholesterol, and intake of dietary vitamin C, vitamin E, folate, and carotene. [d] Sum of (+)-gallocatechin, (-)-epigallocatechin, (-)-epicatechin gallate, and (-)-epigallocatechin gallate.

fourth quintile had a confidence interval that did not include one (risk ratio: 0.54; 95% confidence interval: 0.37-0.80), and the risk ratio was higher in the fifth quintile (risk ratio: 0.76; 95% confidence interval: 0.52-1.12) than in the fourth. Among the women at high risk, (+)-catechin and (-)-epicatechin intake was not clearly associated with CHD death. 'Gallate' intake was not associated with the risk of CHD death in the low risk group, nor in the high risk group.

Discussion

In this population-based prospective study of postmenopausal women who where reportedly free of cardiovascular disease at baseline, the intake of (+)-catechin and (-)-epicatechin was inversely associated with CHD mortality, whereas the intake of 'gallates', catechins characteristic of tea, was not. As a consequence, the inverse trend between the total intake of catechins and CHD mortality was not statistically significant. Adjustment for lifestyle and dietary potential confounders attenuated the observed associations, but the inverse trend with (+)-catechin plus (-)-epicatechin remained. These data do not support the reported strong inverse association between total catechin intake and CHD mortality among elderly men in the Netherlands[9] (Chapter 5.1). In the Dutch study, no separate analyses for subgroups of catechins were reported, but 87 percent of the catechins were derived from tea, and the data showed that both tea and catechins not from tea were independently associated with a reduced risk of CHD death. When we entered catechins from tea and catechins not from tea in our models, or stratified the analysis by tea drinking, the results were similar to those for the catechin subgroups reported in Table 5.2.2. The average intake of catechins in the Iowa women was 25.4 mg/day (range: 0-278 mg/day), compared to 72 mg/day (range: 0-355.4 mg/day) in the Dutch men. The lower catechin intake in our study may partially explain the lack of an association, however, the range of intake among the women is wide, the intake in the highest quintile is comparable to the level at which an effect was observed in the Dutch study, and the women's intake of (+)-catechin and (-)-epicatechin was similar to the Dutch men's intake of non-tea catechins.

Potential explanations for the distinct associations of (+)-catechin and (-)-epicatechin versus 'gallates' with CHD mortality include differences in bioavailability, microbial degradation, metabolism, or bioactivity. Studies reporting on pharmacokinetic parameters of catechins were reviewed recently.[23] Plasma levels of (-)-epigallocatechin gallate and (-)-epicatechin are generally comparable, but no (-)-epicatechin gallate has been detected in plasma so far, indicating that it is either degraded, metabolized or not absorbed.[23] Moreover, diet may affect the

bioavailability of various catechins differentially. *In vitro* antioxidant activities of the different catechins do not suggest that 'gallates' have lower bioactivity,[5] but the relevance of these tests to the *in vivo* situation remains to be established. More comparative studies regarding *in vivo* bioactivity and bioavailability of the various catechins are necessary in order to explain the observed differences.

Tea is not only the major source of 'gallates', it is a good source of (-)-epicatechin as well: tea-drinkers obtained on average 29 percent (SD: 25.5) of their (-)-epicatechin from tea. The correlation between the sum of (+)-catechin and (-)-epicatechin and the 'gallates' was 0.53. If (+)-catechin and (-)-epicatechin were the only bioactive catechins, then tea would still be expected to be inversely associated with CHD mortality because of its high (-)-epicatechin content. The data from Table 5.2.4 show that this is not the case: catechins from tea were not associated with CHD mortality in the present study. Several studies support our findings: three American cohort studies reported that consumption of approximately 1 cup of tea daily compared to no tea was not associated with myocardial infarction incidence,[24] myocardial infarction death,[25] or CHD death.[16] Two studies from the United Kingdom reported that tea consumption was not associated with CHD,[14] or that CHD mortality actually increased with increasing tea consumption.[10] Tea consumption in these studies was very high: at least 5 cups daily in the highest consumption category. Contrary to this, two Dutch,[11,12] and one American study[13] reported strong inverse associations between tea consumption and coronary disease, and one study from Norway[26] showed that the consumption of 1 cup of tea daily was inversely associated with CHD mortality. A possible explanation offered previously for the observed discrepancies between studies was inadequate adjustment for confounders. Whereas tea consumption is generally associated with a healthy lifestyle, the opposite is the case in the two studies from United Kingdom, the only studies where there was a suggestion of an increased risk with high tea consumption. In our study, tea consumption was associated with a healthy lifestyle, but not with CHD mortality. Another potential explanation for the lack of an association of a high tea consumption with CHD mortality could be that women with subclinical disease had changed their lifestyle and dietary habits. However, exclusion of the deaths that occurred during the first two years of follow-up did not modify the reported results.

Two of the five other sources of catechins, apples and wine, were inversely associated with CHD death. A high intake of apples has been associated with a reduced risk of cardiovascular disease in two European studies,[12,27] but not in one American study.[16] The strong inverse association with apple consumption in our study raises the question whether (+)-catechin and (-)-epicatechin are merely

indicators of a diet high in fruits and vegetables. Adjustment for folate, vitamin C, vitamin E, and carotenoids (which adjusts to a large extent for fruits and vegetables) did not essentially alter the observed associations, nor did additional adjustment for total fruit and vegetable intake (data not shown). Moreover, of the antioxidant vitamins only vitamin E from foods was previously shown to be associated with CHD death in our dataset.[28] Apples are a good source of flavonols as well, and estimated flavonol intake has previously been shown to be inversely associated with CHD death in this study[29] and in other studies.[12,27] We were unable to adjust for flavonol intake because of the high correlation between catechins and flavonols in our study (0.87), which was partly due to the fact that the intake of onions, a major source of flavonols in the US[16] was not ascertained. There is an ongoing debate whether the often observed inverse association between wine consumption and cardiovascular disease is stronger than could be explained solely by its alcohol content.[30-33] In our study, the inverse association of CHD with wine consumption remained after adjustment for alcohol intake. However, wine intake was generally very low, and it is questionable whether the relatively small contribution of wine to the total catechin intake would have any physiological significance.

Several limitations of our study should be pointed out. Misclassification of dietary exposure could have occurred at both the dietary assessment level, and in the assignment of catechin levels to the foods reported. The ability of the questionnaire to determine intakes of various foods,[18] and nutrients[34] was validated previously and found to be satisfactory. Moreover, of all the catechin containing foods, tea was estimated most accurately.[18] Data on the catechin contents of foods were primarily limited to analyses conducted in the Netherlands,[20,21] but catechin levels determined in foods purchased in Minnesota were within the same range. However, catechin contents are known to vary greatly by variety,[20,23] thus individual preferences for particular varieties of foods would lead to misclassification. Also, the catechin content of tea infusions depends, among other things, on the brewing time.[21] The standard brewing method used to determine catechin values for tea was as follows: a 2-g tea bag was placed in 200 mL of boiled tap water for 5 minutes, and stirred through the brew before removing. Five minutes of infusion may be too long for US habits, and we therefore may have overestimated the catechin content of US tea. These limitations all most likely would have attenuated true effects, but there is no reason to assume they attenuated more severely the relation between CHD death and tea intake than that with other catechin containing foods.

There is evidence suggesting that dietary antioxidants may be particularly beneficial in subjects with an otherwise less healthy lifestyle.[15] Epidemiological studies that

appear to support this hypothesis include the study by Rimm and coworkers[16] who reported that a suggestion of an inverse trend between flavonols and CHD death was limited to men with prevalent cardiovascular disease, and the study by Geleijnse and coworkers[11] that showed that tea drinking was inversely associated with severe aortic atherosclerosis and not with moderate or mild atherosclerosis. Tea drinking in our study was associated with a relatively healthy lifestyle, e.g., less smoking, more physical activity, and healthier diet, which, we hypothesize, could partly explain the absence of a relation between tea catechins and CHD death. In our study, however, tea catechins were unrelated to CHD death among low risk or high risk subjects. And, if anything, the intake of (+)-catechin and (-)-epicatechin was inversely associated with CHD death in low risk subjects only. The CHD death rate was more than four times greater in our high than low risk group, which validates our definition of high risk. The high risk group was composed of women with very diverse profiles: smokers, diabetics, and women with prevalent cardiovascular disease. Analyses by each of these groups individually gave similar but less clear results due to smaller numbers (data not shown).

In summary, the intake of (+)-catechin and (-)-epicatechin was inversely associated with CHD mortality, particularly in women that were considered to be at low risk of CHD death, i.e. free of cardiovascular disease and diabetes and not smoking at baseline. There was no indication that catechins were particularly beneficial in subjects at high risk. The intake of 'gallates', catechins derived primarily from tea, was not associated with CHD mortality. The results from this study suggest that possible preventive effects of catechins might be limited to certain types of catechins, or that these types of catechins are indicators for a healthy lifestyle in general or certain components of the diet that are as yet unknown or unavailable for epidemiological evaluation.

Acknowledgments

The authors would like to thank Betty van de Putte and Ching-Ping Hong for excellent technical assistance and Dr Feskens, Dr Hollman, Dr Bueno de Mesquita, and Dr Kromhout for valuable comments on the manuscript. This work was supported in part by grant CA-39742 from the National Cancer Institute, and by the Commission of the European Communities Agriculture and Fisheries (FAIR) specific RTD Programme CT95 0653. Ilja Arts was also supported by a travel grant from the Netherlands Organization for Scientific Research (NWO).

References

1. Middleton E, Kandaswami C. The impact of plant flavonoids on mammalian biology: implications for immunity, inflammation and cancer. In: Harborne JB, ed. *The Flavonoids: advances in research since 1986*. London: Chapman & Hall;1994:619-652.

2. Zhu QY, Huang Y, Tsang D, Chen ZY. Regeneration of alpha-tocopherol in human low-density lipoprotein by green tea catechin. *J Agric Food Chem* 1999;47:2020-2025.
3. Packer L. Vitamin E and the antioxidant network: protection of human Low Density Lipoprotein from oxidation. In: Ohigashi H, Osawa T, Terao J, Watanabe S, Yoshikawa T, eds. *Food factors for cancer prevention*. Tokyo: Springer-Verlag;1997:452-459.
4. Wiseman SA, Balentine DA, Frei B. Antioxidants in tea. *Crit Rev Food Sci Nutr* 1997;37:705-718.
5. Rice-Evans CA, Miller NJ, Paganga G. Structure-antioxidant activity relationships of flavonoids and phenolic acids. *Free Radical Biol Med* 1996;20:933-956.
6. Middleton E. Effect of plant flavonoids on immune and inflammatory cell function. *Adv Exp Med Biol* 1998;439:175-182.
7. Stein JH, Keevil JG, Wiebe DA, Aeschlimann S, Folts JD. Purple grape juice improves endothelial function and reduces the susceptibility of LDL cholesterol to oxidation in patients with coronary artery disease. *Circulation* 1999;100:1050-1055.
8. Wollny T, Aiello L, Di Tommaso D, Bellavia V, Rotilio D, Donati MB, De Gaetano G, Iacoviello L. Modulation of haemostatic function and prevention of experimental thrombosis by red wine in rats: a role for increased nitric oxide production. *Br J Pharmacol* 1999;127:747-755.
9. Arts ICW, Hollman PCH, Feskens EJM, Bueno de Mesquita HB, Kromhout D. Catechin intake is inversely associated with coronary heart disease mortality; results from the Zutphen Elderly Study. Circulation 2000;101:711(abstract).
10. Hertog MGL, Sweetnam PM, Fehily AM, Elwood PC, Kromhout D. Antioxidant flavonols and ischemic heart disease in a Welsh population of men: the Caerphilly Study. *Am J Clin Nutr* 1997;65:1489-1494.
11. Geleijnse JM, Launer LJ, Hofman A, Pols HA, Witteman JC. Tea flavonoids may protect against atherosclerosis: the Rotterdam Study. *Arch Intern Med* 1999;159:2170-2174.
12. Hertog MGL, Feskens EJ, Hollman PCH, Katan MB, Kromhout D. Dietary antioxidant flavonoids and risk of coronary heart disease: the Zutphen Elderly Study. *Lancet* 1993;342:1007-1011.
13. Sesso HD, Gaziano JM, Buring JE, Hennekens CH. Coffee and tea intake and the risk of myocardial infarction. *Am J Epidemiol* 1999;149:162-167.
14. Woodward M, Tunstall-Pedoe H. Coffee and tea consumption in the Scottish Heart Health Study follow up: conflicting relations with coronary risk factors, coronary disease, and all cause mortality. *J Epidemiol Community Health* 1999;53:481-487.
15. Diplock AT, Charleux JL, Crozier-Willi G, Kok FJ, Rice-Evans C, Roberfroid M, Stahl W, Vina-Ribes J. Functional food science and defence against reactive oxidative species. *Br J Nutr* 1998;80 Suppl 1:S77-112.
16. Rimm EB, Katan MB, Ascherio A, Stampfer MJ, Willett WC. Relation between intake of flavonoids and risk for coronary heart disease in male health professionals. *Ann Intern Med* 1996;125:384-389.
17. Willett WC, Sampson L, Browne ML, Stampfer MJ, Rosner B, Hennekens CH, Speizer FE. The use of a self-administered questionnaire to assess diet four years in the past. *Am J Epidemiol* 1988;127:188-199.
18. Feskanich D, Rimm EB, Giovannucci EL, Colditz GA, Stampfer MJ, Litin LB, Willett WC. Reproducibility and validity of food intake measurements from a semiquantitative food frequency questionnaire. *J Am Diet Assoc* 1993;93:790-796.
19. Putnam JJ, Allshouse JE. *Food consumption, prices, and expenditures, 1970-1995. Statistical Bulletin number 939*. Washington (DC): Economic Research Service/USDA; 1997.
20. Arts ICW, Van de Putte B, Hollman PCH. Catechin contents of foods commonly consumed in The Netherlands. 1. Fruits, vegetables, staple foods, and processed foods. *J Agric Food Chem*

2000;48:1746-1751.

21. Arts ICW, Van de Putte B, Hollman PCH. Catechin contents of foods commonly consumed in The Netherlands. 2. Tea, wine, fruit juices, and chocolate milk. *J Agric Food Chem* 2000;48:1752-1757.
22. World Health Organization. *Manual of the international statistical classification of diseases, injuries, and causes of death. Ninth Revision, 1975.* Geneva: WHO; 1977.
23. Hollman PCH, Arts ICW. Flavonols, flavones and flavanols - nature, occurrence and dietary burden. *J Sci Food Agric* 2000;80:1081-1093.
24. Klatsky AL, Friedman GD, Armstrong MA. Coffee use prior to myocardial infarction restudied: heavier intake may increase the risk. *Am J Epidemiol* 1990;132:479-488.
25. Klatsky AL, Armstrong MA, Friedman GD. Coffee, tea, and mortality. *Ann Epidemiol* 1993;3:375-381.
26. Stensvold I, Tverdal A, Solvoll K, Foss OP. Tea consumption. relationship to cholesterol, blood pressure, and coronary and total mortality. *Prev Med* 1992;21:546-553.
27. Knekt P, Järvinen R, Reunanen A, Maatela J. Flavonoid intake and coronary mortality in Finland: A cohort study. *BMJ* 1996;312:478-481.
28. Kushi LH, Folsom AR, Prineas RJ, Mink PJ, Wu Y, Bostick RM. Dietary antioxidant vitamins and death from coronary heart disease in postmenopausal women. *N Engl J Med* 1996;334:1156-1162.
29. Yochum L, Kushi LH, Meyer K, Folsom AR. Dietary flavonoid intake and risk of cardiovascular disease in postmenopausal women. *Am J Epidemiol* 1999;149:943-949.
30. Renaud S, de Lorgeril M. Wine, alcohol, platelets, and the French paradox for coronary heart disease. *Lancet* 1992;339:1523-1526.
31. Rimm EB, Klatsky A, Grobbee D, Stampfer MJ. Review of moderate alcohol consumption and reduced risk of coronary heart disease: Is the effect due to beer, wine, or spirits? *BMJ* 1996;312:731-736.
32. Renaud SC, Guéguen R, Siest G, Salamon R. Wine, beer, and mortality in middle-aged men from eastern France. *Arch Intern Med* 1999;159:1865-1870.
33. Wannamethee SG, Shaper AG. Type of alcoholic drink and risk of major coronary heart disease events and all-cause mortality. *Am J Publ Health* 1999;89:685-690.
34. Munger RG, Folsom AR, Kushi LH, Kaye SA, Sellers TA. Dietary assessment of older Iowa women with a food frequency questionnaire: nutrient intake, reproducibility, and comparison with 24-hour dietary recall interviews. *Am J Epidemiol* 1992;136:192-200.

Dietary catechins and epithelial cancer incidence: the Zutphen Elderly Study

In this study we evaluated the association between intake of catechins and incidence of epithelial cancers with data from the Zutphen Elderly Study, a prospective cohort study among 728 men aged 65-84 years in 1985. The average catechin intake at baseline was 72 mg/day (range: 0-355 mg/day). After 10 years of follow-up, 96 incident epithelial cancers were recorded, including 42 cases of lung cancer. After multivariate adjustment, catechin intake was not associated with epithelial cancer (risk ratio from lowest to highest tertile: 1.00, 0.75, 0.94; P for trend: 0.82), or lung cancer (risk ratio from lowest to highest tertile: 1.00, 0.72, 0.92; P for trend: 0.80). Catechins not from tea were borderline significantly inversely associated with lung cancer incidence (risk ratio and 95% confidence interval (CI) for a 7.5 mg increase in intake: 0.66, 0.42-1.05), whereas catechins from tea were not. Catechins from apple, the major source of non-tea catechins, were also related to lung cancer incidence (risk ratio and 95% CI for a 7.5 mg catechin increase: 0.67, 0.38-1.17). Because tea, the major catechin source in this population, was not associated with cancer risk, it seems unlikely that catechins are responsible for the observed inverse trend between non-tea catechins and lung cancer incidence. However, differences in bioavailibility of the various catechins may play a role, and effects on individual cancer sites cannot be excluded and merit further investigation.

Arts ICW, Hollman PCH, Bueno de Mesquita HB, Feskens EJM, Kromhout D. Dietary catechins and epithelial cancer incidence: the Zutphen Elderly Study. *Int J Cancer, in press.*

INTRODUCTION

Diets rich in fruits and vegetables are associated with reduced risks of many cancers, in particular those of epithelial origin such as cancer of the mouth and pharynx, esophagus, stomach, colon, rectum and lung.[1] The flavonoids, a large group of more than 4000 different polyphenolic antioxidants, have been identified as potential cancer preventive components of fruits and vegetables.[2,3] Flavonoids occur ubiquitously in plant foods, and can be categorized into six major subclasses: catechins, flavonols, flavones, flavanones, anthocyanidins, and isoflavonoids.[4] A high intake of flavonols and flavones has previously been associated with a decreased risk of lung cancer.[5,6]

Tea is a rich source of flavonols, but catechins are its main components; they constitute about 30% of the dry weight of green tea, and 9% of the dry weight of black tea.[7] Tea has been shown to inhibit tumorigenesis at the initiation, promotion and progression stages, and it could potentially reduce cancers of all sites.[3,8,9] Epidemiological studies regarding the association between tea and cancer have been inconclusive, but point to the possibility of lowered risks of digestive tract cancers among tea drinkers.[10,11]

Tea, however, is not the sole source of catechins. In particular in countries with a relatively low level of tea consumption, epidemiological studies may suffer from misclassification if other catechin sources are neglected. Up to now, an epidemiological evaluation of catechins was impossible, because reliable data on the catechin content of foods were lacking. We have developed a method to determine catechins in foods[12] and we have determined six major catechins: (+)-catechin, (+)-gallocatechin, (-)-epicatechin, (-)-epigallocatechin, (-)-epicatechin gallate, and (-)-epigallocatechin gallate in foods and beverages commonly consumed in the Netherlands.[13,14] Using these data we evaluated for the first time the association between a high intake of catechins and the incidence of epithelial cancers in a cohort of elderly men.

METHODS

Study population

The Zutphen Elderly Study is a prospective cohort study on risk factors for chronic diseases in elderly men. It is an extension of the Zutphen Study, the Dutch contribution to the Seven Countries Study. In 1985, 555 men of the original Zutphen cohort were still alive, and were invited to participate in the Zutphen Elderly Study. A

random sample of all other men of the same age also living in Zutphen but not belonging to the original cohort, was invited additionally. This resulted in a total target population of 1266 men aged 65-84 years. Of these 1266 men, 939 men (74%) participated in the study, and 876 men completed a dietary questionnaire. Complete information on both diet and other risk factors was available for 788 men. After excluding all prevalent cancer cases, 728 men remained in the analysis.

Data collection

Dietary and medical examinations were conducted between March and June 1985. Medical examinations were carried out by trained physicians and included anthropometry, blood sampling, blood pressure measurement, and detailed questionnaires on smoking behavior and physical activity. The latter was assessed by a validated questionnaire designed for retired men.[15] The habitual diet in the month preceding the interview was determined using a cross-check dietary history method adapted to the Dutch situation, which was extensively described by Bloemberg and coworkers.[16] Participants were interviewed at home by an experienced dietitian in the presence of the person usually preparing meals. First, their average pattern of food intake during weekdays and on weekends was recorded. Based on these daily patterns, a checklist of foods was presented to the respondents so they could indicate the frequency of consumption and the serving size of each food. These data were subsequently compared to the average estimated weekly consumption, and to the quantities of foods bought per week. Any discrepancies were discussed with the respondent. The food intake data were encoded by the dietitians, and converted into energy and nutrient data using the 1985 release of the Dutch Food Table,[17] updated with 1993 data for beta-carotene and vitamin E, with flavonol and flavone data, and with catechin data. Flavonol and flavone contents of foods were previously determined by Hertog and colleagues.[18,19] Flavones are a minor group of flavonoids compared to the flavonols; the term flavonols will be used here for the sum of both groups. Catechin, or total catechin, is defined as the sum of (+)-catechin, (+)-gallocatechin, (-)-epicatechin, (-)-epigallocatechin, (-)-epicatechin gallate, and (-)-epigallocatechin gallate. We determined these six catechins by Reversed-Phase High Performance Liquid Chromatography with ultraviolet and fluorescence detection in sequence[12] in more than 120 commonly consumed plant foods and beverages.[13,14] Each food was purchased at three outlets: a nation-wide supermarket chain, an open-air street market, and a grocery. To take into account seasonal and year-to-year variability, each food was purchased in August and December 1997 and in April and August 1998, if available in that period. Different brands and varieties of the most important

catechin sources were analyzed, and combined into average values taking into account consumption levels.

Case ascertainment

Information on the vital status of the participants until January 1995 was obtained from municipal population registries. Three men were lost to follow-up in 1991. Of these, two had moved abroad, and one had moved with unknown destination. These men were included in the analyses, but censored at 31 December 1990. The prevalence of disease at baseline, and the first clinical diagnosis of disease during follow-up (further referred to as incidence) were recorded at the examinations in 1985, 1990, and 1995 using standardized questionnaires. Data on baseline prevalence and incidence of all cancers were verified with hospital discharge data and written information from the general practitioner. Incidence data were uniformly coded by three physicians according to ICD-guidelines.[20] Our endpoint of interest, incident epithelial cancer, included tumors of the oropharynx, esophagus, stomach, colon, rectum, liver, gallbladder, pancreas, kidney, bladder, and bronchus and lung. At baseline, there were 60 men (8%) with prevalent cancer (40 epithelial cancer cases, of which 13 lung cancer cases). All prevalent cases were excluded from the analyses, resulting in a study population of 728 cancer-free men at baseline.

Statistical analysis

Baseline characteristics of the participants were compared between tertiles of catechin intake using a Chi-square test for categorical variables, one-way analysis of variance for normally distributed variables and Kruskal-Wallis test for skewed variables. Risk ratios of epithelial cancer incidence were estimated by Cox proportional hazards analysis, using the SAS procedure PHREG (SAS, release 6.12, SAS Institute, North Carolina, USA). In addition, separate analyses were performed for lung cancer and for epithelial cancers other than lung cancer. Following crude analysis, a multivariate model was adjusted for age, smoking status, pack-years of smoking, total energy intake, alcohol intake, physical activity, body mass index, and dietary factors: coffee consumption, vitamin C, vitamin E, beta-carotene, and fiber. Foods containing apple or chocolate were combined into 'catechins from apple' or 'catechins from chocolate' by converting each individual's consumption of these foods into milligrams of catechins per day, and taking the sum of these values. Probability values for a linear trend were derived from tertile medians.

RESULTS

The average daily catechin intake among the 728 elderly men who participated in the

Zutphen Elderly Study was 72 mg (standard deviation: 47.5 mg/day). It ranged from 0 (in 1 participant) to 355.4 mg/day. At baseline, participants that belonged to the highest tertile of catechin intake were less likely to be current smokers, and more likely to have never smoked (Table 6.1.1). Catechin intake was inversely associated with pack-years of cigarette smoking, and positively associated with physical activity. Men with a high catechin intake tended to also have a higher intake of total energy, fiber, vitamin C, vitamin E and beta-carotene. They ate more fruits and vegetables, but drank less coffee.

Table 6.1.1 Baseline characteristics of 728 elderly men by tertile of total catechin intake, the zutphen elderly study, 1985

	Tertile of total catechin intake			
	low	middle	high	P-value
Range of total catechin intake (mg/day)	0-49.0	49.1-85.7	85.8-355.4	
Number of men	242	243	243	
Current smokers (%)	42.6	29.2	20.6	0.001
Never smokers (%)	16.9	16.9	24.7	0.04
	mean (standard deviation)			
Catechin intake (mg/day)	25.2 (15.6)	66.8 (10.4)	123.7 (39.1)	
Flavonol + flavone intake (mg/day)	14.4 (9.0)	24.4 (7.9)	39.1 (13.1)	0.0001
Age in 1985 (yrs)	70.6 (5.0)	71.6 (5.4)	71.1 (5.2)	0.15
Pack-years of cigarette smoking	23.7 (22.2)	19.6 (20.1)	17.0 (18.2)	0.001
Energy intake (MJ/day)	9.3 (2.2)	9.3 (2.1)	9.9 (2.1)	0.004
Alcohol (g/day)	14.7 (18.5)	12.5 (17.5)	12.5 (15.0)	0.66
Physical activity (min/week)	674 (670)	653 (631)	751 (656)	0.04
Body mass index (kg/m^2)	25.7 (3.5)	25.4 (2.9)	25.7 (3.1)	0.55
Tea (mL/day)	122 (106)	389 (85)	766 (267)	0.0001
Coffee (mL/day)	555 (342)	409 (215)	372 (230)	0.0001
Fruits (g/day)	176 (146)	203 (138)	223 (137)	0.0001
Vegetables (g/day)	171 (75)	173 (69)	187 (75)	0.02
Vitamin C (mg/day)	87.8 (49.0)	95.6 (43.2)	104.7 (47.3)	0.0001
Vitamin E (mg/day)	8.2 (2.6)	8.4 (2.6)	8.9 (2.6)	0.003
Beta-carotene (mg/day)	1.3 (0.7)	1.4 (0.6)	1.5 (0.6)	0.01
Fiber (g/day)	24.0 (7.7)	25.0 (6.5)	28.0 (8.2)	0.0001

During 10 years of follow-up 96 men reported having a newly diagnosed epithelial cancer, including cancers of the oropharynx (7 cases), esophagus (2 cases), stomach (7 cases), colon (16 cases), rectum (3 cases), liver, gallbladder and pancreas (9 cases), kidney and bladder (15 cases), and lung and bronchus (42 cases). Numbers do not add up to 96 because 5 men had more than one incident tumor. Risk ratios of incidence of total epithelial cancer, lung cancer, and epithelial

cancers other than lung cancer are presented in Table 6.1.2. Dietary catechin intake was not associated with the risk of epithelial cancer in this population. After multivariate adjustment, the crude risk ratio in the highest tertile of intake of 0.71 (95% confidence interval (CI): 0.44-1.14) was attenuated to 0.94 (95% CI: 0.56-1.59). The separate analyses for lung cancer and other epithelial cancers again did not suggest an association between cancer risk and catechin intake, but these analyses suffer from small numbers and resulting wide confidence intervals.

Table 6.1.2 Risk ratios of cancer incidence in 728 elderly men according to tertiles of catechin intake, the zutphen elderly study

	Tertile of total catechin intake			P for trend
	low	middle	high	
Number of men	242	243	243	
Epithelial cancer				
Number of person years	1664	1721	1759	
Cases	40	26	30	
Crude RR (95% CI)[a]	1.00	0.63 (0.38-1.03)	0.71 (0.44-1.14)	0.16
Adjusted RR (95% CI)[b]	1.00	0.75 (0.45-1.26)	0.94 (0.56-1.59)	0.82
Lung cancer				
Number of person years	1696	1765	1798	
Cases	19	11	12	
Crude RR (95% CI)	1.00	0.56 (0.26-1.17)	0.60 (0.29-1.23)	0.15
Adjusted RR (95% CI)	1.00	0.72 (0.33-1.56)	0.92 (0.41-2.07)	0.80
Other epithelial cancers				
Number of person years	1682	1724	1764	
Cases	22	17	18	
Crude RR (95% CI)	1.00	0.75 (0.40-1.42)	0.78 (0.42-1.46)	0.45
Adjusted RR (95% CI)	1.00	0.91 (0.47-1.77)	1.02 (0.52-2.03)	0.94

[a] RR: risk ratio; CI: confidence interval. [b] Adjusted for age, physical activity, total energy intake, alcohol intake, smoking status, pack-years of smoking, body mass index, coffee, fiber, vitamin C, vitamin E, beta-carotene.

Tea is the sole source of (-)-epigallocatechin gallate, and the major source of (-)-epicatechin and (-)-epicatechin gallate. Other catechin-rich foods contain mainly (+)-catechin and (-)-epicatechin. Most catechin containing foods, except chocolate, also contain flavonols. The correlation coefficients between tea and catechins (r_s=0.98), tea and flavonols (r_s=0.82) and catechins and flavonols (r_s=0.84) were consequently high. In order to discern the individual associations of these compounds with the risk of cancer, and at the same time avoid problems of multicollinearity, we tested a multivariate adjusted model including catechins from tea, catechins from other sources than tea, and flavonols from other sources than tea (Table 6.1.3). A 7.5 mg increase in intake of catechins from other sources than tea was associated with a borderline significant 34% lower risk of lung cancer (95% CI:

0.42-1.05). Neither catechins from tea, nor flavonols from sources other than tea were significantly associated with cancer risk.

Table 6.1.3 Intake of catechins from tea, other sources than tea, and flavonols and flavones from other sources than tea, and mutually independent risk ratios of cancer incidence for one standard deviation increase in intake in 728 men, the zutphen elderly study

	mean intake (standard deviation) (mg)	Lung cancer	Other epithelial cancers
		RR (95% CI)[a]	RR (95% CI)
Catechins from tea	62.3 (46.2)	1.06 (0.76-1.47)	0.97 (0.74-1.29)
Catechins not from tea	9.7 (7.2)	0.66 (0.42-1.05)	0.95 (0.69-1.33)
Flavonols + flavones not from tea	10.9 (7.8)	0.80 (0.52-1.22)	0.76 (0.52-1.10)

[a] RR: risk ratio; CI: confidence interval, for a standard deviation increase in intake (46 mg catechins from tea, 7.5 mg catechins or flavonols not from tea); adjusted for other flavonoids listed, age, physical activity, total energy intake, alcohol intake, smoking status, pack-years of smoking, body mass index, coffee, fiber, vitamin C, vitamin E, beta-carotene.

Table 6.1.4 Risk ratios of cancer incidence for 7.5 mg catechin intake increases from major catechin sources in 728 men, the zutphen elderly study

Catechin source	% consumers	% contribution to total catechin intake	Lung cancer	Other epithelial cancers
			RR (95% CI)[a]	RR (95% CI)
Tea	89	86.6	1.01 (0.96-1.07)	1.00 (0.95-1.04)
Apple	86	8.0	0.67 (0.38-1.17)	1.06 (0.72-1.57)
Chocolate	80	2.9	0.76 (0.29-2.02)	0.89 (0.45-1.80)

[a] RR: risk ratio; CI: confidence interval, for a 7.5 mg increase in catechin intake; adjusted for other sources listed, age, physical activity, total energy intake, alcohol intake, smoking status, pack-years of smoking, body mass index, coffee, fiber, vitamin C, vitamin E, beta-carotene.

In Table 6.1.4 the major catechin sources in our study are presented. Except for tea, which was by far the major source (87%), apple (8%), and chocolate (3%) contributed substantially to the total catechin intake. These foods were moreover consumed by a substantial number of participants (≥ 80%). In order to determine whether one particular catechin source was more strongly associated with lung cancer risk, we estimated risk ratios for the major catechin sources after controlling for other risk factors and each other; both consumers and non-consumers were included in the analyses. After multivariate adjustment, catechins from apple (risk ratio for a 7.5 mg increase: 0.67), rather than catechins from chocolate (risk ratio for a 7.5 mg increase: 0.76), tended to be inversely associated with lung cancer risk, but these associations were not statistically significant. The intake of catechins from apple or chocolate was not associated with incidence of epithelial cancers other than lung cancer.

DISCUSSION

This prospective cohort study among elderly men in The Netherlands shows that the intake of catechins is not associated with epithelial cancer incidence, or with incidence of lung cancer or epithelial cancers other than those of the lung. The number of cases was too small to consider other individual sites. To our knowledge, no previous studies regarding catechin intake and cancer incidence or mortality have been reported.

We also studied independent associations of tea, and of two major flavonoid subgroups present in tea, catechins and flavonols, with lung cancer incidence and with incidence of epithelial cancers other than lung cancer. Because of the high correlations between the tea and flavonoid variables, we modeled catechins from tea, catechins from other sources than tea, and flavonols from other sources than tea. None of the studied variables was associated with epithelial cancer incidence other than lung cancer. Neither catechins from tea nor flavonols not from tea were associated with lung cancer incidence, but there was a suggestion of an inverse association between catechins not from tea and lung cancer incidence. Our data regarding tea confirm three previous studies where tea consumption was found to be unrelated to total cancer mortality or incidence.[21] One study with high intakes of green tea (10 or more cups/day) reported inverse associations between tea intake and total cancer incidence that were significant for women only.[22] Studies on the relation between tea and lung cancer have found results similar to ours: four cohort studies and three case-control studies reported that there was no association between tea intake and lung cancer risk.[6,10] One cohort study found an increased risk in lung cancer with a high intake of tea, which was ascribed to confounding because of the limited adjustment for smoking and the reported positive association between smoking and tea consumption.[10] One case-control study, on the other hand, reported a 50% reduction in lung cancer risk when consuming 1 cup of black tea per day.[23] Data for the two major other epithelial cancers, colon and kidney/bladder, generally show no association for tea intake and kidney/bladder cancer, and inconsistent data for colon cancer.[10]

We are aware of five studies that reported on the relation between intake of flavonols and cancer risk. Of these, three found no association between total flavonols and lung cancer,[6,24,25] one Finnish study reported a statistically significant 47% reduction in lung cancer risk,[5] and a previous report on five-year follow-up data from the Zutphen Elderly Study found no association between total flavonol intake and incidence of cancer of the alimentary and respiratory tract.[26] With regard to

subgroups of flavonols, a non-significant inverse trend (P for trend: 0.06) was found between flavonoids from vegetables and fruits and cancer of the respiratory and alimentary tract in the study by Hertog and colleagues[26] and in the study by Le Marchand and colleagues, intake of quercetin tended to be inversely related to lung cancer risk (P for trend: 0.07).[6] None of the studies found flavonol intake to be related to colorectal or stomach cancer.[5,24,25] Interestingly, in the Finnish study apple was the main flavonol source and the only flavonol rich food that was inversely associated with lung cancer incidence. A similar finding was reported by Le Marchand and coworkers: consumption of apple was associated with lung cancer incidence in their study, even though total flavonol intake was not.[6] In both studies catechins were not addressed, because data on catechin contents of foods were not available at the time. These results are in line with our finding of an inverse trend between intake of catechins from apple and lung cancer incidence.

The main catechins present in foods other than tea, such as apples, are (+)-catechin and (-)-epicatechin, whereas tea contains mainly (-)-epigallocatechin gallate and (-)-epicatechin gallate. Differences in bioavailability, microbial degradation, or metabolism of the different catechins may partly explain the observed differences in their relation to cancer. A number of studies reporting on pharmacokinetic parameters of catechins were reviewed recently.[27] Plasma levels of (-)-epigallocatechin gallate and (-)-epicatechin were generally comparable, but (-)-epicatechin gallate was not detectable in plasma or urine in a number of studies,[27] suggesting that at least for this compound differences in bioavailability, metabolism, or degradation exist. Moreover, no data whatsoever are available on interactions of catechins with other dietary compounds. Information is too limited at this point to derive from the pharmacokinetic studies conclusions regarding the interpretation of our epidemiological data. However, tea is a good source of (-)-epicatechin as well, in particular because of the high level of tea-consumption in this population. If (+)-catechin and (-)-epicatechin were the only bioactive catechins, then tea would still be expected to be inversely associated with cancer incidence due to its high (-)-epicatechin content. Because this is not the case, it appears that not catechins, but an as yet unknown compound or group of compounds, is responsible for the suggested inverse association between apple consumption and lung cancer incidence.

Some limitations of our study should be pointed out. Misclassification of dietary exposure could have occurred at both the dietary assessment level, and in the assignment of catechin levels to the foods reported. A validation study performed 12 months after the baseline examination showed that the reproducibility of the dietary

history method used for dietary assessment in this study was satisfactory.[16] Catechin contents are known to vary greatly by variety, thus individual preferences for particular varieties of foods would lead to misclassification. Also, the catechin content of tea infusions depends, among other things, on the brewing time.[14] The standard brewing method used to determine catechin values for tea was as follows: a 2-g tea bag was placed in 200 mL of boiled tap water for 5 minutes, and stirred through the brew before removing. This procedure is quite common in the Netherlands. Since tea was by far the major source of catechins in this population, misclassification of catechin intake due to large individual differences in brewing methods would have attenuated our results, in particular those pertaining to tea. Residual confounding needs to be considered as a potential explanation for the inverse trend we found between catechins from apple and lung cancer incidence. We feel, however, that this is unlikely to have severely affected our results, because we were able to adjust for all known risk factors and because tea intake was strongly related to a healthy lifestyle but not to lung cancer risk after adjusting for these risk factors. Finally, even though ours is a prospective study, cases could have changed their dietary habits and lifestyle as a result of subclinical disease present at baseline. However, exclusion of incident cases that occurred during the first year of follow-up did not essentially modify our results.

In this prospective cohort of elderly men, the intake of catechins was not associated with epithelial cancer incidence, lung cancer incidence, or epithelial cancers other than lung cancer. We did, however, observe an inverse trend between catechins obtained from other sources than tea, mainly apple, and the risk of lung cancer. Because tea, the major catechin source in this population, was not associated with cancer risk, it appears unlikely that catechins are responsible for the observed effect of apples. However, ours is a small study and misclassification of a few cases may have obscured any true relations. Although it seems unlikely that catechins have a major impact on cancer risk in general, effects on individual sites cannot be excluded and merit further investigation.

ACKNOWLEDGMENTS

This work was carried out with financial support from the Commission of the European Communities Agriculture and Fisheries (FAIR) specific RTD Programme CT95 0653, and The Netherlands Prevention Foundation.

REFERENCES

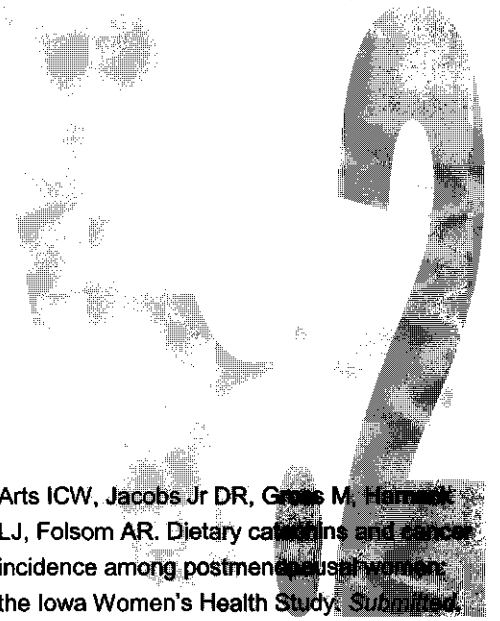
1. World Cancer Research Fund. Vegetables and fruits. In: WCRF/AICR. *Food, nutrition and the prevention of cancer: a global perspective.* Washington (DC): World Cancer Research

Fund/American Institute for Cancer Research;1997:436-446.
2. World Cancer Research Fund. Other bioactive compounds. In: WCRF/AICR. *Food, nutrition and the prevention of cancer: a global perspective*. Washington (DC): World Cancer Research Fund/American Institute for Cancer Research;1997:421-427.
3. Middleton E, Kandaswami C. The impact of plant flavonoids on mammalian biology: implications for immunity, inflammation and cancer. In: Harborne JB, ed. *The Flavonoids: advances in research since 1986*. London: Chapman & Hall;1994:619-652.
4. Harborne JB. *The Flavonoids: Advances in research since 1986*. London: Chapman & Hall; 1994.
5. Knekt P, Järvinen R, Seppänen R, Helliövaara M, Teppo L, Pukkala E, Aromaa A. Dietary flavonoids and the risk of lung cancer and other malignant neoplasms. *Am J Epidemiol* 1997;146:223-230.
6. Le Marchand L, Murphy SP, Hankin JH, Wilkens LR, Kolonel LN. Intake of flavonoids and lung cancer. *J Natl Cancer Inst* 2000;92:154-160.
7. Harbowy ME, Balentine DA. Tea chemistry. *Crit Rev Plant Sciences* 1997;16:415-480.
8. Dreosti IE, Wargovich MJ, Yang CS. Inhibition of carcinogenesis by tea: The evidence from experimental studies. *Crit Rev Food Sci Nutr* 1997;37:761-770.
9. Katiyar SK, Mukhtar H. Tea in chemoprevention of cancer: epidemiologic and experimental studies (review). *Int J Oncol* 1996;8:221-238.
10. Blot WJ, Chow WH, McLaughlin JK. Tea and cancer: a review of the epidemiological evidence. *Eur J Cancer Prev* 1996;5:425-438.
11. Bushman JL. Green tea and cancer in humans: a review of the literature. *Nutr Cancer* 1998;31:151-159.
12. Arts ICW, Hollman PCH. Optimization of a quantitative method for the determination of catechins in fruits and legumes. *J Agric Food Chem* 1998;46:5156-5162.
13. Arts ICW, Van de Putte B, Hollman PCH. Catechin contents of foods commonly consumed in The Netherlands. 1. Fruits, vegetables, staple foods, and processed foods. *J Agric Food Chem* 2000;48:1746-1751.
14. Arts ICW, Van de Putte B, Hollman PCH. Catechin contents of foods commonly consumed in The Netherlands. 2. Tea, wine, fruit juices, and chocolate milk. *J Agric Food Chem* 2000;48:1752-1757.
15. Caspersen CJ, Bloemberg BP, Saris WH, Merritt RK, Kromhout D. The prevalence of selected physical activities and their relation with coronary heart disease risk factors in elderly men: the Zutphen Study, 1985. *Am J Epidemiol* 1991;133:1078-1092.
16. Bloemberg BP, Kromhout D, Obermann-De Boer GL, Van Kampen-Donker M. The reproducibility of dietary intake data assessed with the cross-check dietary history method. *Am J Epidemiol* 1989;130:1047-1056.
17. Kommissie UCV. *UCV Tabel: Uitgebreide voedingsmiddelentabel 1985 [in Dutch]*. Den Haag: Voorlichtingsbureau voor de Voeding (Nutrition Education Bureau); 1985.
18. Hertog MGL, Hollman PCH, Van de Putte B. Content of potentially anticarcinogenic flavonoids of tea infusions, wines, and fruit juices. *J Agric Food Chem* 1993;41:1242-1246.
19. Hertog MGL, Hollman PCH, Katan MB. Content of potentially anticarcinogenic flavonoids of 28 vegetables and 9 fruits commonly consumed in the Netherlands. *J Agric Food Chem* 1992;40:2379-2383.
20. World Health Organization. *Manual of the international statistical classification of diseases, injuries, and causes of death. Ninth Revision, 1975*. Geneva: WHO; 1977.
21. Kohlmeier L, Weterings KGC, Steck S, Kok FJ. Tea and cancer prevention: an evaluation of the epidemiologic literature. *Nutr Cancer* 1997;27:1-13.

22. Imai K, Suga K, Nakachi K. Cancer-preventive effects of drinking green tea among a japanese population. *Prev Med* 1997;26:769-775.
23. Mendilaharsu M, De Stefani E, Deneo-Pellegrini H, Carzoglio JC, Ronco A. Consumption of tea and coffee and the risk of lung cancer in cigarette-smoking men: a case-control study in Uruguay. *Lung Cancer* 1998;19:101-107.
24. Goldbohm RA, Hertog MGL, Brants HAM, Van Poppel G, Van den Brandt PA. Intake of flavonoids and cancer risk: a prospective cohort study. *Cost 916 Polyphenols In Food* 1998;159-166.
25. Hertog MGL, Kromhout D, Aravanis C, Blackburn H, Buzina R, Fidanza F, Giampaoli S, Jansen A, Menotti A, Nedeljkovic S, Pekkarinen M, Simic B, Toshima H, Feskens EJM, Hollman PCH, Katan MB. Flavonoid intake and long-term risk of coronary heart disease and cancer in the seven countries study. *Arch Intern Med* 1995;155:381-386.
26. Hertog MGL, Feskens EJM, Hollman PCH, Katan MB, Kromhout D. Dietary flavonoids and cancer risk in the Zutphen Elderly Study. *Nutr Cancer* 1994;22:175-184.
27. Hollman PCH, Arts ICW. Flavonols, flavones and flavanols - nature, occurrence and dietary burden. *J Sci Food Agric* 2000;80:1081-1093.

Dietary catechins and cancer incidence: the Iowa Women's Health Study

To determine whether a high intake of catechins was associated with cancer incidence, we studied a cohort of 34,651 postmenopausal cancer-free women aged 55-69 years that were followed from 1986-1998. At baseline, data on diet, medical history and lifestyle were collected. Incident cancers were obtained through linkage with a cancer registry. Cox proportional hazards analysis was used to estimate risk ratios. After adjustment for potential confounders, catechin intake was inversely associated with rectal cancer incidence only (risk ratios from lowest to highest quartile 1.00, 0.93, 0.73, 0.55; P for trend 0.02). Non-significant inverse trends were found for cancer of the upper digestive tract, pancreas, and for hematopoietic cancers. Catechins derived primarily from fruits, (+)-catechin and (-)-epicatechin, tended to be inversely associated with upper digestive tract cancer, whereas catechins derived from tea were inversely associated with rectal cancer. Among several cancers studied, our data suggest that catechin intake may protect against rectal cancer. The distinct effects found for catechins derived from solid foods (fruits) and beverages (tea) may be due to differences in bioavailability and metabolism of catechins, and their interactions with other dietary components, but further studies are needed to elucidate this hypothesis.



Arts ICW, Jacobs Jr DR, Gross M, Harnack LJ, Folsom AR. Dietary catechins and cancer incidence among postmenopausal women: the Iowa Women's Health Study. *Submitted.*

INTRODUCTION

Diets rich in fruits and vegetables are associated with a reduced risk of cancer, in particular cancers of epithelial origin such as those of the mouth and pharynx, esophagus, stomach, colon, rectum and lung.[1] The flavonoids, a group of more than 4000 different polyphenolic compounds, have been identified as potential cancer preventive components of fruits and vegetables.[2,3] Flavonoids occur ubiquitously in plant foods, and can be categorized into six major subclasses: catechins, flavonols, flavones, flavanones, anthocyanidins, and isoflavonoids.[4] Tea is a rich source of catechins; they constitute about 30% of the dry weight of green tea, and 9% of the dry weight of black tea.[5] In animal studies, tea has been shown to inhibit tumorigenesis at the initiation, promotion and progression stages, and it could potentially prevent cancers of many sites.[3,6,7] Epidemiological studies regarding the association between tea and cancer have been inconclusive, but point to the possibility of a lowered risk of digestive tract cancers among tea drinkers.[8,9] Because tea is not the sole source of catechins, including other sources of these bioactive components may clarify reported associations.

We have determined levels of six major catechins [(+)-catechin, (+)-gallocatechin, (-)-epicatechin, (-)-epigallocatechin, (-)-epicatechin gallate, and (-)-epigallocatechin gallate] in commonly consumed foods and beverages.[10,11] The objective of the current study was to explore the relation between catechin intake and incident cancers at all sites in postmenopausal women in Iowa.

METHODS

Study population

The Iowa Women's Health Study was approved by the Committee on the Use of Human Subjects in Research, University of Minnesota. In January 1986, a 16-page survey was sent to a random sample of 99,826 women aged 55-69 years who had a valid Iowa driver's license. 41,836 women who returned the questionnaire were enrolled in this prospective cohort study. Women were excluded from the analyses if they were not postmenopausal at baseline (n = 569), or if they left $\geq$ 30 items blank on the food frequency questionnaire or had improbably high ($\geq$ 5,000 Kcal) or low (< 600 Kcal) energy intakes (n = 3096). Also, women who reported having been diagnosed by a physician as having any form of cancer other than skin cancer were excluded from the analyses (n = 3896). This left 34,651 women for analysis; due to missing covariates this number was reduced to 33,339 in the multivariate analysis. Women who had undergone a mastectomy (n = 314), hysterectomy (n = 11,332), or

oophorectomy (n = 8997) were additionally excluded from the analyses concerning hormone-related cancers (breast, uterus, ovary), leaving 21,502 women for these analyses. Numbers excluded are not mutually exclusive.

Data collection

The baseline questionnaire included a 127-item semiquantitative food frequency questionnaire.[12] Although this questionnaire was not validated for its ability to assess catechin intake, Feskanich and co-authors[13] assessed the ability of the questionnaire to determine the intake of foods compared to a 28-day diet record among male health professionals. Correlation coefficients for the main sources of catechins were: 0.77 for tea, 0.70 for apples, 0.45 – 0.83 for chocolate containing foods, and 0.83 for red and 0.78 for white wine. In our Iowa cohort, the reproducibility of the food frequency questionnaire and estimates of nutrient intakes compared to five 24-hour dietary recalls were satisfactory.[14] Also, the questionnaire did not omit important dietary sources of catechins. Catechin contents of questionnaire items that referred to more than one food, e.g. 'fresh apples or pears', were weighted using US food disappearance data for the year 1986.[15] Data on catechin contents of foods and beverages were taken from recently published data from the Netherlands,[10,11] supplemented with analyses of US tea, apples, chocolate, beans, and lentils done at the same laboratory in the Netherlands. Catechin contents of US foods were similar to comparable Dutch foods, except for US apples, which tended to have higher catechin levels than those from the Netherlands.

Case ascertainment

Vital status of the participants was determined annually through the State Health Registry of Iowa (for deaths in Iowa), through follow-up questionnaires mailed in 1987, 1989, 1992, and 1997 (for deaths outside Iowa), or through the National Death Index (for nonresponders). Incident cancer cases were identified through annual linkage of the participants with the State Health Registry of Iowa, part of the National Cancer Institute's Surveillance, Epidemiology, and End Results (SEER) Program. Data on primary site, morphology, and date of diagnosis were obtained for all cancers from 1986 through 1998. During that period, 5038 incident cancers were recorded. Upper-digestive tract cancers, including cancer of the mouth and pharynx (International Classification of Diseases for Oncology (ICD-O) codes C00-C14; n = 78), esophagus (ICD-O code C15; n = 19), stomach (ICD-O code C16; n = 53), and small intestine (ICD-O code C17; n = 26), were combined for analysis because of the relative rarity of these cancers and because the mode of exposure to catechins appears similar for these sites.

Statistical analysis

Total catechin was defined as the sum of six major catechins: (+)-catechin, (+)-gallocatechin, (-)-epicatechin, (-)epigallocatechin, (-)-epicatechin gallate, and (-)-epigallocatechin gallate. Two subgroups of catechins were created, the first reflecting catechins derived mainly or solely from tea (the sum of (+)-gallocatechin, (-)-epigallocatechin, (-)-epicatechin gallate, and (-)-epigallocatechin gallate), further referred to as 'gallates', and the second reflecting catechins derived mainly from sources other than tea (the sum of (+)-catechin and (-)-epicatechin). The intake of catechins, 'gallates' and the sum of (+)-catechin and (-)-epicatechin was calculated for each woman, with participants stratified by quintile, quartile, or tertile of catechin intake, depending on the number of cases for each cancer site. Baseline characteristics of the participants were compared between quintiles of catechin intake using linear regression or analysis of variance. Dietary intakes were compared after adjustment for total energy intake.

The follow-up time for each participant was calculated from completion of the baseline questionnaire until diagnosis of the cancer of interest, the day of death or December 31, 1998, whichever came first. Risk ratios of cancer incidence were estimated by Cox proportional hazards analysis, using the SAS procedure PHREG (SAS, release 6.12, SAS Institute, North Carolina, USA). Initial analyses were adjusted for age and total energy intake only. The multivariate models were also adjusted for measured cancer risk factors: body mass index (quintiles), waist-to-hip ratio (quintiles), physical activity (low, moderate, or high, based on the frequency and intensity of leisure activity), pack-years of smoking, smoking status (current or former/never), number of years since quit smoking, alcohol intake, and total intake of fruits and vegetables. The models concerning hormone-related cancers (breast, uterus, ovary) were also adjusted for use of estrogen replacement therapy (current, former, never), age at menarche, age at menopause, and age at first life-birth. Other covariates that were considered included marital status, educational attainment, self reported diabetes, use of vitamin supplements, intake of whole grains, red meat, and coffee, and intake of saturated fatty acids, polyunsaturated fatty acids, cholesterol, fiber, and dietary vitamin C, vitamin E, folate, carotene, and calcium. Even though some of these variables were related to cancer risk, they did not appreciably alter the reported associations between catechin intake and cancer incidence, and were therefore not included in the multivariate models. Two-sided probability values for trends in risk ratios were based on category medians.

Table 6.2.1 Baseline characteristics by quintiles of catechin intake for 34,651 cancer free postmenopausal women, 1986. Iowa Women's Health Study

	Catechin intake quintile					
	1	2	3	4	5	P value[a]
Flavonoid intake						
Total catechins (mg/day)[b]	3.6 (1.5)[c]	8.7 (1.3)	14.8 (2.2)	24.7 (5.1)	75.1 (42.9)	
(+)-catechin	1.4 (0.7)	2.5 (0.9)	3.6 (1.2)	4.5 (2.1)	6.1 (3.2)	
(-)-epicatechin	1.9 (0.9)	5.0 (1.7)	8.9 (3.0)	11.2 (4.2)	18.2 (10.1)	
(+)-gallocatechin	0.0 (0.1)	0.1 (0.1)	0.2 (0.2)	0.6 (0.5)	3.5 (2.6)	
(-)-epigallocatechin	0.0 (0.1)	0.1 (0.2)	0.2 (0.3)	1.0 (0.8)	5.5 (4.1)	
(-)-epicatechin gallate	0.2 (0.4)	0.6 (0.7)	1.1 (1.5)	4.3 (3.5)	24.0 (18.1)	
(-)-epigallocatechin gallate	0.1 (0.3)	0.4 (0.5)	0.8 (1.1)	3.1 (2.6)	17.8 (13.5)	
Sum of (+)-catechin and (-)-epicatechin (mg/day)	3.2 (1.4)	7.5 (2.0)	12.5 (3.5)	15.7 (5.6)	24.3 (12.8)	
'Gallates' (mg/day)[d]	0.4 (0.8)	1.2 (1.5)	2.3 (3.2)	8.9 (7.4)	50.8 (38.3)	
Flavonols + flavones (mg/day)	5.3 (3.6)	7.8 (4.2)	10.7 (4.5)	14.5 (6.2)	31.4 (15.7)	0.0001
Demographics						
Age (y)	61.4 (4.1)	61.5 (4.2)	61.5 (4.1)	61.8 (4.2)	61.7 (4.2)	0.0001
Education (% > high school)	33.8	38.5	41.4	42.5	42.8	0.0001
Anthropometry						
Body mass index (kg/m^2)	26.9 (5.2)	27.0 (5.1)	27.0 (5.1)	27.0 (5.0)	27.0 (5.1)	0.17
Waist-to-hip ratio	0.845 (0.091)	0.837 (0.084)	0.834 (0.084)	0.834 (0.082)	0.836 (0.082)	0.0001
Self-reported illness						
Diabetes (%)	6.4	6.7	6.7	6.2	7.4	0.05
Lifestyle behaviors						
Current smoker (%)	25.0	16.5	11.6	10.1	11.8	0.0001
Pack-years of smoking	14.3 (21.0)	9.7 (17.9)	7.9 (16.4)	7.0 (15.3)	8.1 (17.1)	0.0001
Vitamin supplement use (%)	29.7	32.5	34.2	34.9	33.0	0.0001
Alcohol (% never drink)	53.1	54.5	55.2	54.0	58.0	0.0001
Physical activity (% *in moderate to high category*)	42.8	50.7	56.2	58.3	54.5	0.0001
Gynecological history						
Age at menarche (y)	12.9 (1.5)	12.9 (1.4)	12.8 (1.4)	12.8 (1.5)	12.8 (1.4)	0.0001
Age at first pregnancy (y)	20.5 (7.6)	20.7 (7.6)	20.6 (7.6)	20.9 (7.5)	20.8 (7.8)	0.06
Age at menopause (y)	47.1 (6.6)	47.7 (6.3)	47.9 (6.3)	47.8 (6.3)	47.9 (6.4)	0.0001
Hormone replacement therapy (% ever)	38.2	38.7	38.7	39.0	39.0	0.83
Diet[e]						
Energy intake (kcal/day)	1,529 (493.2)	1,709 (543.0)	1,844 (583.8)	1,967 (636.4)	1,946 (653.5)	0.0001
Alcohol intake (g/day)	5.9	3.8	3.3	2.8	3.0	0.0001
Saturated fat intake (g/day)	25.5	24.5	23.6	23.1	23.3	0.0001
Polyunsaturated fat intake (g/day)	12.1	12.1	12.1	12.0	12.1	0.71
Total fruit and vegetable intake (servings/week)	35.8	41.3	46.5	48.9	48.1	0.0001
Whole grain intake (servings/week)	9.9	11.0	11.8	12.1	11.8	0.0001

[a] Two sided test for any difference between catechin intake *quintiles*. [b] Sum of: (+)-catechin, (-)-epicatechin, (+)-gallocatechin, (-)-epigallocatechin, (-)-epicatechin gallate, and (-)-epigallocatechin gallate. [c] Values are means (standard deviation) or percentages where indicated. [d] Sum of: (+)-gallocatechin, (-)-epigallocatechin, (-)-epicatechin gallate, and (-)-epigallocatechin gallate. [e] All diet means, except energy intake, were adjusted for total energy intake.

RESULTS

The average intake of catechins at baseline among the 34,651 female postmenopausal participants was 25.4 mg/day (standard deviation: 32.3), it ranged from 0 to 277.7 mg/day, and only 26 participants had an estimated catechin intake of zero. The distribution of catechin intakes in the population was skewed, with the median intake (14.8 mg/day) considerably lower than the average intake. Major contributors to the total intake of catechins were tea (59%), and apples and pears (26%). Chocolate, fruits other than apples and pears, wine, and legumes were minor catechin sources. The median intake of 'gallates', catechins present mainly in tea, was 2.1 mg/day (range: 0-176.5). The median intake of catechins present predominantly in other foods, (+)-catechin and (-)-epicatechin, was 10.3 mg/day (range: 0-124.8). As shown in Table 6.2.1, catechin intake was associated with a healthy lifestyle.

Over 13 years of follow-up, 5038 incident primary cancers were recorded, including cancers of the upper digestive tract (n = 176), colon (n = 635), rectum (n = 132), bronchus and lung (n = 549), and breast (n = 1069). After adjustment for potential confounders (Table 6.2.2), only cancer of the rectum was statistically significantly inversely related to catechin intake (risk ratios (RRs) from lowest to highest quartile: 1.00, 0.93, 0.73, and 0.55; P for trend: 0.02). Inverse, but nonsignificant, associations were seen for cancer of the upper digestive tract (RRs across quartiles: 1.00, 0.71, 0.72, and 0.71; P for trend: 0.31), pancreas cancer (RRs across quartiles: 1.00, 0.56, 0.53, and 0.74; P for trend: 0.77), and hematopoietic cancer (RRs across quartiles: 1.00, 0.65, 0.83, and 0.65; P for trend: 0.14). Colon cancer was clearly not associated with catechin intake. Many of the crude RR estimates were severely confounded, particularly through the inverse relation between catechin intake and smoking. After adjustment for potential confounders, catechin intake was therefore not associated with total cancer incidence, smoking-related cancers other than those mentioned above , or hormone-related cancers.

Table 6.2.3 shows the RRs of digestive tract cancers with (+)-catechin plus (-)-epicatechin (top panel) and with 'gallates' (bottom panel). After adjustment for confounders, and for (+)-catechin and (-)-epicatechin intake, intake of 'gallates', catechins derived mainly from tea, was inversely associated with the risk of rectal cancer (RRs across quartiles: 1.00, 0.56, 0.66, and 0.39; P for trend: 0.02). The intake of (+)-catechin and (-)-epicatechin, food derived catechins, was not associated with rectal cancer risk. In line with this finding, we found RRs smaller than the reference value for distal colon cancer risk with 'gallate' intake (RRs across quintiles:

Table 6.2.2 Risk ratios (RR) and 95% confidence intervals (CI) of cancer incidence by site according to categories of catechin intake for 34,651 cancer free postmenopausal women, 1986-1998

	Catechin intake category													
	1		2			3			4			5		
Cancer site (ICD-O codes)	n[a]	RR	n	RR	95% CI	n	RR	95% CI	n	RR	95% CI	n	RR	95% CI	P for trend
Any cancers combined															
age and energy adjusted	1103	1.00	971	0.86	(0.79-0.94)	1041	0.92	(0.85-1.01)	948	0.82	(0.75-0.89)	975	0.85	(0.78-0.93)	0.009
multivariate adjusted[b]		1.00		0.92	(0.84-1.01)		1.05	(0.96-1.15)		0.94	(0.85-1.03)		0.97	(0.88-1.06)	0.65
Upper digestive tract (C0-C17)															
age and energy adjusted	59	1.00	39	0.59	(0.40-0.90)	38	0.55	(0.36-0.83)	40	0.57	(0.38-0.86)				0.06
multivariate adjusted		1.00		0.71	(0.46-1.09)		0.72	(0.46-1.12)		0.71	(0.46-1.11)				0.31
Colon (C18)															
age and energy adjusted	123	1.00	128	1.05	(0.82-1.34)	128	1.07	(0.83-1.38)	134	1.12	(0.87-1.44)	122	1.03	(0.79-1.33)	0.99
multivariate adjusted		1.00		1.06	(0.82-1.37)		1.15	(0.89-1.50)		1.19	(0.91-1.55)		1.10	(0.85-1.44)	0.63
Proximal colon (C18.0-4)															
age and energy adjusted	74	1.00	57	0.78	(0.55-1.11)	67	0.95	(0.68-1.33)	76	1.08	(0.77-1.50)	78	1.12	(0.80-1.55)	0.15
multivariate adjusted		1.00		0.80	(0.56-1.15)		1.04	(0.73-1.47)		1.10	(0.78-1.57)		1.18	(0.84-1.66)	0.11
Distal colon (C18.5-7)															
age and energy adjusted	45	1.00	65	1.43	(0.98-2.10)	61	1.36	(0.92-2.01)	55	1.21	(0.81-1.81)	42	0.93	(0.61-1.43)	0.15
multivariate adjusted		1.00		1.44	(0.97-2.13)		1.46	(0.97-2.19)		1.37	(0.90-2.09)		1.04	(0.67-1.62)	0.37
Rectum (C20)															
age and energy adjusted	43	1.00	37	0.84	(0.54-1.31)	30	0.67	(0.42-1.09)	22	0.49	(0.29-0.83)				0.008
multivariate adjusted		1.00		0.93	(0.59-1.46)		0.73	(0.44-1.21)		0.55	(0.32-0.95)				0.02
Pancreas (C25)															
age and energy adjusted	45	1.00	25	0.52	(0.32-0.86)	24	0.49	(0.29-0.81)	36	0.73	(0.46-1.14)				0.72
multivariate adjusted		1.00		0.56	(0.33-0.93)		0.53	(0.31-0.90)		0.74	(0.46-1.20)				0.77
Bronchus and lung (C34)															
age and energy adjusted	182	1.00	99	0.52	(0.41-0.67)	92	0.48	(0.37-0.62)	78	0.40	(0.30-0.52)	98	0.50	(0.39-0.65)	0.0003
multivariate adjusted		1.00		0.78	(0.60-1.01)		0.97	(0.74-1.27)		0.85	(0.63-1.13)		0.94	(0.72-1.23)	0.94

Table 6.2.2 Continued

	Catechin intake category													
	1		2			3			4			5			
Cancer site (ICD-O codes)	n[a]	RR	n	RR	95% CI	n	RR	95% CI	n	RR	95% CI	n	RR	95% CI	P for trend
Hematopoietic (C42)															
age and energy adjusted	69	1.00	46	0.62	(0.43-0.90)	58	0.76	(0.53-1.08)	45	0.58	(0.40-0.86)				0.04
multivariate adjusted		1.00		0.65	(0.44-0.98)		0.83	(0.56-1.23)		0.65	(0.43-0.98)				0.14
Breast (C50)[c]															
age and energy adjusted	209	1.00	201	0.95	(0.78-1.16)	245	1.16	(0.96-1.39)	199	0.92	(0.75-1.12)	215	1.00	(0.83-1.22)	0.87
multivariate adjusted[d]		1.00		0.98	(0.80-1.20)		1.23	(1.00-1.50)		0.98	(0.79-1.22)		1.04	(0.84-1.28)	1.00
Uterus (C54-C55)[c]															
age and energy adjusted	87	1.00	77	0.89	(0.66-1.22)	89	1.04	(0.77-1.41)	100	1.17	(0.87-1.57)				0.13
multivariate adjusted[d]		1.00		0.81	(0.59-1.12)		0.92	(0.67-1.27)		1.00	(0.73-1.36)				0.54
Ovary (C56)[c]															
age and energy adjusted	38	1.00	38	0.99	(0.63-1.56)	44	1.14	(0.73-1.78)	31	0.80	(0.49-1.30)				0.30
multivariate adjusted[d]		1.00		0.92	(0.57-1.48)		1.08	(0.67-1.74)		0.73	(0.44-1.24)				0.21
Kidney and renal pelvis (C64-C65)															
age and energy adjusted	27	1.00	38	1.37	(0.83-2.25)	25	0.89	(0.51-1.55)	24	0.85	(0.48-1.49)				0.23
multivariate adjusted		1.00		1.24	(0.74-2.08)		0.82	(0.46-1.46)		0.73	(0.40-1.32)				0.12
Bladder (C67)															
age and energy adjusted	36	1.00	39	1.10	(0.70-1.75)	28	0.80	(0.48-1.32)							0.27
multivariate adjusted		1.00		1.56	(0.95-2.56)		1.12	(0.65-1.93)							0.93
Non-Hodgkin's lymphoma (M959,M967-M971)															
age and energy adjusted	51	1.00	63	0.90	(0.62-1.31)	58	0.91	(0.63-1.33)	68	1.12	(0.78-1.60)				0.29
multivariate adjusted		1.00		1.00	(0.68-1.48)		0.98	(0.65-1.46)		1.26	(0.87-1.85)				0.13

[a] n: number of cases. [b] Adjusted for age, total energy intake, body mass index, waist-to-hip ratio, physical activity, pack-years of smoking, smoking status, number of years since quit smoking, alcohol intake, and total fruit and vegetable consumption (n = 33,339). [c] Exclusion of women with baseline mastectomy, hysterectomy, or oophorectomy left 21,502 women for analysis. [d] Adjusted for use of estrogen replacement therapy, age at menarche, age at menopause, age at first pregnancy, and other covariates listed under [b].

Table 6.2.3 Risk ratios (RR) and 95% confidence intervals (CI) of colon and rectal cancer incidence according to categories of intake of the sum of (+)-catechin and (-)-epicatechin and of the sum of (+)-gallocatechin, (-)-epigallocatechin, (-)-epicatechin gallate, and (-)-epigallocatechin gallate ('gallates') for 34,651 cancer free postmenopausal women, 1986-1998

	Intake category													
	1		2			3			4			5		
Cancer site (ICD-O codes)	n[a]	RR	n	RR	95% CI	n	RR	95% CI	n	RR	95% CI	n	RR	95% CI	P for trend
Sum of (+)-catechin and (-)-epicatechin[b]															
Upper digestive tract (C0-C17)															
age and energy adjusted	55	1.00	45	0.78	(0.52-1.16)	36	0.58	(0.37-0.91)	40	0.60	(0.37-0.97)				0.02
multivariate adjusted[c]		1.00		0.89	(0.58-1.35)		0.74	(0.46-1.19)		0.77	(0.45-1.30)				0.27
Colon (C18)															
age and energy adjusted	128	1.00	139	1.11	(0.86-1.42)	122	0.99	(0.76-1.29)	126	1.01	(0.77-1.32)	120	0.96	(0.71-1.29)	0.44
multivariate adjusted		1.00		1.12	(0.87-1.44)		1.01	(0.76-1.33)		1.04	(0.79-1.38)		1.04	(0.76-1.43)	0.90
Proximal colon (C18.0-4)															
age and energy adjusted	75	1.00	62	0.82	(0.58-1.16)	63	0.85	(0.59-1.21)	78	1.02	(0.73-1.45)	74	0.96	(0.65-1.42)	0.69
multivariate adjusted		1.00		0.86	(0.60-1.22)		0.88	(0.61-1.29)		1.08	(0.75-1.55)		1.06	(0.70-1.61)	0.39
Distal colon (C18.5-7)															
age and energy adjusted	48	1.00	71	1.56	(1.07-2.27)	59	1.33	(0.89-2.00)	46	1.04	(0.67-1.59)	44	1.01	(0.63-1.63)	0.19
multivariate adjusted		1.00		1.51	(1.03-2.21)		1.31	(0.86-2.00)		1.06	(0.68-1.66)		1.09	(0.65-1.80)	0.42
Rectum (C20)															
age and energy adjusted	40	1.00	34	0.95	(0.60-1.52)	34	1.03	(0.63-1.68)	24	0.80	(0.45-1.43)				0.36
multivariate adjusted		1.00		1.03	(0.64-1.66)		1.12	(0.67-1.87)		0.92	(0.50-1.71)				0.75
'Gallates'[b]															
Upper digestive tract (C0-C17)															
age and energy adjusted	49	1.00	46	0.76	(0.50-1.14)	42	0.79	(0.51-1.21)	39	0.84	(0.52-1.35)				0.96
multivariate adjusted		1.00		0.85	(0.56-1.30)		0.94	(0.61-1.45)		0.91	(0.56-1.49)				0.95
Colon (C18)															
age and energy adjusted	132	1.00	128	0.79	(0.62-1.01)	111	0.80	(0.62-1.04)	132	0.90	(0.70-1.15)	132	0.93	(0.71-1.23)	0.40
multivariate adjusted		1.00		0.81	(0.63-1.05)		0.84	(0.64-1.09)		0.94	(0.72-1.21)		0.95	(0.72-1.25)	0.44
Proximal colon (C18.0-4)															
age and energy adjusted	65	1.00	75	0.97	(0.69-1.36)	56	0.85	(0.59-1.23)	73	1.06	(0.75-1.51)	83	1.17	(0.81-1.69)	0.17
multivariate adjusted		1.00		1.03	(0.73-1.45)		0.89	(0.61-1.29)		1.09	(0.76-1.56)		1.18	(0.81-1.72)	0.25
Distal colon (C18.5-7)															
age and energy adjusted	63	1.00	51	0.63	(0.43-0.91)	52	0.74	(0.51-1.08)	56	0.73	(0.50-1.07)	46	0.68	(0.45-1.04)	0.65
multivariate adjusted		1.00		0.63	(0.42-0.92)		0.78	(0.53-1.15)		0.79	(0.54-1.16)		0.71	(0.46-1.09)	0.76
Rectum (C20)															
age and energy adjusted	48	1.00	31	0.53	(0.33-0.83)	33	0.61	(0.39-0.97)	20	0.40	(0.22-0.71)				0.04
multivariate adjusted		1.00		0.56	(0.35-0.89)		0.66	(0.41-1.04)		0.39	(0.22-0.71)				0.02

[a] n: number of cases. [b] All risk ratios also adjusted for other catechins listed in Table. [c] Adjusted for age, total energy intake, body mass index, waist-to-hip ratio, physical activity, pack-years of smoking, smoking status, number of years since quit smoking, alcohol intake, and total fruit and vegetable consumption (n = 33,339).

1.00, 0.63, 0.78, 0.79, and 0.71; P for trend: 0.76), but not with (+)-catechin plus (-)-epicatechin intake. Proximal colon cancer risk was not associated with intake of either groups of catechins. Contrary to our findings for the lower digestive tract, the risk of cancer of the upper digestive tract tended to be inversely related to intake of (+)-catechin and (-)-epicatechin (RRs across quartiles: 1.00, 0.89, 0.74, and 0.77; P for trend: 0.27), but not of 'gallates'. There were no clearly distinct associations for the two groups of catechins with other cancers (data not shown).

DISCUSSION

In this cohort of postmenopausal women, total catechin intake was inversely and significantly associated with rectal cancer incidence. There were inverse, but nonsignificant, associations with upper digestive tract, pancreatic, and hematopoietic cancers. This is the first study of catechin intake and cancer incidence at sites other than the lung. Among 728 elderly Dutch men, catechin intake was not associated with total epithelial cancer incidence, lung cancer or non-lung epithelial cancers (Chapter 6.1). Our data generally do not support evidence from animal experiments which suggests that catechins may prevent a number of cancers.[6,7] Proposed anti-cancer mechanisms include inhibition of the metabolic activation of procarcinogens to DNA-reactive species, induction of enzyme systems involved in detoxification, and protection against DNA damaging free radicals through their antioxidant activity.[3,16]

The intake of (+)-catechin and (-)-epicatechin, a subgroup of catechins derived primarily from apple and other fruits, was inversely though not significantly associated with upper digestive tract cancer incidence only. The evidence for a protective effect of diets high in fruits and vegetables against cancer of the mouth and pharynx, esophagus, and stomach was considered convincing according to the 1997 World Cancer Research Fund report on nutrition and cancer.[17] Our suggestive finding for fruit-derived catechins is in line with this large body of evidence, even though one study found no inverse association of apple consumption with stomach cancer incidence.[18]

On the other hand, the intake of 'gallates', catechins derived from tea, was inversely associated with rectal cancer, but not with any other sites. The RRs for distal colon cancer were smaller than the reference value although there was no clear trend. Most literature does not support an inverse association between tea consumption and rectal cancer. Of four prospective cohort studies, one found an increased rectal cancer risk with increasing black tea consumption,[19] and three found no association.[20-22] Of nine case-control studies,[8,23,24] only one[25] found an inverse

association with rectal cancer, and two found a positive association.[26,27] A major drawback of all these studies, except for the cohort studies and the one case-control study that reported an inverse association, is that they were conducted in countries were black tea consumption is extremely low (Japan and Italy) and therefore unlikely to exert any physiological effects. Data on colon cancer and tea consumption are equally unconvincing.[8] The one case-control study reporting on colon subsites found an inverse association between green tea consumption and distal colon adenomas but not proximal colon adenomas,[28] consistent with our findings. Even though colon and rectal cancer share many environmental risk factors, there are reported differences in incidence rates,[29] risk factors,[30] and tumor morphology.[31,32] These differences may explain our finding of distinct effects of tea consumption on colorectal tumors in different parts of the intestine.

There are several possible explanations for our results. The first explanation relates to limitations of the study possibly leading to attenuation of true associations. Although the questionnaire has reasonable validity,[13,14] misclassification of dietary exposure could have occurred at both the dietary assessment level, and in the assignment of catechin levels to the foods reported. Catechin content of foods was assessed primarily in the Netherlands,[10,11] supplemented by a few foods purchased in Minnesota. Catechin contents vary greatly by variety;[10,33] thus individual preferences for particular varieties of foods would lead to misclassification. Also, the catechin content of tea infusions depends, among other things, on the brewing time.[11] The standard brewing method used to determine catechin values for tea involved a 2-g tea bag in 200 mL of boiled tap water for 5 minutes, which may be too long for US habits. We therefore may have overestimated the catechin content of US tea. Some characteristics of our study population may have contributed to the lack of hypothesized associations: the Iowa Women's Health Study included largely healthy women with a relatively low intake of catechins, as evidenced by the median intake of only 14.8 mg/day. Perhaps higher intakes are required for physiological effects. It has been hypothesized that antioxidants, such as catechins, are most beneficial in subjects with an otherwise unhealthy lifestyle.[34] However, analyses among current smokers did not yield stronger associations (data not shown).

A second possible interpretation is that the few inverse associations that we observed were artefactual. Clearly, some of the statistically significant findings may have been due to chance, given the large number of comparisons made. Moreover, intake of (+)-catechin plus (-)-epicatechin is correlated with total fruit consumption (Pearson r=0.50), and with total vegetable consumption (r=0.27). Fruit and vegetable consumption has been associated with reduced risks of digestive tract cancers,[17] and

consumption levels of fruits and vegetables are notoriously difficult to estimate reliably. Therefore, even after multivariate adjustment, the inverse association between (+)-catechin and (-)-epicatechin intake and upper digestive tract cancers may have been due to residual confounding by fruit and vegetable consumption. However, this is at odds with the evidence from animal experiments, which strongly points towards protective effects of catechins against various cancers through biologically plausible pathways.[7] And, on the other hand, overadjustment by fruit and vegetable consumption is a concern because fruits are catechin sources. Adjustment for vegetables only did not substantially change reported associations (data not shown).

Alternatively, differences in bioavailability and metabolism between catechins contained in foods and beverages may potentially explain our data. Catechins are absorbed from the small intestine into the blood,[35] but until now, the absorption process is not very well characterized. Regarding our findings for the upper digestive tract, a relevant difference between (+)-catechin plus (-)-epicatechin versus 'gallates' is that the major sources of the former are solid foods, while the major source of the latter is tea. Catechins from tea will have a lower contact time with the upper digestive tract than catechins from solid foods, which require chewing and move more slowly through the gastrointestinal tract. A recent study showed that catechin concentrations in saliva were increased four to five times when a tea solution was held in the mouth for 5 minutes compared to 1 minute.[36] This time-dependent absorption might result in higher exposure of endothelial cells of the gastrointestinal tract to catechins from slow moving solid foods compared to liquids. Some of the ingested catechins will not be absorbed in the upper digestive tract and will proceed into the colon, where bacteria have been shown to degrade catechins into compounds that have *in vitro* antioxidant activity themselves.[16] Only approximately 50% of radioactively labeled (+)-catechin was recovered in urine after oral administration to rodents, monkeys, and man.[35] Also, polymerization products of catechins found in black tea (e.g. thearubigins) are too large to be absorbed in the small intestine, and are important substrates for colonic fermentation.[37,38] Therefore, the reduced risk of rectal cancer we found may be due to catechin metabolites or metabolites of other tea components, rather than the compounds themselves. Catechins from tea, as compared to those from foods, might yield higher concentrations of antioxidant metabolites in the fecal mass because of the high concentration of catechins and catechin polymers present in black tea beverage solids (up to 40%),[39] and the low amount of matrix material in tea. Further studies on the bioavailability and metabolism of catechins, and their interactions with other dietary components, are necessary to substantiate whether the fluid or solid state of

a catechin source is a discriminating feature of its functionality, which may have implications for the design of future intervention trials.

ACKNOWLEDGMENTS

The authors would like to thank Betty van de Putte and Ching-Ping Hong for excellent technical assistance and Drs. Feskens, Hollman, Bueno de Mesquita, and Kromhout for valuable comments on the manuscript. This work was supported in part by grant CA-39742 from the National Cancer Institute, and by the Commission of the European Communities Agriculture and Fisheries (FAIR) specific RTD Programme CT95 0653. Ilja Arts was also supported by a travel grant from the Netherlands Organization for Scientific Research (NWO).

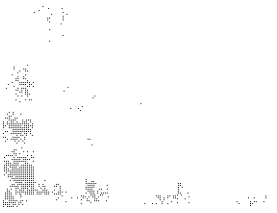
REFERENCES

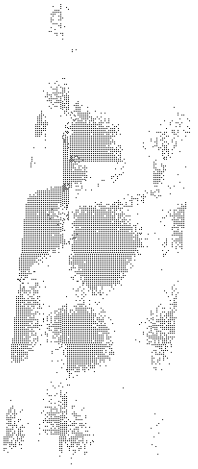
1. World Cancer Research Fund. Vegetables and fruits. In: WCRF/AICR. *Food, nutrition and the prevention of cancer: a global perspective*. Washington (DC): World Cancer Research Fund/American Institute for Cancer Research;1997:436-446.
2. World Cancer Research Fund. Other bioactive compounds. In: WCRF/AICR. *Food, nutrition and the prevention of cancer: a global perspective*. Washington (DC): World Cancer Research Fund/American Institute for Cancer Research;1997:421-427.
3. Middleton E, Kandaswami C. The impact of plant flavonoids on mammalian biology: implications for immunity, inflammation and cancer. In: Harborne JB, ed. *The Flavonoids: advances in research since 1986*. London: Chapman & Hall;1994:619-652.
4. Harborne JB. *The Flavonoids: Advances in research since 1986*. London: Chapman & Hall; 1994.
5. Harbowy ME, Balentine DA. Tea chemistry. *Crit Rev Plant Sciences* 1997;16:415-480.
6. Dreosti IE, Wargovich MJ, Yang CS. Inhibition of carcinogenesis by tea: The evidence from experimental studies. *Crit Rev Food Sci Nutr* 1997;37:761-770.
7. Katiyar SK, Mukhtar H. Tea in chemoprevention of cancer: epidemiologic and experimental studies (review). *Int J Oncol* 1996;8:221-238.
8. Blot WJ, Chow WH, McLaughlin JK. Tea and cancer: a review of the epidemiological evidence. *Eur J Cancer Prev* 1996;5:425-438.
9. Bushman JL. Green tea and cancer in humans: a review of the literature. *Nutr Cancer* 1998;31:151-159.
10. Arts ICW, Van de Putte B, Hollman PCH. Catechin contents of foods commonly consumed in The Netherlands. 1. Fruits, vegetables, staple foods, and processed foods. *J Agric Food Chem* 2000;48:1746-1751.
11. Arts ICW, Van de Putte B, Hollman PCH. Catechin contents of foods commonly consumed in The Netherlands. 2. Tea, wine, fruit juices, and chocolate milk. *J Agric Food Chem* 2000;48:1752-1757.
12. Willett WC, Sampson L, Browne ML, Stampfer MJ, Rosner B, Hennekens CH, Speizer FE. The use of a self-administered questionnaire to assess diet four years in the past. *Am J Epidemiol* 1988;127:188-199.
13. Feskanich D, Rimm EB, Giovannucci EL, Colditz GA, Stampfer MJ, Litin LB, Willett WC. Reproducibility and validity of food intake measurements from a semiquantitative food frequency questionnaire. *J Am Diet Assoc* 1993;93:790-796.
14. Munger RG, Folsom AR, Kushi LH, Kaye SA, Sellers TA. Dietary assessment of older Iowa women with a food frequency questionnaire: nutrient intake, reproducibility, and comparison with

24-hour dietary recall interviews. *Am J Epidemiol* 1992;136:192-200.
15. Putnam JJ, Allshouse JE. *Food consumption, prices, and expenditures, 1970-1995. Statistical Bulletin number 939.* Washington (DC): Economic Research Service/USDA; 1997.
16. Rice-Evans CA, Miller NJ, Paganga G. Structure-antioxidant activity relationships of flavonoids and phenolic acids. *Free Radical Biol Med* 1996;20:933-956.
17. World Cancer Research Fund. *Food, nutrition and the prevention of cancer: a global perspective.* Washington (DC): World Cancer Research Fund/American Institute for Cancer Research; 1997.
18. Botterweck AAM, Van den Brandt PA, Goldbohm RA. A prospective cohort study on vegetable and fruit consumption and stomach cancer risk in the Netherlands. *Am J Epidemiol* 1998;148:842-853.
19. Heilbrun LK, Nomura A, Stemmermann GN. Black tea consumption and cancer risk: a prospective study. *Br J Cancer* 1986;54:677-683.
20. Hartman TJ, Tangrea JA, Pietinen P, Malila N, Virtanen M, Taylor PR, Albanes D. Tea and coffee consumption and risk of colon and rectal cancer in middle-aged Finnish men. *Nutr Cancer* 1998;31:41-48.
21. Goldbohm RA, Hertog MGL, Brants HA, Van Poppel G, Van den Brandt PA. Consumption of black tea and cancer risk: a prospective cohort study. *J Natl Cancer Inst* 1996;88:93-100.
22. Kinlen LJ, Willows AN, Goldblatt P, Yudkin J. Tea consumption and cancer. *Br J Cancer* 1988;58:397-401.
23. Inoue M, Tajima K, Hirose K, Hamajima N, Takezaki T, Kuroishi T, Tominaga S. Tea and coffee consumption and the risk of digestive tract cancers: data from a comparative case-referent study in Japan. *Cancer Causes Control* 1998;9:209-216.
24. Tavani A, Pregnolato A, LaVecchia C, Negri E, Talamini R, Franceschi S. Coffee and tea intake and risk of cancers of the colon and rectum: a study of 3,530 cases and 7,057 controls. *Int J Cancer* 1997;73:193-197.
25. Baron JA, Gerhardsson de Verdier M, Ekbom A. Coffee, tea, tobacco, and cancer of the large bowel. *Cancer Epidemiol Biomarkers Prev* 1994;3:565-570.
26. La Vecchia C, Negri E, Franceschi S, D'Avanzo B, Boyle P. Tea consumption and cancer risk. *Nutr Cancer* 1992;17:27-31.
27. La Vecchia C, Negri E, Decarli A, D'Avanzo B, Gallotti L, Gentile A, Franceschi S. A case-control study of diet and colo-rectal cancer in northern Italy. *Int J Cancer* 1988;41:492-498.
28. Kato I, Tominaga S, Matsuura A, Yoshii Y, Shirai M, Kobayashi S. A comparative case-control study of colorectal cancer and adenoma. *Jpn J Cancer Res* 1990;81:1101-1108.
29. Schottenfeld D, Winawer SJ. Cancers of the large intestine. In: Schottenfeld D, Fraumeni Jr JF, eds. *Cancer epidemiology and prevention.* New York: Oxford University Press;1996:813-840.
30. Potter JD. Colorectal cancer: molecules and populations. *J Natl Cancer Inst* 1999;91:916-932.
31. Konishi K, Fujii T, Boku N, Kato S, Koba I, Ohtsu A, Tajiri H, Ochiai A, Yoshida S. Clinicopathological differences between colonic and rectal carcinomas: are they based on the same mechanism of carcinogenesis? *Gut* 1999;45:818-821.
32. Crawford JM. The gastrointestinal tract. In: Cotran RS, Kumar V, Collins T, eds. *Robbins pathologic basis of disease* . Philadelphia: W.B. Saunders Company;1999:775-843.
33. Risch B, Herrmann K. Die Gehalte an Hydroxyzimtsäure-Verbindungen und Catechinen in Kern- und Steinobst (Contents of hydroxycinnamic acid derivatives and catechins in pome and stone fruit). *Z Lebensm Unters Forsch* 1988;186:225-230.
34. Diplock AT, Charleux JL, Crozier-Willi G, Kok FJ, Rice-Evans C, Roberfroid M, Stahl W, Vina-Ribes J. Functional food science and defence against reactive oxidative species. *Br J Nutr* 1998;80 Suppl 1:S77-112.

35. Hollman PCH, Arts ICW. Flavonols, flavones and flavanols - nature, occurrence and dietary burden. *J Sci Food Agric* 2000;80:1081-1093.
36. Yang CS, Lee MJ, Chen LS. Human salivary tea catechin levels and catechin esterase activities: Implication in human cancer prevention studies. *Cancer Epidemiol Biomarkers Prev* 1999;8:83-89.
37. Bailey RG, Nursten HE, McDowell I. Comparative study of the reversed-phase high-performance liquid chromatography of black tea liquors with special reference to the thearubigins. *J Chromatogr* 1991;542:115-128.
38. Clifford MN, Copeland EL, Bloxsidge JP, Mitchell LA. Hippuric acid as a major excretion product associated with black tea consumption. *Xenobiotica* 2000;30:317-326.
39. Balentine DA, Wiseman SA, Bouwens LCM. The chemistry of tea flavonoids. *Crit Rev Food Sci Nutr* 1997;37:693-704.

Discussion and conclusions





INTRODUCTION

The aim of the work described in this thesis was to examine the associations between dietary catechin intake and risk of coronary heart disease (CHD), stroke, and cancer. In order to do this, we first had to develop an analytical method to determine catechin contents of foods, and with this method to compile a catechin food composition table. Food composition tables are a prerequisite for epidemiological studies on (non)nutrients and diseases, but the importance of high-quality food composition data is often underestimated. After recapitulating our main findings, a number of methodological issues related to the work described in this thesis will be discussed. Given our findings, but also considering broader developments in the field of (non)nutrients and chronic diseases, we have attempted to provide directions for further research.

MAIN FINDINGS

Catechins in foods and beverages

We first optimized an analytical method for the quantification of catechins in foods (Chapter 2). Adequate extraction was attained with 40-100% methanol in water, while extraction time and sample quantity, within given limits, did not determine yield. Catechins do not occur as glycosides; it was therefore not necessary to hydrolyze the food samples. The catechins were separated with gradient reversed phase HPLC within 37 minutes. The specific method of detection, UV and fluorescence in series, made sample clean up superfluous. Recovery (>94%), reproducibility (relative standard deviation <5%), and limits of detection were satisfactory, and comparable to or better than those of previously published methods.

Most fruits, some legumes, and chocolate contained catechins, while vegetables and staple foods such as potatoes and rice did not (Chapter 3.1). Concentrations in solid foods varied from low (4.5 mg/kg in kiwi fruit) to very high (610 mg/kg in black chocolate). Tea and red wine contained substantial quantities of catechins (up to 418 mg/L in Ceylon blend black tea), while low to negligible amounts were found in white wine, commercially available fruit juices, ice-tea, and chocolate milk (Chapter 3.2). Catechins were not detected in beer and coffee. Tea was the only food that contained all six catechins studied; only (+)-catechin and (-)-epicatechin were present in other beverages, and these were also the most common catechins in solid foods. Preparation of foods caused a decrease in catechin concentrations, but since most catechin-containing foods are consumed raw, this is not likely to be an important determinant of catechin intake. In contrast to flavonols, which are present mainly in

the peel of apples,[1] catechins were found in both apple peel and pulp. Catechin concentrations decreased by only 23% when the peel was removed. Seasonal variation in catechin concentrations was small for some fruits, yet large for others, and was probably related in part to maturity and variety of the fruit available in a given season. Catechin concentrations of tea brews were mainly determined by tea blend and the amount of tea used for brewing, and less by brand and infusion time.

The average intake of catechins in a representative sample of Dutch men and women aged 1-97 years was 50 mg/day (Chapter 4). Women had a higher catechin intake (60 mg/day) than men (40 mg/day), and the intake increased with age among both sexes. The major catechin sources in children, adults and elderly were tea, apples plus pears, and chocolate. Of the individual catechins, (-)-epicatechin gallate (ECg) contributed most to the total catechin intake, followed by (-)-epigallocatechin gallate (EGCg) and (-)-epicatechin. (+)-Gallocatechin (GC), (-)-epigallocatechin (EGC), and (+)-catechin were minor contributors. Subjects with a high intake of catechins tended to also have a high intake of vitamin C, beta-carotene, and fiber, consumed less alcohol, and were more often non-smokers. There was an increase in catechin intake with increasing socio-economic status.

Catechins and cardiovascular diseases

The association between catechin intake and risk of cardiovascular diseases was studied in elderly men in the Netherlands and in postmenopausal women in the USA. Findings have been summarized in Table 7.1. The textbox on page 133 gives a brief description of the definition of exposure variables. Among men that participated in the Zutphen Elderly Study (Chapter 5.1), catechin intake was inversely associated with CHD mortality (risk ratio in highest tertile of intake: 0.49; P for trend: 0.02), but not with myocardial infarction incidence, after adjusting for cardiovascular disease risk

Table 7.1 Summary of main findings on the relation between dietary catechins and cardiovascular diseases

	coronary heart disease mortality	myocardial infarction incidence	stroke
Zutphen Elderly Study			
total catechins	↓↓	0	0
tea catechins	↓	-	-
non-tea catechins	↓	-	-
Iowa Women's Health Study			
total catechins	0	-	-
tea catechins	0	-	-
non-tea catechins	↓↓	-	-

↓↓: P<0.05; ↓: non-significant trend; 0: no association; -: not determined.

factors including diet. Stroke mortality or incidence was not related to the intake of catechins. Tea supplied 87% of the population's catechin intake. After adjusting for cardiovascular disease risk factors including dietary factors like flavonols and flavones, both tea and non-tea catechins were inversely associated with CHD mortality risk, albeit with borderline significance.

Among postmenopausal women that participated in the Iowa Women's Health Study, total catechin intake was not clearly associated with CHD mortality (Chapter 5.2). Tea supplied 59% of the catechin intake of the participants. The intake of tea catechins was not associated with CHD mortality, whereas the intake of non-tea catechins was inversely associated with CHD mortality (risk ratio in highest quintile of intake: 0.76; P for trend: 0.02). Apples plus pears, and wine were the foods responsible for the observed inverse association. We found no evidence for a protective effect among women with a high baseline risk of developing CHD. The inverse association with non-tea catechins was limited to apparently healthy non-smoking women free of cardiovascular disease and diabetes at baseline (risk ratio in highest quintile of intake: 0.76; P for trend: 0.03).

Catechins and cancer

Again, we used data from the Netherlands and the USA to evaluate the association between catechin intake and cancer. Among Dutch elderly men we studied epithelial cancers and lung cancer, while among American women we studied cancers at individual sites. The main findings have been summarized in Table 7.2. Catechin intake was not associated with the incidence of epithelial cancer in the Zutphen Elderly Study, nor with the incidence of lung cancer, or epithelial cancers other than lung cancer (Chapter 6.1). Tea catechins were not associated with these cancers either, but we did observe an inverse trend between non-tea catechins and lung

Table 7.2 Summary of main findings on the relation between dietary catechins and cancer incidence [a]

	any cancer	lung	epithelial other than lung	rectum
Zutphen Elderly Study				
total catechins	-	0	0	-
tea catechins	-	0	0	-
non-tea catechins	-	↓	0	-
Iowa Women's Health Study				
total catechins	0	0	-	↓↓
tea catechins	0	0	-	↓↓
non-tea catechins	0	0	-	0

[a] No statistically significant associations were found for cancers studied but not listed in this table.

↓↓: P<0.05; ↓: non-significant trend; 0: no association; -: not determined.

cancer incidence (risk ratio for one standard deviation (7.2 mg) increase in intake: 0.66; 95% confidence interval: 0.42-1.05). Apple appeared the catechin source primarily responsible for the inverse association with lung cancer.

Our findings from the Iowa Women's Health Study did not confirm the Zutphen data: catechin intake was not associated with lung cancer incidence in Iowa women In Chapter 6.2, data for tea and non-tea catechins were tabulated for only a limited number of cancer sites; results for all other sites did not differ between these catechin subgroups. Among several cancers studied, we found a significant inverse association of catechin intake with rectal cancer incidence (risk ratio in highest quartile of intake: 0.55; P for trend: 0.02), and inverse, but nonsignificant, associations with upper digestive tract, pancreatic and hematopoietic cancers. The intake of tea catechins was inversely associated with rectal cancer, whereas the intake of non-tea catechins showed an inverse trend with upper-digestive tract cancer.

DEFINITION OF EXPOSURE VARIABLES

Statistical analyses were performed depending on the limitations and opportunities offered by the two epidemiological cohort studies, and are therefore not exactly the same. The effect of total catechin intake was assessed in all studies reported. Total catechin intake, or catechin intake, was defined as the sum of (+)-catechin, (-)-epicatechin, (+)-gallocatechin (GC), (-)-epigallocatechin (EGC), (-)-epicatechin gallate (ECg), and (-)-epigallocatechin gallate (EGCg). The high degree of correlation between each individual's intake of these catechins, prevented us from determining independent effects of each catechin. Instead, in the Zutphen Elderly Study, we created two new variables: catechins from tea and catechins from sources other than tea (Chapter 6.1). These variables were created by converting each individual's consumption of tea on the one hand, and all other foods on the other hand, into milligrams of catechins per day. The variable tea in Chapter 5.1 is therefore identical to the variable catechins from tea in Chapter 6.1. In the Iowa Women's Health Study, a slightly different approach was followed (Chapters 5.2 and 6.2). There, we created two variables as well: catechins derived mainly from tea (defined as the sum of GC, EGC, ECg, and EGCg) and catechins derived mainly from sources other than tea (defined as the sum of (+)-catechin and (-)-epicatechin). These variables were created by first calculating each individual's intake of these catechins, and then adding them up. The major difference between the Zutphen and the Iowa approach is that tea contains considerable quantities of (-)-epicatechin, in the Iowa Women's Health Study it provided 29% of the average (-)-epicatechin intake. Thus, strictly speaking, the variables used in the Iowa Women's Health Study cannot be referred to as tea catechins and non-tea catechins. Nevertheless, when we entered catechins from tea and catechins from sources other than tea in the models tested in the Iowa Women's Health study, the results were essentially the same. Therefore, in this discussion, we will use the terms tea catechins and non-tea catechins when referring to the subgroup analyses reported in Chapters 5.1, 5.2, 6.1, and 6.2 of this thesis. Apple was the main non-tea catechin source in both the Zutphen Elderly Study (8% of total catechin intake) and the Iowa Women's Health Study (26% of total catechin intake), followed by chocolate (3% and 6%, respectively).

CATECHIN INTAKE ASSESSMENT

To avoid misinterpretation of our findings, it is essential to examine in more detail some methodological issues related to studies on diet and chronic diseases. First, misclassification of exposure may severely bias the results. We used three different methods to estimate the intake of catechins: a two-day dietary record (Dutch Food Consumption Survey, Chapter 4), a cross-check dietary history reflecting the diet in the month preceding the interview (Zutphen Elderly Study, Chapter 5.1 and 6.1), and a semiquantitative food frequency questionnaire on the average food use in the year before the interview (Iowa Women's Health Study, Chapter 5.2 and 6.2). The catechin intake estimates obtained by these three methods have been summarized in Table 7.3. The average intake among men in Zutphen was in line with the average intake for men aged 65 and older in the Dutch Food Consumption Survey. The wider intake range in the Dutch Food Consumption Survey (0-439 mg/day versus 0-355 mg/day in Zutphen) probably reflects the fact that a two-day dietary record method is less representative of the usual diet, and, at the individual level, is more likely to measure the intake on an unusual day. It was previously shown that, compared to a three-day dietary record, the two-day method gave similar average intake values, but a wider distribution in intake.[2,3] The catechin intake of Iowa women was approximately one third of the intake of women of the same age in the Netherlands. The entire difference in catechin intake between Iowa and Zutphen, and between Iowa and elderly participants of the Dutch Food Consumption Survey was due to a difference in tea consumption. The intake of catechins from sources other than tea was virtually identical in all these populations, approximately 10 mg/day.

Table 7.3 Mean intake of catechins in the Dutch Food Consumption Survey, the Zutphen Elderly Study, and the Iowa Women's Health Study

		Catechin intake (mg/day)	
	Age (yrs)	men	women
Dutch Food Consumption Survey, 1998	50-65	47	69
	65-75	62	78
	75-97	79	80
Zutphen Elderly Study, 1985	65-84	72	
Iowa Women's Health Study, 1986	55-69		25

The questionnaires used in the cohort studies were not tested for their ability to determine catechin intake, but their reproducibility and relative validity with regard to foods[4,5] and nutrients[4,6] were assessed and considered satisfactory. For catechin containing foods, the correlation coefficients between the food frequency questionnaire used in Iowa and a 28-day diet record ranged from 0.45 for chocolate

containing candy bars to 0.83 for red wine, and the correlation was 0.77 for tea, the major catechin source in Iowa.[5] In Zutphen, the rankorder correlation coefficient between baseline examination and the repeated examination twelve months later was 0.63 for fruits; no data were provided for tea.[4] While in epidemiological studies correct ranking of subjects is of the greatest importance, the goal of a food consumption survey is to correctly estimate absolute intake levels. Underestimation of energy intake appears to be a common problem of recording-based diet measurement instruments.[7,8] When validated against the estimated energy expenditure, total energy intake was underestimated in the Dutch Food Consumption Survey. [2] Expressed as energy percentages, the intake of fat tended to be underreported, and the intake of protein tended to be overreported among energy-underreporters. On the other hand, protein intake, compared to urinary nitrogen, was estimated reliably.[2] Although catechin intake may thus have been underreported in the Dutch Food Consumption Survey, the fact that most catechin sources except chocolate are low in fat may have diminished the problem of underreporting.

All three dietary assessments took into account variation in intake between weekdays and weekends. The Zutphen Elderly Study was the only study in which seasonal variation was not taken into account: all data were collected between March and June and the average intake in the month preceding the interview was assessed. Although additional information on summer fruit consumption at the time of the largest supply was collected with a food frequency questionnaire, we did not use these data because information obtained with the two methods was different. Fruits such as berries and strawberries contain high concentrations of catechins, but none of the studies we used collected data in July and August, the months in which these fruits are primarily consumed. Even though no data were collected during the summer months in the Iowa Women's Health study, the food frequency questionnaire included several summer fruit items, and the questionnaire aimed to determine the average intake in the entire past year. In the Zutphen Elderly Study and in the Dutch Food Consumption Survey inadequate assessment of summer fruit consumption may have led to an underestimation of the catechin intake. This would have caused misclassification of individuals in the Zutphen cohort if intake levels of these fruits vary to a large extent between individuals. Overall, no important catechin sources were omitted and information on the consumption of catechin containing foods was fairly complete. In addition to seasonal variation in consumption, seasonal and year-to-year variation in catechin contents of foods may have caused error in intake estimates. Relative to differences in catechin content of varieties of fruits, however, seasonal and year-to-year variation appeared to be modest, and no consistently higher or lower levels were found in one particular season (Chapter 3.1).

Thus, we are fairly confident that, at the population level, our catechin intake estimates are reliable. The average intake estimates in the Zutphen Elderly Study and the Dutch Food Consumption Survey were comparable, and energy underreporting and missing data on seasonal variation and summer fruit consumption have probably caused modest underestimation only. At the individual level, however, misclassification may have occurred in the assignment of catechin levels to the foods reported. We used supply data (e.g. US food disappearance data, Dutch auction supply data) to weigh catechin levels of foods that were not reported as separate items on the questionnaires. For example, red and white wine were not reported separately in the Dutch Food Consumption Survey and the Zutphen Elderly Study, and apples and pears were combined on the Iowa questionnaire. Catechin levels in red wine and in apples are substantially higher than levels in white wine and pears, respectively. Our population average estimate is probably fair, given the use of supply data to estimate average consumption levels. Individuals, however, do not consume averages, they often preferentially consume either red or white wine. The same applies to apples and pears. We thus underestimated the catechin intake of red wine and of apple consumers, and overestimated the intake of white wine and of pear consumers. Other potential sources of variation are preferences for certain varieties of fruits, and individual variations in tea brewing methods. The catechin content of a tea infusion depends heavily on the amount of tea used for brewing a given amount of tea beverage, and less on the brewing time. Misclassification probably has occurred here as well, because individuals tend to consistently drink either strong or weak tea. This type of nondifferential misclassification leads to attenuation of associations in epidemiological studies, and may thus have caused an underestimation of true effects in our data.

RESIDUAL CONFOUNDING

A second methodological concern in observational studies is that healthy behaviors tend to cluster. Smoking, the single most important risk factor for many cancers, and an important determinant of cardiovascular diseases, is associated with a higher intake of energy, alcohol, and fat, with a lower intake of fruits and vegetables, lower socio-economic status, and with physical inactivity.[9-11] Previous studies have shown that consumption of tea in the Netherlands[12] and in Japan,[13] and consumption of wine in Denmark,[14] is associated with a healthy dietary pattern. In addition, wine-drinkers are less likely to smoke, tend to have a higher level of education, and a lower body mass index in the USA[15] and in Denmark.[14] Even though clustering of healthy behaviors may be culturally dependent, our findings from the Netherlands and the USA agree with the data published so far: individuals with a high catechin

intake tended to have a higher intake of vitamin C, vitamin E, beta-carotene, and fiber, tended to smoke less, exercise more, and were of higher socio-economic class. These data strongly suggest that catechin-rich foods are part of a healthy lifestyle and this creates difficulties in interpreting our findings because of residual confounding.

Residual confounding occurs if confounders, extraneous factors that are associated with both the outcome and the exposure under study, are not or insufficiently accounted for in the statistical analysis. Insufficient control for confounders can occur as a result of misclassification of the confounding factor, and depends thus on the quality and amount of detail with which the confounders are measured. In particular if the confounding is strong, as is the case for smoking in our data, misclassification of the confounder can yield spurious associations.[16,17] Studying associations in lifelong nonsmokers is an effective way of ruling out residual confounding by smoking. In the Zutphen Elderly Study, it was impossible to do this because the proportion of never smokers was less than 20% in this elderly male population. In the Iowa Women's Health Study, we performed analyses stratified by baseline CHD risk. Contrary to what would be expected if residual confounding by smoking underlies the reported associations, among women at low risk (not smoking at baseline, and no prevalent diabetes or cardiovascular disease), the intake of non-tea catechins was inversely associated with CHD death, while there was no association among high-risk women that were smoking. Tea catechins were not related to CHD death in either group. With respect to cancer, the reported associations were not stronger among a subgroup of smoking participants of the Iowa Women's Health Study. Thus, although due to the limited number of cases among never smokers we did not perform analyses in this group only, our data do not appear to support the hypothesis that residual confounding due to inaccurately measured smoking underlies the reported associations between catechin intake and chronic diseases in the Iowa Women's Health Study. Based on our analyses, we cannot rule this out with regard to the Zutphen Elderly Study. Also, we cannot rule out that factors other than smoking that were either insufficiently adjusted for or not taken into account, may have caused residual confounding.

CATECHINS IN RELATION TO OTHER DIETARY COMPONENTS

The intake of catechins is correlated with the intake of other components of an individual's diet. Each food contains a large number of different compounds, some known and quantified, some less well characterized, and some as yet unknown or unmeasurable. Many compounds tend to be present in the same foods or families of

foods. The intake of catechins was positively correlated with the intake of fruits and vegetables, and their constituents, e.g. vitamin C, vitamin E, carotenoids, folate, and fiber. For the intake of vitamin C, beta-carotene and fiber, correlations in several European populations were reported of the order of 0.40 to 0.70.[18] However, catechin intake was only weakly related to these dietary components, up to 0.21 for fiber (Chapter 4). When the correlation is too high, it is impossible to ascertain independent effects of catechins because of multicollinearity. In the work described in this thesis, this was the case for flavonols and for individual catechins. To circumvent multicollinearity problems, but still disentangle effects of catechins from those of flavonols, we defined subgroups of catechins derived from tea, catechins from sources other than tea, and flavonols from sources other than tea. While in this way we could perform analyses adjusted for flavonols in Zutphen, in Iowa the high correlation between catechin and flavonol intake still did not permit adjustment. The latter was partly due to the fact that the intake of onions, a major source of flavonols in the USA[19] was not ascertained in the Iowa Women's Health Study. The data from the Zutphen Elderly Study suggest that catechins, rather than flavonols, were responsible for the inverse trends with CHD mortality and with lung cancer incidence. Because the correlations between individual catechins were extremely high, ranging from 0.71 to 0.99 in the Zutphen Elderly Study, we could not assess their separate effects. Instead, we defined two subgroups of catechins, as described in the textbox on page 133.

A diet can be described not only in terms of compounds, but also in terms of foods. The obvious advantage of describing diet in terms of compounds, is that such information can be related directly to biological mechanisms. Describing the diet in terms of foods can strengthen compound findings if their major sources are associated with the outcome in a similar manner, in particular if the foods are different otherwise. With regard to CHD mortality, the inverse association with catechin intake in Zutphen was observed for both catechins from tea and for catechins from sources other than tea (mostly apple), albeit that the reduction in risk appeared larger for non-tea catechins. In Iowa, apples plus pears and wine, but not tea and chocolate were inversely associated with CHD mortality. Thus, these data do not support a general protective effect of catechins against CHD death, but they do not rule out that certain catechins, in particular (+)-catechin and (-)-epicatechin, may be inversely related to CHD death, even though we found no inverse association for the (-)-epicatechin containing chocolate. With regard to cancer, in Zutphen we found a nonsignificant inverse trend with lung cancer incidence for catechins from apples only. Catechins from tea or chocolate were not associated with lung cancer risk, and none of the catechin sources were associated with other epithelial cancers. The

inverse association of apple catechins with lung cancer risk could not be confirmed in the Iowa Women's Health Study. Although we did not perform food-based analyses, the subgroups of mainly apple-derived non-tea catechins on the one hand, and tea catechins on the other, were not both inversely associated with one single type of cancer. Inverse trends were observed for upper digestive tract cancer incidence with non-tea catechins, and for rectal and distal colon cancer with tea catechins. Again, these data do not support a protective effect of catechins from all sources against one single type of cancer. The data suggest that apples may protect against CHD and perhaps lung and upper digestive tract cancer. Whether catechins or other compounds in apples are responsible for the observed associations remains to be investigated. Tea or tea catechins may play a role in the prevention of distal colon or rectal cancer, but we found no evidence for a further role in the prevention of cardiovascular diseases or cancer.

Apart from the fact that different foods contain different catechins, variation in the food matrix may also explain part of our findings. For beta-carotene, it has been shown that the matrix in which this compound is incorporated, strongly affects its bioavailability. [20,21] Catechins are absorbed from the small intestine into the blood,[22] but until now, the absorption process is not very well characterized. Some of the ingested catechins will not be absorbed in the upper digestive tract and will proceed into the colon, where bacteria have been shown to degrade catechins. These degradation products have been found in urine, and can thus be absorbed as well.[22,23] A determinant of catechin bioavailability could be the type of matrix: liquid (tea, wine) or solid (fruits, chocolate). However, studies on the pharmacokinetics of catechins published so far, do not suggest major differences between tea, chocolate, and wine in time to reach peak plasma concentrations or in elimination half-life.[22,24] The habit of drinking tea with milk, which was suggested to explain the absence of a protective effect of tea consumption against CHD risk in the UK,[25,26] also did not impede the absorption of catechins from the gut.[27] Other components of the diet may influence the bioavailability of catechins, but no further data are available.

ANTIOXIDANTS

Catechins were first considered as mediators of cardiovascular disease and cancer risk because they are strong antioxidants *in vitro*.[28] Antioxidants scavenge free radicals and reactive oxygen species, and may thus prevent cancer-causing DNA damage and inhibit LDL oxidation involved in atherosclerosis.[29] With ongoing research on antioxidants, an apparent contradiction has emerged: while antioxidant laden plant foods appear to protect against CHD and cancer, and laboratory data

continue to confirm the importance of free radicals and reactive oxygen species in disease pathology, large-scale randomized supplementation trials with individual antioxidants largely fail to demonstrate beneficial effects.[30-32] The initial enthusiasm for antioxidant vitamins faded after a number of trials failed to show beneficial effects of beta-carotene and vitamin E in well-nourished populations.[33-35] In fact, among heavy smokers and asbestos workers,[36,37] the incidence of lung cancer appeared to be higher among those taking beta-carotene supplements. The CARET trial was terminated early,[34] and since then editorials with titles such as "Antioxidant vitamins and cardiovascular disease: Dr Jekyll or Mr Hyde?"[38] or "Beta-carotene and lung cancer promotion in heavy smokers – a plausible relationship?"[39] have started to appear. Certainly, a null finding for one antioxidant does not mean that other antioxidants could not be effective. Moreover, finding an association in intervention trials depends on giving not only the appropriate compound, but also supplying an appropriate dose, for an appropriate period of time, to an appropriate population.[40] In retrospect, a number of weaknesses of the intervention trials can be pointed out. First, supplementation covered a limited time-span, between 4 and 12 years,[33-35] which may not have been enough to detect benefits. Second, supplements were given at doses that clearly exceeded those attainable by diet only. The hypothesis has been put forward that under certain circumstances, such as concurrent exposure to high concentrations of oxidants in cigarette smoke, high doses of single antioxidants may exert prooxidant instead of antioxidant effects.[39,41] A third weakness is that in the intervention trials only one or two antioxidant vitamins were supplemented simultaneously. Chain-breaking antioxidants inhibit oxidation by scavenging free radicals. In the process, they are converted into less reactive, but not completely unreactive radicals themselves.[29,42] Dietary antioxidants have been proposed to interact in what can be considered a network, to continuously restore antioxidant ability. For example, vitamin C and catechins have been shown to recycle alpha-tocopherol radical back to alpha-tocopherol.[43,44] Thus, it may be necessary to consume a balanced combination of a variety of antioxidants to attain beneficial effects. Interestingly, a situation similar in some respects to the antioxidant vitamin intervention trials might in fact have been encountered in the Caerphilly Study.[26] In this population, predominated by heavily smoking miners, high tea consumption was associated with an increased risk of CHD. Residual confounding from smoking and social class was suggested as an explanation for the apparent incongruent finding, but a prooxidant effect of catechins from tea (the level of tea consumption was twice that in the Zutphen Elderly Study) in combination with a high degree of oxidative stress, and a diet low in vegetables and fruits, may hypothetically provide an alternative explanation. We did not observe increased risks with a high catechin intake for any of the endpoints studied, but then we studied dietary catechins instead

of supplements, our cohort members did not consume diets that were extremely low in plant foods, and the catechin intake in our cohorts was probably not as high as that in the Caerphilly study.[26]

IN VITRO AND EX VIVO EXPERIMENTS

Part of the confusion about beneficial effects of antioxidants may be due to the inadequacy of currently available tests that measure oxidative damage and the capacity of antioxidants to reduce this damage to reflect the complex *in vivo* situation.[45-47] While this problem is most prominent in antioxidant research, it can be generalized to many *in vitro* and *ex vivo* test that have generated data on other proposed biological mechanisms for catechins. For example, in most studies of antioxidant effects on LDL oxidation, LDL is separated from plasma during its isolation.[48] Aqueous antioxidants, such as catechins, are not located in the LDL particle but in the surrounding plasma, and it is therefore not very well possible to assess directly the effects of catechins on LDL oxidation with these tests. In addition to the urgent need for better tests, two other areas of concern can be identified. First, most studies on catechin mechanisms have been performed with unphysiologically high concentrations of catechins. Free catechin concentrations in the human body are extremely low because they are rapidly metabolized, both before absorption by gut microbes and after absorption in the liver, and are rapidly excreted. Because of the short elimination half-life of less than 6 hours, no accumulation of catechins in plasma occurs.[22] Thus, the bioavailability of catechins and possible differences in bioavailability are not taken into account in these studies. In rats, (-)-epicatechin and EGC were absorbed much more efficiently than EGCg from green tea,[23] suggesting that indeed differences in absorption between the individual catechins exist. A second concern is that almost all *in vitro* studies have examined catechins as such, while their metabolites, which also have *in vitro* antioxidant activity,[28] have been disregarded. Due to the rapid and extensive metabolism of catechins, they may contribute substantially to any biological effects of dietary catechins in the human body. Thus, even though a large body of literature exists on potential protective effects of catechins against chronic diseases, the relevance of many of these findings remains to be established. Until there is a better understanding of the complex actions of catechins in the human body, it is difficult to relate our epidemiological findings to *in vitro* or *ex vivo* data.

FUTURE RESEARCH DIRECTIONS

Ten years ago, a workshop entitled "Non-nutritive anticarcinogens in the diet: state of the art and future developments" brought on innovative research in the area of

flavonoids and chronic diseases through a collaboration between RIKILT and RIVM. The two-step approach of first chemically analyzing a large number of foods to compile a food composition table and thereafter evaluating associations with chronic diseases in epidemiological cohort studies proved successful for the flavonoid classes of flavonols and flavones. We now added information for the major class of catechins. As is often the case, the work done so far, has generated more questions than it has answered. First, it would be useful to accumulate more evidence on the relation between dietary catechins and chronic diseases from well designed prospective cohort studies. Emphasis should be laid on studies in which analyses can be stratified by smoking status, to eliminate residual confounding by smoking, and on populations with a low intake of tea or with a substantial number of non-drinkers of tea in the population. With observational studies it will not be possible to disentangle effects of individual catechins, but only effects of the subgroups of tea and non-tea catechins.

Short-term intervention studies with individual catechins could theoretically assess their separate effects, but would be useful mostly if intermediate endpoints of disease would be available. Although a number of such intermediates are available now, in particular in the field of cardiovascular diseases, it is hard to choose the relevant intermediates, given the lack of knowledge on the relevance of the many suggested mechanisms through which catechins could affect human health. Atherosclerosis measured by for example intima media thickness,[49] could serve as a more general intermediate endpoint for cardiovascular disease, but still then potential health effects could be missed if catechins act by influencing for example vasodilation, thrombosis, or cardiac arrhythmias. Until more knowledge regarding relevant biological mechanisms has been gained, long-term intervention studies with hard endpoints would be the main alternative. The current evidence, however, does not justify such a huge and costly undertaking.

In order to study biological mechanisms of catechins, it is essential also to determine their bioavailability and metabolism. Catechins are metabolized extensively, both in the colon by bacteria and after absorption in the liver, and these metabolites could contribute to biological effects.[22] Important questions are which metabolites are formed, to what extent, and what is their biological effect. The fact that urinary catechin metabolites identified thus far are simple phenolic acids,[50] leads to the interesting situation that bioactive catechin metabolites circulating in the body could be derived from parent molecules other than catechins. There are still very few data available on the fate of catechins in the human body, and more research effort should be targeted at identifying catechin metabolites, determining target tissues and

concentrations, and determining factors, for example matrix effects, that influence the bioavailability of catechins.

CONCLUSIONS

Catechins are quantitatively important minor constituents of the diet. Major catechin sources in the Netherlands and the USA are tea, apples, and chocolate, but other fruits, wine and legumes also contribute to the catechin intake. Non-tea catechins, derived mainly from apple, were inversely related to coronary heart disease mortality. For tea catechins, on the other hand, we found a nonsignificant inverse trend with coronary heart disease mortality in elderly men but not in postmenopausal women. Inverse, but nonsignificant trends were found for non-tea catechin intake and upper-digestive tract cancer in postmenopausal women and lung cancer in elderly men. We found indications for a protective effect of tea catechins against rectal cancer in postmenopausal women. Catechin intake was not related to stroke risk among elderly men. Misclassification of dietary exposure may have attenuated true inverse associations between catechin intake and risk of chronic diseases. Differences in bioavailability and *in vivo* activity among individual catechins may hypothetically explain our findings, but more data are needed to substantiate this. Overall, our data do not support a strong protective effect of the studied catechins against coronary heart disease, stroke and cancer. However, certain catechins may lower the risk of CHD mortality and possibly certain cancers. Further research has to reveal if, and if so, why this is the case. At this point, there is not enough evidence to offer recommendations regarding the consumption of catechin-rich foods.

REFERENCES

1. Escarpa A, Gonzalez MC. High-performance liquid chromatography with diode-array detection for the determination of phenolic compounds in peel and pulp from different apple varieties. *J Chromatogr A* 1998;823:331-337.
2. Voorrips LE, Bemelmans W, Brussaard JH, Hulshof KFAM. *Vooronderzoek VCP-3 validering en methodologie [in Dutch]*. Zeist: TNO Voeding; 1997.
3. Hulshof KFAM, Van Staveren WA. The Dutch National Food Consumption Survey: design, methods and first results. *Food Policy* 1991;16:257-260.
4. Bloemberg BP, Kromhout D, Obermann-De Boer GL, Van Kampen-Donker M. The reproducibility of dietary intake data assessed with the cross-check dietary history method. *Am J Epidemiol* 1989;130:1047-1056.
5. Feskanich D, Rimm EB, Giovannucci EL, Colditz GA, Stampfer MJ, Litin LB, Willett WC. Reproducibility and validity of food intake measurements from a semiquantitative food frequency questionnaire. *J Am Diet Assoc* 1993;93:790-796.
6. Munger RG, Folsom AR, Kushi LH, Kaye SA, Sellers TA. Dietary assessment of older Iowa women with a food frequency questionnaire: nutrient intake, reproducibility, and comparison with

24-hour dietary recall interviews. *Am J Epidemiol* 1992;136:192-200.
7. Zhang J, Temme EH, Sasaki S, Kesteloot H. Under- and overreporting of energy intake using urinary cations as biomarkers: relation to body mass index. *Am J Epidemiol* 2000;152:453-462.
8. Bingham SA, Nelson M. Assessment of food consumption and nutrient intake. In: Margetts BM, Nelson M, eds. *Design concepts in nutritional epidemiology.* Oxford: Oxford University Press;1991:153-191.
9. Burke V, Milligan RA, Beilin LJ, Dunbar D, Spencer M, Balde E, Gracey MP. Clustering of health-related behaviors among 18-year-old Australians. *Prev Med* 1997;26:724-733.
10. McPhillips JB, Eaton CB, Gans KM, Derby CA, Lasater TM, McKenney JL, Carleton RA. Dietary differences in smokers and non-smokers from two southern New England communities. *J Am Diet Assoc* 1994;94:287-292.
11. Veenstra J, Schenkel JA, Erp-Baart AM, Brants HA, Hulshof KF, Kistemaker C, Schaafsma G, Ockhuizen T. Alcohol consumption in relation to food intake and smoking habits in the Dutch National Food Consumption Survey. *Eur J Clin Nutr* 1993;47:482-489.
12. Hulshof KFAM, Wedel M, Löwik MRH, Kok FJ, Kistemaker C, Hermus RJJ, ten Hoor F, Ockhuizen T. Clustering of dietary variables and other lifestyle factors (Dutch Nutritional Surveillance System). *J Epidemiol Community Health* 1992;46:417-424.
13. Tsubono Y, Takahashi T, Iwase Y, Iitoi Y, Akabane M, Tsugane S. Dietary differences with green tea intake among middle-aged japanese men and women. *Prev Med* 1997;26:704-710.
14. Tjonneland A, Gronbaek M, Stripp C, Overvad K. Wine intake and diet in a random sample of 48763 Danish men and women. *Am J Clin Nutr* 1999;69:49-54.
15. Klatsky AL, Armstrong MA, Kipp H. Correlates of alcoholic beverage preference: traits of persons who choose wine, liquor or beer. *Br J Addict* 1990;85:1279-1289.
16. Rothman KJ, Greenland S. Precision and validity in epidemiologic studies. In: Rothman KJ, Greenland S, eds. *Modern epidemiology.* Philadelphia: Lippincott-Raven;1998:115-134.
17. Marshall JR, Hastrup JL. Mismeasurement and the resonance of strong confounders: uncorrelated errors. *Am J Epidemiol* 1996;143:1069-1078.
18. Riboli E, Slimani N, Kaaks R. Identifiability of food components for cancer chemoprevention. *IARC Sci Publ* 1996;23-31.
19. Rimm EB, Katan MB, Ascherio A, Stampfer MJ, Willett WC. Relation between intake of flavonoids and risk for coronary heart disease in male health professionals. *Ann Intern Med* 1996;125:384-389.
20. Van het Hof KH, West CE, Weststrate JA, Hautvast JG. Dietary factors that affect the bioavailability of carotenoids. *J Nutr* 2000;130:503-506.
21. Castenmiller JJ, West CE. Bioavailability and bioconversion of carotenoids. *Annu Rev Nutr* 1998;18:19-38.
22. Hollman PCH, Arts ICW. Flavonols, flavones and flavanols - nature, occurrence and dietary burden. *J Sci Food Agric* 2000;80:1081-1093.
23. Kim SB, Lee MJ, Hong JI, Li C, Smith TJ, Yang GY, Seril DN, Yang CS. Plasma and tissue levels of tea catechins in rats and mice during chronic consumption of green tea polyphenols. *Nutr Cancer* 2000;37:41-48.
24. Bell JRC, Donovan JL, Wong R, Waterhouse AL, German JB, Walzem RL, Kasim-Karakas SE. (+)-Catechin in human plasma after ingestion of a single serving of reconstituted red wine. *Am J Clin Nutr* 2000;71:103-108.
25. Woodward M, Tunstall-Pedoe H. Coffee and tea consumption in the Scottish Heart Health Study follow up: conflicting relations with coronary risk factors, coronary disease, and all cause mortality. *J Epidemiol Community Health* 1999;53:481-487.

26. Hertog MGL, Sweetnam PM, Fehily AM, Elwood PC, Kromhout D. Antioxidant flavonols and ischemic heart disease in a Welsh population of men: the Caerphilly Study. *Am J Clin Nutr* 1997;65:1489-1494.
27. Van het Hof KH, Kivits GAA, Weststrate JA, Tijburg LBM. Bioavailability of catechins from tea: the effect of milk. *Eur J Clin Nutr* 1998;52:356-359.
28. Rice-Evans CA, Miller NJ, Paganga G. Structure-antioxidant activity relationships of flavonoids and phenolic acids. *Free Radical Biol Med* 1996;20:933-956.
29. Halliwell B, Gutteridge JMC. *Free radicals in biology and medicine*. Oxford: Clarendon Press; 1989.
30. Marchioli R. Antioxidant vitamins and prevention of cardiovascular disease: laboratory, epidemiological and clinical trial data. *Pharmacol Res* 1999;40:227-238.
31. Virtamo J. Vitamins and lung cancer. *Proc Nutr Soc* 1999;58:329-333.
32. Diplock AT, Charleux JL, Crozier-Willi G, Kok FJ, Rice-Evans C, Roberfroid M, Stahl W, Vina-Ribes J. Functional food science and defence against reactive oxidative species. *Br J Nutr* 1998;80 Suppl 1:S77-112.
33. Hennekens CH, Buring JE, Manson JE, Stampfer M, Rosner B, Cook NR, Belanger C, LaMotte F, Gaziano JM, Ridker PM, Willett W, Peto R. Lack of effect of long-term supplementation with beta carotene on the incidence of malignant neoplasms and cardiovascular disease. *N Engl J Med* 1996;334:1145-1149.
34. Omenn GS, Goodman GE, Thornquist MD, Balmes J, Cullen MR, Glass A, Keogh JP, Meyskens FL, Valanis B, Williams JH, Barnhart S, Hammar S. Effects of a combination of beta carotene and vitamin A on lung cancer and cardiovascular disease. *N Engl J Med* 1996;334:1150-1155.
35. The Alpha-Tocopherol Beta Carotene Cancer Prevention Study Group. The effect of vitamin E and beta carotene on the incidence of lung cancer and other cancers in male smokers. *N Engl J Med* 1994;330:1029-1035.
36. Albanes D, Heinonen OP, Taylor PR, Virtamo J, Edwards BK, Rautalahti M, Hartman AM, Palmgren J, Freedman LS, Haapakoski J, Barrett MJ, Pietinen P, Malila N, Tala E, Liippo K, Salomaa ER, Tangrea JA, Teppo L, Askin FB, Taskinen E, Erozan Y, Greenwald P, Huttunen JK. Alpha-Tocopherol and beta-carotene supplements and lung cancer incidence in the alpha-tocopherol, beta-carotene cancer prevention study: effects of base-line characteristics and study compliance. *J Natl Cancer Inst* 1996;88:1560-1570.
37. Omenn GS, Goodman GE, Thornquist MD, Balmes J, Cullen MR, Glass A, Keogh JP, Meyskens FL, Jr., Valanis B, Williams JH, Jr., Barnhart S, Cherniack MG, Brodkin CA, Hammar S. Risk factors for lung cancer and for intervention effects in CARET, the Beta-Carotene and Retinol Efficacy Trial. *J Natl Cancer Inst* 1996;88:1550-1559.
38. Hercberg S, Galan P, Preziosi P. Antioxidant vitamins and cardiovascular disease: Dr Jekyll or Mr Hyde? *Am J Public Health* 1999;89:289-291.
39. Mayne ST, Handelman GJ, Beecher G. Beta-Carotene and lung cancer promotion in heavy smokers--a plausible relationship? *J Natl Cancer Inst* 1996;88:1513-1515.
40. Hankinson S, Stampfer MJ. All that glitters is not beta carotene. *JAMA* 1994;272:1455-1456.
41. Halliwell B. The antioxidant paradox. *Lancet* 2000;355:1179-1180.
42. Halliwell B. Antioxidants in human health and disease. *Annu Rev Nutr* 1996;16:33-50.
43. Zhu QY, Huang Y, Tsang D, Chen ZY. Regeneration of alpha-tocopherol in human low-density lipoprotein by green tea catechin. *J Agric Food Chem* 1999;47:2020-2025.
44. Packer L. Vitamin E and the antioxidant network: protection of human Low Density Lipoprotein from oxidation. In: Ohigashi H, Osawa T, Terao J, Watanabe S, Yoshikawa T, eds. *Food factors for cancer prevention*. Tokyo: Springer-Verlag;1997:452-459.

45. Halliwell B. Why and how should we measure oxidative DNA damage in nutritional studies? how far have we come? *Am J Clin Nutr* 2000;72:1082-1087.
46. Halliwell B. Lipid peroxidation, antioxidants and cardiovascular disease: how should we move forward? *Cardiovasc Res* 2000;47:410-418.
47. Duthie GG. Determination of activity of antioxidants in human subjects. *Proc Nutr Soc* 1999;58:1015-1024.
48. Esterbauer H, Gebicki J, Puhl H, Jurgens G. The role of lipid peroxidation and antioxidants in oxidative modification of LDL. *Free Radic Biol Med* 1992;13:341-390.
49. Persson J, Formgren J, Israelsson B, Berglund G. Ultrasound-determined intima-media thickness and atherosclerosis. Direct and indirect validation. *Arterioscler Thromb* 1994;14:261-264.
50. Hollman PCH, Tijburg LBM, Yang CS. Bioavailability of flavonoids from tea. *Crit Rev Food Sci Nutr* 1997;37:719-738.

Summary

Catechins are compounds that belong to the family of the flavonoids. Six major subclasses of flavonoids, all with a common diphenylpyran skeleton, can be distinguished: catechins, flavonols, flavones, flavanones, anthocyanidins, and isoflavones. At present, more than 4000 naturally occurring flavonoids have been identified, and this list is still growing. Catechins are found in a multitude of plant foods, they have strong *in vitro* antioxidant activity and they have shown to influence a large variety of mammalian enzyme systems. The aim of the work described in this thesis was to develop a method for the quantification of catechins in foods, to determine the catechin contents of commonly consumed plant foods, and to evaluate the associations between dietary catechin intake and risk of cardiovascular diseases and cancer in prospective cohort studies.

First, we optimized the extraction and quantification of catechins in three model foods: apples, black grapes and canned kidney beans (Chapter 2). Catechins [(+)-catechin, (-)-epicatechin, (-)-epigallocatechin (EGC), (-)-epicatechin gallate (ECg), (-)-epigallocatechin gallate (EGCg)] were separated with gradient reversed phase HPLC and quantified by UV (270 nm) or fluorescence (280-310 nm excitation-emission) detection in series. Type (ethanol, methanol or acetone) and concentration (40-100% in water) of extraction solvent influenced catechin yield, whereas extraction time (10-60 min) did not. Adequate extraction was attained with 60-100% methanol for apple and grapes, and 40-80% methanol for beans. Recovery (>94%), within-run repeatability (1-5%), between-run reproducibility (3-9%) and detection limits (0.1-3.9 mg/kg fresh apple or 0.01-0.29 µg/mL extract) were satisfactory.

With this analytical method, we determined the levels of (+)-catechin, (-)-epicatechin, (+)-gallocatechin (GC), EGC, ECg, and EGCg in 24 types of fruits, 27 types of vegetables and legumes, some staple foods, and processed foods commonly consumed in the Netherlands (Chapter 3.1). Most fruits, chocolate, and some legumes contained catechins. Levels varied to a large extent: from 4.5 mg/kg in kiwi fruit to 610 mg/kg in black chocolate. (+)-Catechin and (-)-epicatechin were the predominant catechins; GC, EGC, and ECg were detected in some foods, but none of the foods contained EGCg. We then assessed catechin levels in 8 types of black tea, 18 types of red and white wine, apple juice, grape juice, ice-tea, beer, chocolate milk and coffee (Chapter 3.2). Tea infusions contained high levels of catechins (102-418 mg total catechins/L), and tea was the only beverage which contained GC, EGC,

ECg, and EGCg in addition to (+)-catechin and (-)-epicatechin. Catechin concentrations were still substantial in red wine (27-96 mg/L), but low to negligible amounts were found in white wine, commercially available fruit juices, ice-tea, and chocolate milk. Catechins were absent from beer and coffee.

We then estimated the intake of catechins in the Dutch population and studied associations between catechin intake and other dietary and lifestyle factors (Chapter 4). Data were used from a nation-wide dietary survey carried out in 1998 among a representative sample of 6,200 Dutch men and women aged 1-97 years. Dietary data were collected using a two-day dietary record method. The average daily catechin intake was 50 mg (standard deviation (SD): 56 mg/day). Catechin intake increased with age, and the intake was higher in women (60 mg/day) than in men (40 mg/day). Tea was the main catechin source in all age groups, whereas chocolate was second in children, and apples and pears were second in adults and elderly. Catechin intake was lower in smokers than in non-smokers, and increased with socio-economic status. A high intake was associated with a high intake of fiber (r=0.20), vitamin C (r=0.17) and beta-carotene (r=0.10). Thus, catechins are quantitatively important bioactive components of the daily diet, which should be taken into account when studying the relation between diet and chronic diseases. Catechin intake is only moderately associated with the intake of other nutrients, but much stronger with certain health behaviors such as smoking.

We studied the associations between dietary catechin intake and coronary heart disease (CHD) and stroke incidence and mortality using data from the Zutphen Elderly Study (Chapter 5.1). This is a prospective cohort study among 806 men aged 65-84 years at baseline in 1985. The average catechin intake at baseline was 72 mg/day (SD: 48 mg/day), mainly from black tea, apples, and chocolate. A total of 90 deaths from CHD were documented during 10 years of follow-up. Catechin intake was inversely associated with CHD mortality, the multivariate adjusted risk ratio in the highest tertile of intake was 0.49 (95% confidence interval (CI): 0.27-0.88; P for trend: 0.017). After multivariate adjustment, catechin intake was not associated with myocardial infarction incidence (risk ratio in highest tertile of intake: 0.70; 95% CI: 0.39-1.26; P for trend: 0.232). After adjustment for tea consumption and flavonol intake, a 7.5 mg increase in catechin intake from other sources than tea, was associated with a tendency for a 20% reduction in CHD mortality risk (P-value: 0.114). There was no association between catechin intake and stroke incidence or mortality. These results suggest that catechins, whether from tea or other sources, may reduce risk of CHD mortality, but not stroke.

Next, we studied the association between intake of catechins and risk of CHD death, in a cohort of postmenopausal women with a lower level of tea consumption (Chapter 5.2). Between 1986 and 1998, 767 of 34,492 participants of the Iowa Women's Health Study initially free of cardiovascular diseases died from CHD. There was a strong inverse association between the intake of (+)-catechin and (-)-epicatechin and CHD death, which was somewhat attenuated after multivariate adjustment (risk ratios from lowest to highest quintile: 1.00, 0.95, 0.97, 0.77, 0.76). This inverse association was most pronounced in women at low risk of CHD (non-smokers, free of diabetes and cardiovascular diseases). A high intake of 'gallates', catechins typical of tea, was not associated with CHD death. Of the major catechin sources, apples and wine were inversely associated with CHD death. Our data suggest that preventive effects might be limited to certain types of catechins, or that these catechins are indicators of other dietary components or a healthy lifestyle in general.

The association between intake of catechins and incidence of epithelial cancers was examined in the Zutphen Elderly Study (Chapter 6.1). After excluding all prevalent cancer cases at baseline, 728 men aged 65-84 years remained in the analysis. After 10 years of follow-up, 96 incident epithelial cancers were recorded, including 42 cases of lung cancer. After multivariate adjustment, catechin intake was not associated with epithelial cancer (risk ratios from lowest to highest tertile: 1.00, 0.75, 0.94; P for trend: 0.82), or lung cancer (risk ratios from lowest to highest tertile: 1.00, 0.72, 0.92; P for trend: 0.80). Catechins not from tea were borderline significantly inversely associated with lung cancer incidence (risk ratio and 95% CI for a 7.5 mg increase in intake: 0.66; 0.42-1.05), whereas catechins from tea were not. Catechins from apple, the major source of non-tea catechins, were also related to lung cancer incidence (risk ratio and 95% CI for a 7.5 mg catechin increase: 0.67; 0.38-1.17). Because tea, the major catechin source in this population, was not associated with cancer risk, it seems unlikely that catechins are responsible for the observed inverse trend between non-tea catechins and lung cancer incidence.

Finally, we studied whether a high intake of catechins was associated with cancer incidence at individual sites among postmenopausal women participating in the Iowa Women's Health Study (Chapter 6.2). 34,651 cancer-free women aged 55-69 years were followed from 1986-1998. Incident cancers were obtained through linkage with a cancer registry. After adjustment for potential confounders, catechin intake was inversely associated with rectal cancer incidence only (risk ratios from lowest to highest quartile: 1.00, 0.93, 0.73, 0.55; P for trend: 0.02). Non-significant inverse trends were found for cancer of the upper digestive tract, pancreas, and for hematopoietic cancers. Catechins derived primarily from fruits, (+)-catechin and

(-)-epicatechin, tended to be inversely associated with upper digestive tract cancer, whereas catechins derived from tea were inversely associated with rectal cancer. Among several cancers studied, our data suggest that catechin intake may protect against rectal cancer. The distinct effects found for catechins derived from solid foods (fruits) and beverages (tea) may be due to differences in bioavailability and metabolism of catechins, and their interactions with other dietary components, but further studies are needed to elucidate this hypothesis.

In conclusion, catechins are quantitatively important minor constituents of the diet. Major catechin sources in the Netherlands and the USA are tea, apples, and chocolate, but other fruits, wine and legumes also contribute to the catechin intake. Overall, our data do not support a strong protective effect of the studied catechins against coronary heart disease, stroke and cancer. However, certain catechins may lower the risk of CHD mortality and possibly certain cancers. Misclassification of dietary exposure may have attenuated true inverse associations between catechin intake and risk of chronic diseases. Differences in bioavailability and *in vivo* activity among individual catechins may hypothetically explain our findings, but more data are needed to substantiate this. At this point, there is not enough evidence to offer recommendations regarding the consumption of catechin-rich foods.

Samenvatting

Catechines zijn stoffen die behoren tot de familie van de flavonoïden. Zes belangrijke subklassen van flavonoïden kunnen worden onderscheiden: catechines, flavonolen, flavonen, flavanonen, anthocyanidines en isoflavonen. Alle hebben een diphenylpyran skelet. Meer dan 4000 van nature voorkomende flavonoïden zijn reeds geïdentificeerd. Catechines komen voor in een groot aantal plantaardige voedingsmiddelen. Ze vertonen *in vitro* een sterke antioxidant activiteit. Er zijn aanwijzingen dat ze een groot aantal in mens en dier voorkomende enzymsystemen kunnen beïnvloeden. Het onderzoek dat is beschreven in dit proefschrift had tot doel het ontwikkelen van een analytisch chemische methode ter bepaling van catechines in voedingsmiddelen, het kwantificeren van catechines in plantaardige voedingsmiddelen en het bestuderen van de relaties tussen de inname van catechines en het risico op coronaire hartziekten, beroerte en kanker.

Eerst werd een methode voor de extractie en bepaling van catechinegehaltes in voedingsmiddelen geoptimaliseerd (Hoofdstuk 2). Hiertoe werden catechines [(+)-catechine, (-)-epicatechine, (-)-epigallocatechine (EGC), (-)-epicatechine gallaat (ECg), (-)-epigallocatechine gallaat (EGCg)] bepaald in drie producten: appels, blauwe druiven en bruine bonen uit blik. De catechines werden gescheiden met behulp van HPLC en gekwantificeerd met in serie geschakelde ultraviolet (270 nm) en fluorescentie detectoren (280-310 nm excitatie-emissie). In tegenstelling tot de extractietijd (10-60 min), beïnvloedde zowel het type extractiemiddel (ethanol, methanol, of aceton) als de concentratie extractiemiddel (40-100% in water) de gemeten catechinegehaltes. Efficiënte extractie werd bereikt met 60-100% methanol voor appels en druiven en met 40-80% methanol voor bonen. Deze methode had adequate recovery (>94%), binnen-dag reproduceerbaarheid (1-5%), tussen-dag reproduceerbaarheid (3-9%) en detectiegrenzen (0,1-3,9 mg/kg verse appel of 0,01-0,29 µg/mL extract).

Met deze analytische methode hebben we de gehaltes aan (+)-catechine, (-)-epicatechine, (+)-gallocatechine (GC), EGC, ECg en EGCg bepaald in 24 in Nederland verkrijgbare soorten fruit, 27 soorten groenten, peulvruchten en een aantal bewerkte producten (Hoofdstuk 3.1). De meeste fruitsoorten, chocolade en sommige peulvruchten bevatten catechines, maar de variatie in gehaltes was groot: van 4,5 mg/kg in kiwi tot 610 mg/kg in pure chocolade. (+)-Catechine en (-)-epicatechine kwamen het meest voor. GC, EGC en ECg werden in slechts enkele

voedingsmiddelen gevonden, en EGCg kwam niet voor in deze producten. Vervolgens bepaalden we de catechinegehaltes in 8 soorten thee, 18 soorten rode en witte wijn, appelsap, druivensap, ijsthee, bier, chocolademelk en koffie (Hoofdstuk 3.2). Thee bevatte hoge concentraties catechines (102-418 mg/L) en het was bovendien de enige drank die naast (+)-catechine en (-)-epicatechine ook GC, EGC, ECg en EGCg bevatte. Wijn bevatte ook behoorlijke hoeveelheden catechines (27-96 mg/L), maar de concentraties waren erg laag in witte wijn, vruchtensappen, ijsthee en chocolademelk. Bier en koffie bevatten in het geheel geen catechines.

Vervolgens hebben we voor de Nederlandse bevolking de inname van catechines geschat, en tevens onderzocht welke voedings- en leefstijlfactoren ermee samenhangen (Hoofdstuk 4). Hiertoe werden gegevens gebruikt van de Nederlandse Voedselconsumptiepeiling 1997-1998 die werd uitgevoerd onder een representatieve steekproef van 6200 mannen en vrouwen met een leeftijd van 1-97 jaar. Voedingsgegevens werden verzameld met een tweedaagse opschrijfmethode. De gemiddelde catechine-inname was 50 mg/dag (standaardafwijking 56). De inname van catechines nam toe met hogere leeftijd en was hoger bij vrouwen (60 mg/dag) dan bij mannen (40 mg/dag). De belangrijkste voedingsbron van catechines was thee. Voor kinderen was chocolade de tweede bron van catechines, terwijl dit voor volwassenen en ouderen, appels en peren was. Rokers en mensen uit lagere sociaal-economische klassen hadden een lagere catechine-inname. Personen met een relatief hoge catechine-inname hadden ook een relatief hoge inname van vezel, vitamine C en beta-caroteen.

De relaties tussen de inname van catechines en de incidentie van en sterfte aan coronaire hartziekten (CHZ) en beroerte werden bestudeerd in de Zutphen Ouderen Studie (Hoofdstuk 5.1). Dit is een prospectieve cohortstudie onder 806 mannen, die varieerden in leeftijd van 65-84 jaar aan het begin van de studie in 1985. De gemiddelde inname van catechines was toen 72 mg/dag (standaardafwijking 48), voornamelijk afkomstig uit thee, appels en chocolade. Gedurende de follow-up periode van 10 jaar, stierven 90 mannen aan CHZ. Catechine-inname was invers geassocieerd met CHZ-sterfte. Na correctie voor andere risicofactoren was de risicoratio in het hoogste tertiel van inname 0,49 (95% betrouwbaarheidsinterval: 0,27-0,88; P voor trend: 0,017), terwijl de risicoratio 0,70 was voor het optreden van een myocard infarct (95% betrouwbaarheidsinterval: 0,39-1,26; P voor trend: 0,232). Na correctie voor theeconsumptie en voor de inname van flavonolen, was een toename van 7,5 mg catechines uit andere bronnen dan thee geassocieerd met trend voor een 20% afname in het risico op CHZ-sterfte (P-waarde: 0,114). Er was geen verband tussen de inname van catechines en het risico op beroerte. Deze resultaten

suggereren dat catechines, of ze nu afkomstig zijn uit thee of uit andere bronnen dan thee, het risico op sterfte aan CHZ kunnen verlagen. Dit lijkt niet het geval te zijn voor beroerte.

Daarna bestudeerden we de relatie tussen de inname van catechines en sterfte aan CHZ in een cohort postmenopausale vrouwen in de Verenigde Staten, die een relatief lage theeconsumptie hadden (Hoofdstuk 5.2). Tussen 1986 en 1998 stierven 767 van de 34.492 vrouwen die deelnamen aan deze Iowa Women's Health Study aan CHZ. Er was een sterk invers verband tussen de inname van (+)-catechine en (-)-epicatechine en CHZ-sterfte. Dit verband werd afgezwakt, maar bleef bestaan na correctie voor andere risicofactoren (risicoratio's van het laagste tot het hoogste quintiel: 1,00, 0,95, 0,97, 0,77, 0,76). Het verband was het duidelijkst bij vrouwen die aan het begin van de studie een relatief lagere kans op CHZ hadden (vrouwen die niet rookten, geen diabetes hadden, en geen hartziekten). Een hoge inname van catechines die in thee voorkomen was niet geassocieerd met sterfte aan CHZ. Van de belangrijkste catechinebronnen waren alleen appels en wijn invers geassocieerd met sterfte aan CHZ. De resultaten van deze studie suggereren dat eventueel beschermende effecten van catechines tegen CHZ-sterfte beperkt lijken te zijn tot bepaalde typen catechines, of dat deze stoffen indicatoren zijn voor andere componenten van de voeding of een gezonde leefstijl in het algemeen.

De relatie tussen de inname van catechines en het risico op het krijgen van epitheelkanker hebben we bestudeerd in de Zutphen Ouderen Studie (Hoofdstuk 6.1). Na uitsluiting van mannen die ooit kanker hadden gehad werden analyses uitgevoerd met 728 mannen in de leeftijd van 65-84 jaar. Na 10 jaar was bij 96 deelnemers een diagnose epitheelkanker gesteld, dit betrof in 42 gevallen longkanker. Na correctie voor andere risicofactoren bleek dat de inname van catechines niet geassocieerd was met epitheelkanker incidentie (risicoratio's van het laagste tot het hoogste tertiel: 1,00, 0,75, 0,94; P voor trend: 0,82) of met longkanker incidentie (risicoratio's van het laagste tot het hoogste tertiel: 1,00, 0,72, 0,92; P voor trend: 0,80). Catechines uit andere bronnen dan thee vertoonden een inverse maar niet statistisch significante associatie met longkankerincidentie (risicoratio en 95% betrouwbaarheidsinterval voor een 7,5 mg toename in inname 0,66; 0,42-1,05). De consumptie van catechines uit appels, de belangrijkste bron van catechines na thee, vertoonde eenzelfde verband met longkankerincidentie (risicoratio en 95% betrouwbaarheidsinterval voor een 7,5 mg toename in inname 0,67; 0,38-1,17). Dit was niet het geval voor catechines uit thee. Omdat thee, de belangrijkste bron van catechines in deze populatie, niet gerelateerd was met het risico op kanker, valt te betwijfelen of de gevonden associatie tussen niet-thee catechines en longkanker

causaal is.

Tenslotte bekeken we of de inname van catechines geassocieerd was met de incidentie van een aantal typen kanker in een groep postmenopausale vrouwen die deelnamen aan de Iowa Women's Health Study (Hoofdstuk 6.2). In deze studie werden 34.651 vrouwen zonder kanker in de leeftijd van 55 to 69 jaar gevolgd gedurende de periode 1986-1998. Na correctie voor andere risicofactoren was de inname van catechines alleen invers geassocieerd met het risico op rectaalkanker (risicoratio van het laagste tot het hoogste quartiel: 1,00, 0,93, 0,73, 0,55; P voor trend: 0,02). Niet-significante inverse trends werden gevonden voor kanker aan het bovenste deel van het maagdarmkanaal, de pancreas, en voor leukemieën. De inname van (+)-catechine plus (-)-epicatechine, voornamelijk afkomstig uit fruit, vertoonde een inverse trend met kanker van het bovenste maagdarmkanaal, terwijl catechines uit thee invers geassocieerd waren met rectaalkanker. Onze resultaten suggereren dus dat van een groot aantal bestudeerde kankers catechine-inname alleen invers gerelateerd is met rectaalkanker. De verschillen die we vonden tussen catechines uit thee en uit fruit zijn mogelijk gerelateerd aan verschillen in biobeschikbaarheid en metabolisme van de catechines uit deze producten, of aan interacties met andere componenten van de voeding. Verder onderzoek is echter nodig om dit te onderbouwen.

Concluderend kunnen we stellen dat catechines kwantitatief belangrijke nonnutritieve componenten van de dagelijkse voeding vormen. De belangrijkste bronnen van catechines in Nederland en in de Verenigde Staten zijn thee, appels en chocolade, maar ook andere fruitsoorten, wijn en peulvruchten dragen in bescheiden mate bij aan de totale catechine-inname. In het algemeen suggereren onze gegevens niet dat de bestudeerde catechines een belangrijke bijdrage leveren aan de bescherming tegen coronaire hartziekten, beroerte and kanker. Echter, een aantal catechines, namelijk degene die afkomstig zijn uit andere bronnen dan thee, zouden het risico op sterfte aan coronaire hartziekten en mogelijk de incidentie van een beperkt aantal soorten kanker kunnen verlagen. Misclassificatie van de catechine-inname zou hebben kunnen leiden tot afzwakking van werkelijke verbanden. Verschillen in biobeschikbaarheid en *in vivo* activiteit van de individuele catechines zouden een deel van de gevonden verschillen kunnen verklaren, maar om dit te ondersteunen is meer onderzoek nodig. Op basis van het huidige onderzoek kunnen geen wetenschappelijk onderbouwde aanbevelingen gedaan worden voor de consumptie van catechine-rijke voedingsmiddelen.

Dankwoord

Graag wil ik beginnen met het bedanken van alle mensen die ik heb vergeten te bedanken in het navolgende dankwoord: DANK!!! Vervolgens wil ik zonder daar nog om heen te draaien iedereen bedanken die een meer of minder grote maar altijd belangrijke bijdrage heeft geleverd aan het met zoveel plezier tot stand komen van dit proefschrift.

Eerst Daan Kromhout. Daan, je was altijd zeer betrokken bij en enthousiast over mijn werk. Onze regelmatige overleggen waren zeer inspirerend en ik waardeer het dat je me de ruimte en mogelijkheden gegeven hebt om mijn onderzoek zo in te richten als ik dacht dat het goed was. Peter Hollman, jij bent degene geweest die na mijzelf het intensiefst betrokken is geweest bij dit onderzoek. Ik kon altijd bij je binnenlopen en ik heb ontzettend veel van je geleerd. Hoewel ik nog steeds geen chemisch analyticus ben, heb je me wel enthousiast gemaakt voor de mogelijkheden die dit vak biedt. Onze halfjaarlijkse EU-vergaderingen ergens in Europa waren vooral heel erg leuk (de biertjes smaakten overal even goed!). Ik ben blij dat we onze samenwerking met tenminste een paar jaar kunnen verlengen. Edith Feskens en Bas Bueno de Mesquita, jullie hebben mijn verblijf op het RIVM mede zo prettig gemaakt. Jullie kritische noot bij al mijn artikelen heb ik zeer gewaardeerd. Dankzij jullie bevlogenheid heb ik nog steeds geen keuze kunnen maken tussen hart- en vaatziekten en kanker.

Betty, mijn steun en toeverlaat op het RIKILT. Je wist mijn soms overdreven perfectionisme altijd subtiel te relativeren en toch zeer betrouwbare resultaten te produceren. We hebben heel wat kilo's groenten, fruit en drank versleept. Met name het rammelende karretje vol flessen wijn wat we door het RIKILT reden, overtuigde iedereen ervan dat we met een weliswaar erg leuk, maar onmogelijk serieus te nemen project bezig waren. Dini, John en Michel: zonder jullie was de RIKILT periode een stuk minder prettig geweest. Als totale labnitwit kwam ik aan. Jullie waren altijd bereid om vol geduld dingen uit te leggen die voor jullie ongetwijfeld verbazingwekkend vanzelfsprekend waren. Verder was het ook gewoon erg gezellig en fijn om met jullie te werken. Ik verheug me erop weer deel te worden van het clubje.

Caroline en Rob, mijn kamergenoten op het RIVM. Na de transformatie van archief tot toch best luxueuze kamer, is de bezetting geloof ik nooit compleet geweest. Wij

hebben het er het langst, en wat mij betreft ook met heel veel plezier, uitgehouden. Ik was altijd voorzien van uitgebreide SAS-ondersteuning en zelfs vakinhoudelijke discussies gingen we soms niet uit de weg. Caroline, bedankt voor het veelvuldig doorsturen van telefoontjes als ik weer eens op een andere plek zat dan waar ik behoorde te zijn! Angelika, Claudia, Ina, Margje, Peggy, Saskia en Tommy, mijn collega-AIO's op het RIVM. Dank voor alle input (kant-en-klare SAS-bestanden, geupdate voedingsmiddelentabellen, Zutphen achtergrondmateriaal, NIHES cursusinfo). Wat was het een voordeel om bijna altijd net ná jullie aan iets te beginnen!

Aaron Folsom and David Jacobs, thanks for your hospitality and support during my stay in Minneapolis. I'm still amazed how much I learned and how much I was able to do during those few months. David, you're the first supervisor I've ever met that actually sits down with his students to teach them the art of SAS-analyses. Lisa Harnack and Myron Gross, thanks for being co-authors and for having inspiring discussions. Ching-Ping and Kathy, without you my stay would have been much more troublesome. Thanks for all the support. Maureen, you made my stay not only useful but fun as well! I miss our boatrides.

Verder wil ik bedanken: alle ICT'ers op RIKILT (Ruud en Jan) en RIVM (Jan, Ruud en Hans) voor het altijd razendsnel oplossen van mijn digitale problemen. Anke, Lydia, Els en Cécile voor ondersteuning op het communicatieve vlak in de breedste zin des woords. Huug Boer voor het op vakkundige wijze vriesdrogen van alle monsters. Jan Lenting voor hulp bij het vriesdroogklaar maken van de voedingsmiddelen en natuurlijk voor het gezellige koffiedrinken. Hendriek Boshuizen voor statistische back-up in tijden van nood. Marga Ocké, voor voedingskundige expertise. Vooral bij het VCP artikel heb ik veelvuldig je hulp ingeroepen en het was fijn dat je daar ook altijd tijd voor had. Jaap Seidell, op enkele cruciale momenten was je er nog net op tijd voor me. Ik hoop niet dat de MSc uitreiking in Rotterdam je nog boetes opgeleverd heeft.

Femke en Cora, fijn dat jullie mijn paranimfen willen zijn! Femke, voordat ik begon met mijn promotieonderzoek zei ik nog tegen je dat vier jaar werken aan één onderwerp wel erg lang was, maar dat ik wel zou zien hoe het zou bevallen. Nu is het alweer voorbij.

Pap, mam en Rogier, bedankt voor jullie steun, interesse en vooral voor het iedere keer weer trots zijn op mij! Zonder jullie zou ik hier nu niet hebben gestaan. Oké Maart, tenslotte moet je er toch aan geloven. Ik weet dat je geen kwijlerig bedankje wilt, maar je bent echt heel belangrijk voor me geweest de afgelopen jaren. Nu jij...

About the author

Ilja Catharina Wilhelmina Arts was born on 21 April 1970 in Boxmeer, the Netherlands. After completing secondary school in 1988 (VWO at the Merlet College in Cuijk) she studied Human Nutrition at Wageningen University. She graduated with distinction in 1994 with majors in Human Nutrition, and Epidemiology and Public Health. In November 1994, she obtained a VSB Scholarship for the period of fourteen months to study the biology and treatment of eating disorders at Uppsala University, Sweden. Hereafter, she worked for six months at the Unilever Nutrition Center in Vlaardingen, the Netherlands, on health aspects of fish fatty acids. In December 1996, she started her PhD on dietary catechins and chronic diseases, part of the Commission of the European Communities Programme on Complex Polyphenols and Tannins and Health. The research was carried out at the State Institute for Quality Control of Agricultural Products (RIKILT) in Wageningen, the National Institute of Public Health and the Environment (RIVM) in Bilthoven, and at the School of Public Health, University of Minnesota in Minneapolis, USA. In August 2000 she obtained her MSc in Epidemiology at the Netherlands Institute for Health Sciences (NIHES).

Publications

FULL PAPERS

Arts ICW, Hollman PCH, Bueno de Mesquita HB, Feskens EJM, Kromhout D. Dietary catechins and epithelial cancer incidence: the Zutphen Elderly Study. *Int J Cancer; in press.*

Arts ICW, Hollman PCH, Feskens EJM, Bueno de Mesquita HB, Kromhout D. Catechin intake might explain the inverse relation between tea consumption and coronary heart disease: the Zutphen Elderly Study. *Am J Clin Nutr; in press.*

Arts ICW, Jacobs Jr DR, Harnack LJ, Gross M, Folsom AR. Dietary catechins in relation to coronary heart disease death among postmenopausal women. *Epidemiology; in press.*

Tabak C, Arts ICW, Smit HA, Heederik D, Kromhout D. COPD and intake of catechins, flavonols and flavones: the MORGEN-study. *Am J Respir Crit Care Med; in press.*

Arts ICW, Hollman PCH, Feskens EJM, Bueno de Mesquita HB, Kromhout D. Catechin intake and associated dietary and lifestyle factors in a representative sample of Dutch men and women. *Eur J Clin Nutr* 2001;55:76-81.

Arts ICW, Van de Putte B, Hollman PCH. Catechin contents of foods commonly consumed in The Netherlands. 1. Fruits, vegetables, staple foods, and processed foods. *J Agric Food Chem* 2000;48:1746-1751.

Arts ICW, Van de Putte B, Hollman PCH. Catechin contents of foods commonly consumed in The Netherlands. 2. Tea, wine, fruit juices, and chocolate milk. *J Agric Food Chem* 2000;48:1752-1757.

Hollman PCH, Arts ICW. Flavonols, flavones and flavanols - nature, occurrence and dietary burden. *J Sci Food Agric* 2000;80:1081-1093.

Arts ICW, Hollman PCH, Kromhout D. Chocolate as a source of tea flavonoids. *Lancet* 1999;354:488.

Hollman PCH, Venema D, Khokhar S, Arts ICW. Determination of tea catechins by reversed-phase high performance liquid chromatography. In: Packer L, ed. *Methods in Enzymology, volume 299: Oxidants and Antioxidants Part A*. San Diego: Academic Press;1999:202-206.

Arts ICW, Hollman PCH. Optimization of a quantitative method for the determination of catechins in fruits and legumes. *J Agric Food Chem* 1998;46:5156-5162.

Beridot-Therond ME, Arts I, Fantino M, De La Gueronniere V. Short-term effects of the flavour of drinks on ingestive behaviours in man. *Appetite* 1998;31:67-81.

De Jong N, Van Dusseldorp M, Bergsma JS, Arts ICW, Van Staveren WA. Growth and nutritional status of macrobiotically fed children until 10 years of age. *Aktuelle Ernährungsmedizin* 1996;21:14-15.

Van Dusseldorp M, Arts ICW, Bergsma JS, De Jong N, Dagnelie PC, Van Staveren WA. Catch-up growth in children fed a macrobiotic diet in early childhood. *J Nutr* 1996;126:2977-2983.

Arts ICW, Jacobs Jr DR, Gross M, Harnack LJ, Folsom AR. Dietary catechins and cancer incidence among postmenopausal women: the Iowa Women's Health Study. *Submitted*.

ABSTRACTS

Arts ICW, Hollman PCH, Feskens EJM, Bueno de Mesquita HB, Kromhout D. Catechin intake is inversely associated with coronary heart disease mortality; results from the Zutphen Elderly Study. *Circulation* 2000;101:711.

Arts ICW, Hollman PCH, Van de Putte HM, Bueno de Mesquita HB, Feskens EJM, Kromhout D. Antioxidant catechins: intake and major sources in the Dutch population. In: Johnson IT, Fenwick GR, eds. *Dietary anticarcinogens and antimutagens: chemical and biological aspects*. Royal Society of Chemistry;2000:22-24.

Arts ICW, Hollman PCH. Optimisation of a quantitative method for the determination of catechins in fruits and pulses. *Pathophysiology* 1998;5:268.

The work on which this thesis is based was performed at the State Institute for Quality Control of Agricultural Products (RIKILT), Wageningen, the Department of Chronic Diseases Epidemiology, National Institute of Public Health and the Environment (RIVM), Bilthoven, and the Division of Epidemiology, School of Public Health, University of Minnesota, Minneapolis, USA.

The author gratefully acknowledges the guidance of dr. HB Bueno de Mesquita who was responsible for the supervision of the author during most of the period of epidemiological work conducted at the Department of Chronic Diseases Epidemiology, RIVM, the Netherlands and the guidance of prof. AR Folsom in Minneapolis who supervised the work conducted in the USA.

The project described in this thesis was funded by the Commission of the European Communities, Agriculture and Fisheries (FAIR) specific RTD programme, CT95 0653, and by the Netherlands Institute for Health Sciences (NIHES).

The printing of this thesis was financially supported by the State Institute for Quality Control of Agricultural Products (RIKILT) and the Netherlands Institute for Health Sciences (NIHES). Financial support by the Netherlands Heart Foundation for the printing of this thesis is gratefully acknowledged.

Dietary catechins and their potentially protective role in cardiovascular diseases and cancer
Ilja Catharina Wilhelmina Arts
Thesis Wageningen University – With ref. – With summary in Dutch
ISBN 90-5808-377-2

Cover: Maarten Jacobs
Printing: Grafisch bedrijf Ponsen & Looijen bv Wageningen