Tea Flavonoids May Protect Against Atherosclerosis

The Rotterdam Study
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**Background:** Epidemiological studies have indicated a protective role of dietary flavonoids in cardiovascular disease, but evidence is still conflicting. Tea is the major dietary source for flavonoids in Western populations. We studied the association of tea intake with aortic atherosclerosis in a general population.

**Methods:** The present analysis formed part of the Rotterdam Study, a prospective study of men and women 55 years and older. Dietary intakes were assessed at baseline by a trained dietician who used a semiquantitative food frequency questionnaire. Calcified plaques in the abdominal aorta were radiographically detected after 2 to 3 years of follow-up. Aortic atherosclerosis was classified as “mild,” “moderate,” or “severe,” according to the length of the calcified area (<1 cm, 1-5 cm, and >5 cm, respectively). The association of tea intake with severity of aortic atherosclerosis was studied in 3454 subjects who were free of cardiovascular disease at baseline. Data were analyzed by logistic regression, adjusting for age, sex, body mass index (calculated as weight in kilograms divided by the square of height in meters), smoking, education, and intake of alcohol, coffee, vitamin antioxidants, total fat, and total energy.

**Results:** Multivariable analyses showed a significant, inverse association of tea intake with severe aortic atherosclerosis. Odds ratios decreased from 0.54 (95% confidence interval [CI], 0.32-0.92) for drinking 125 to 250 mL (1-2 cups) of tea to 0.31 (CI, 0.16-0.59) for drinking more than 500 mL/d (4 cups per day). The associations were stronger in women than in men. The association of tea intake with mild and moderate atherosclerosis was not statistically significant.

**Conclusion:** This study indicates a protective effect of tea drinking against ischemic heart disease.

*Arch Intern Med.* 1999;159:2170-2174

**Epidemiological** studies have reported a reduced risk of coronary heart disease in subjects with a high flavonoid intake.¹ ² The protective effect of flavonoids, in particular the subgroup of flavonols, has been attributed to antioxidative activity.³ ⁴ Quercetin, for example, has been shown to inhibit oxidative modification of low-density lipoproteins and the development of fatty streaks in animals.⁵ ⁶ About half of the flavonol intake in Western populations is derived from black tea. We investigated the association of aortic atherosclerosis, a strong indicator of cardiovascular risk,⁷ ⁸ with intake of tea in 3454 participants of the population-based Rotterdam Study.

**Table 1** presents characteristics of the population for analysis. The proportion of tea drinkers was high in men (84%) and women (91%). Female tea drinkers on average had a higher daily tea intake than male drinkers (438 vs 375 mL [3.5 vs 3 cups]). Tea drinking, adjusted for age and sex, was significantly inversely correlated with body mass index, smoking, and intake of alcohol and coffee. Subjects with higher educations tended to drink more tea. Tea drinking was significantly and positively associated with the intake of vitamin antioxidants and inversely associated with total fat and energy intake. Aortic calcification was present in 1900 subjects, classified as “mild” in 641, “moderate” in 1061, and “severe” in 198. In 1554 subjects, no calcified plaques could be detected. The different categories of aortic atherosclerosis were about equally distributed in men and women (Table 1).

The intake of tea adjusted for age and sex, by atherosclerosis status, is presented in the Figure. Subjects without aortic calcification drank more tea (385 mL/d) than...
PARTICIPANTS AND METHODS

THE ROTTERDAM STUDY

The Rotterdam Study is a population-based follow-up study that aims to assess the occurrence of chronic diseases in an aging population and to clarify their determinants.12

A total of 7983 men and women 55 years and older, living in a defined district of Rotterdam, entered the study. During the baseline examination from August 1990 to June 1993, participants were interviewed at home by a trained research assistant. Information was obtained on subjects’ current and past health, medication, lifestyle, and risk indicators for chronic diseases. The participants subsequently visited the study center twice for clinical examination and assessment of diet. A total of 6315 subjects (88% of those alive) participated in the first follow-up phase from September 1993 to December 1995, which comprised a self-administered questionnaire and clinical examination at the study center. The median duration of follow-up was 1.9 years.

ASSESSMENT OF DIET

Before the baseline center visits, the participants received a checklist on which they indicated all foods and drinks that they consumed more than once a month during the preceding year. The completed checklist formed the basis of an interview at the study center by a trained dietician. An extensive, validated semiquantitative food frequency questionnaire was used.13,14 More than half of the dietary interviews were performed with the help of a computer that simultaneously carried out multiple checks on the data. The questionnaire comprised 170 food items and all relevant beverages, including tea, coffee, and alcohol.14 Seasonal variations in consumption were taken into account. Participants quantified their habitual tea intake as number of cups per day, week, or month. One cup of tea was equal to 125 mL. The intake of green tea is negligible in the Netherlands and was therefore not assessed. From the questionnaire data, the intake of total energy, fat, protein, carbohydrates, subtypes of these macronutrients, and a large number of minerals, vitamins, and other micronutrients was calculated with the use of Dutch food composition tables.15 No dietary data were collected during follow-up.

ASSESSMENT OF ATHEROSCLEROSIS

At baseline and during follow-up, lateral radiographic films of the abdomen were made from a fixed distance, while the participant was seated. Atherosclerosis in the abdominal aorta was diagnosed off-line by detecting linear calcified densities in an area parallel and anterior to the lumbar spine (vertebrae L1-L4).16,17 Mild, moderate, and severe aortic atherosclerosis were defined according to the length of the calcified area (<1 cm, 1-5 cm, and >5 cm, respectively). Necropsy has shown that aortic calcification represents an advanced stage of atherosclerosis and that radiographic assessment of calcified plaques has a high sensitivity.18

Baseline dietary interviews were performed in independently living subjects (n = 6250), except in those participating in the pilot phase of the Rotterdam Study. Subjects having a possible diagnosis of dementia (n = 122) were not interviewed because of expected difficulties in dietary recall. For a random group of 482 subjects, no dietary data could be obtained due to logistic reasons. Based on the judgment of the dietician, 212 unreliable dietary reports were excluded. Dietary data were thus available for 5434 subjects.

At the time of the present analysis, radiographic atherosclerosis scores were only available for subjects who participated in the first follow-up phase (1993-1995). Atherosclerosis scores were missing for 638 follow-up participants who did not visit the study center, 944 participants who were not radiographically examined due to absence of the device or refusal, a random group of 577 participants because the x-ray films were temporarily not available for atherosclerosis assessment, and 19 participants for whom the x-ray film did not allow scoring of atherosclerosis. Radiographic scores of aortic atherosclerosis were thus obtained in 4137 subjects, 3837 of whom had baseline dietary data. The eventual study population did not differ with regard to age, sex, tea consumption, and total energy intake from the remainder of follow-up participants with dietary data who lacked atherosclerosis scores.

We excluded from the analysis 383 subjects (of those having their baseline dietary data available) with a history of cardiovascular disease (ie, myocardial infarction, stroke, coronary artery bypass grafting, or percutaneous transluminal coronary angioplasty), because they could intentionally have changed their diet or lifestyle. A total of 3454 subjects remained for the present analysis.

DATA ANALYSIS

Daily tea intake was classified into 4 categories ranging from “0 mL” to “more than 500 mL.” Partial correlations of age- and sex-adjusted tea intake with lifestyle factors and energy-adjusted nutrient intakes were computed to identify potential confounders.

Categories of tea intake were entered into a multivariable logistic regression model with aortic atherosclerosis as the dependent variable. Age-adjusted odds ratios (ORs) and 95% confidence intervals (CIs) were estimated in men and women for mild, moderate, and severe atherosclerosis separately, using subjects free of aortic calcification as the reference group in all analyses. The analysis was repeated with the additional adjustment for body mass index (calculated as weight in kilograms divided by the square of height in meters), pack-years of smoking, education (5 categories), and intake of alcohol (g/d), coffee (mL/d), vitamin E (mg/d), vitamin C (mg/d), β-carotene (mg/d), total fat (g/d), and total energy (kJ/d). With regard to intake of vitamins C and E and β-carotene, indicator variables for use of specific vitamin supplements were also added to the model.

To study whether blood pressure or serum cholesterol levels could be intermediary factors in the relation of tea drinking with atherosclerosis, we examined changes in ORs after entering these parameters one at a time into the multivariable model.
We observed an inverse association between tea drinking and advanced stages of aortic atherosclerosis. The risk reductions were most pronounced in female tea drinkers. Before accepting these findings, several methodological issues need to be discussed.

Tea drinking in Western populations is generally associated with a healthy lifestyle and diet. Also in our study, the intake of tea was somewhat higher in lean, educated people who smoked less and had a relatively low intake of alcohol, coffee, and fat. The ORs increased up to 20% after adjustment for these parameters, which indeed indicates confounding of the crude association between tea drinking and atherosclerosis.

The estimates in the present study could be biased in subjects who were aware of an increased risk of cardiovascular disease and intentionally changed their diet toward a more healthy one. We largely avoided this type of bias by excluding from the analysis subjects with a history of cardiovascular disease. Furthermore, we used a measure of subclinical disease, making it less likely that subjects would have changed their behavior in response to cardiac disease. Had bias occurred, however, it would likely be in the direction of attenuating the OR because a healthier lifestyle is associated with a higher tea intake.

When studying atherosclerosis, long-term diet rather than recent intakes is the relevant exposure. The semiquantitative food frequency questionnaire that we used aims to assess diet over the preceding year. It has been shown that adults have a stable nutrient intake for many years, such as a medically prescribed diet for diabetes or a diet for diabetes mellitus. Because the proportion of subjects for whom misclassification of long-term dietary intakes occurred is small, we do not think that this has had a large effect on our findings.
Atherosclerosis was assessed by detection of calcified deposits in the aorta on x-ray films of the abdomen. In a validation study, all 20 subjects with calcified deposits on the x-ray film were found to have atherosclerosis at necropsy, indicating a high sensitivity. In 5 of 31 subjects in whom no calcification was diagnosed radiographically, atherosclerotic plaques were found in the aorta at necropsy. False-negative misclassification in our study, however, would mean only that our calculated risk estimate is conservative. Previous studies have shown that aortic atherosclerosis is related to the classical cardiovascular risk factors and that it predicts cardiovascular mortality and morbidity. Furthermore, it has been shown to reflect atherosclerosis in other vessel beds. The assessment and classification of aortic atherosclerosis in these studies was similar to our method.

Whereas strong inverse associations of tea drinking with severe aortic atherosclerosis were observed in the pre-

### Table 2. Risk of Aortic Atherosclerosis With Tea Intake*

<table>
<thead>
<tr>
<th>Tea Intake, mL/d (Cups/d)</th>
<th>0</th>
<th>≤250 (1-2)</th>
<th>&gt;250–≤500 (3-4)</th>
<th>&gt;500 (&gt;4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mild Atherosclerosis</td>
<td>OR, adjusted for age and sex</td>
<td>1.03 (0.74-1.42)</td>
<td>0.88 (0.63-1.23)</td>
<td>0.97 (0.67-1.39)</td>
</tr>
<tr>
<td></td>
<td>OR, adjusted for all confounders</td>
<td>1.15 (0.82-1.61)</td>
<td>1.05 (0.73-1.49)</td>
<td>1.15 (0.78-1.69)</td>
</tr>
<tr>
<td>Moderate Atherosclerosis</td>
<td>OR, adjusted for age and sex</td>
<td>0.69 (0.53-0.91)</td>
<td>0.58 (0.44-0.76)</td>
<td>0.63 (0.47-0.86)</td>
</tr>
<tr>
<td></td>
<td>OR, adjusted for all confounders</td>
<td>0.78 (0.59-1.03)</td>
<td>0.72 (0.54-0.97)</td>
<td>0.79 (0.57-1.10)</td>
</tr>
<tr>
<td>Severe Atherosclerosis</td>
<td>OR, adjusted for age and sex</td>
<td>0.46 (0.28-0.77)</td>
<td>0.36 (0.21-0.61)</td>
<td>0.24 (0.13-0.45)</td>
</tr>
<tr>
<td></td>
<td>OR, adjusted for all confounders</td>
<td>0.54 (0.32-0.92)</td>
<td>0.47 (0.27-0.81)</td>
<td>0.31 (0.16-0.59)</td>
</tr>
</tbody>
</table>

*OR indicates odds ratio obtained by logistic regression analysis with 95% confidence interval. The confounders include age, sex, body mass index, smoking, education, and intake of alcohol, coffee, vitamin antioxidants, fat, and total energy.

### Table 3. Risk of Aortic Atherosclerosis With Tea Intake in Men*

<table>
<thead>
<tr>
<th>Tea Intake, mL/d (Cups/d)</th>
<th>0</th>
<th>≤250 (1-2)</th>
<th>&gt;250–≤500 (3-4)</th>
<th>&gt;500 (&gt;4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mild Atherosclerosis</td>
<td>OR, adjusted for age</td>
<td>1.00 (0.64-1.57)</td>
<td>1.07 (0.66-1.75)</td>
<td>0.82 (0.46-1.46)</td>
</tr>
<tr>
<td></td>
<td>OR, adjusted for all confounders</td>
<td>1.09 (0.68-1.75)</td>
<td>1.25 (0.74-2.13)</td>
<td>0.94 (0.50-1.75)</td>
</tr>
<tr>
<td>Moderate Atherosclerosis</td>
<td>OR, adjusted for age</td>
<td>0.70 (0.48-1.01)</td>
<td>0.82 (0.55-1.23)</td>
<td>0.81 (0.51-1.29)</td>
</tr>
<tr>
<td></td>
<td>OR, adjusted for all confounders</td>
<td>0.71 (0.48-1.06)</td>
<td>0.90 (0.59-1.39)</td>
<td>0.89 (0.54-1.46)</td>
</tr>
<tr>
<td>Severe Atherosclerosis</td>
<td>OR, adjusted for age</td>
<td>0.60 (0.27-1.35)</td>
<td>0.87 (0.37-2.00)</td>
<td>0.41 (0.13-0.83)</td>
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<tr>
<td></td>
<td>OR, adjusted for all confounders</td>
<td>0.67 (0.28-1.59)</td>
<td>1.17 (0.47-2.92)</td>
<td>0.44 (0.13-1.48)</td>
</tr>
</tbody>
</table>

*OR indicates odds ratio obtained by logistic regression analysis with 95% confidence interval. The confounders include age, body mass index, smoking, education, and intake of alcohol, coffee, vitamin antioxidants, fat, and total energy.

### Table 4. Risk of Aortic Atherosclerosis With Tea Intake in Women*

<table>
<thead>
<tr>
<th>Tea Intake, mL/d (Cups/d)</th>
<th>0</th>
<th>≤250 (1-2)</th>
<th>&gt;250–≤500 (3-4)</th>
<th>&gt;500 (&gt;4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mild Atherosclerosis</td>
<td>OR, adjusted for age</td>
<td>1.09 (0.68-1.74)</td>
<td>0.84 (0.53-1.34)</td>
<td>1.06 (0.65-1.73)</td>
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<td></td>
<td>OR, adjusted for all confounders</td>
<td>1.28 (0.78-2.09)</td>
<td>1.05 (0.64-1.71)</td>
<td>1.29 (0.76-2.19)</td>
</tr>
<tr>
<td>Moderate Atherosclerosis</td>
<td>OR, adjusted for age</td>
<td>0.72 (0.49-1.07)</td>
<td>0.48 (0.32-0.71)</td>
<td>0.55 (0.36-0.83)</td>
</tr>
<tr>
<td></td>
<td>OR, adjusted for all confounders</td>
<td>0.93 (0.61-1.41)</td>
<td>0.71 (0.46-1.07)</td>
<td>0.83 (0.52-1.31)</td>
</tr>
<tr>
<td>Severe Atherosclerosis</td>
<td>OR, adjusted for age</td>
<td>0.38 (0.20-0.76)</td>
<td>0.22 (0.11-0.44)</td>
<td>0.17 (0.08-0.37)</td>
</tr>
<tr>
<td></td>
<td>OR, adjusted for all confounders</td>
<td>0.46 (0.23-0.96)</td>
<td>0.29 (0.13-0.60)</td>
<td>0.23 (0.09-0.54)</td>
</tr>
</tbody>
</table>

*OR indicates odds ratio obtained by logistic regression analysis with 95% confidence interval. The confounders include age, sex, body mass index, smoking, education, and intake of alcohol, coffee, vitamin antioxidants, fat, and total energy.
sent study, risk reductions for moderate and mild atherosclerosis were only weak or absent. This finding could be explained by the larger contrast between index and reference group, when studying severe atherosclerosis. Also, the discrepancy in findings may be caused by the high prevalence of mild and moderate atherosclerosis at old age. The relative contribution of tea drinking compared with age to the development these atherosclerotic stages will consequently be lower and more difficult to detect. Therefore, the fact that tea drinking appeared only related to advanced and not to milder stages of aortic atherosclerosis in our study could be due to methodological issues, rather than the biological mechanisms.

Flavonoids have been shown to protect against oxidation of low-density lipoproteins and the development of fatty streaks. Considerable levels of flavonoids with antioxidative properties, such as quercetin (10-25 mg/L), kaempferol (7-17 mg/L), and myricetin (2-5 mg/L), have been detected in black tea. In the Zutphen Elderly Study of aged men, tea drinkers had a more than 50% reduced risk of 5-year coronary mortality. However, no protective effect of tea and flavonoids against coronary events was observed in the male cohorts of the Health Professionals Follow-up Study and the Caerphilly Study. The absence of a relationship in the latter study has partly been attributed to the fact that participants took milk with their tea, which may reduce the bioavailability of flavonoids. We could not study effect modification by milk, because this way of tea drinking is rather uncommon in the Netherlands, and necessary data to address this issue were not collected.

In conclusion, our data provide evidence for a protective effect of tea drinking against severe atherosclerosis, especially in women. Further investigation into the mechanisms that explain the protective effect of tea flavonoids in the cardiovascular system is warranted.

Accepted for publication February 15, 1999.

Financial support for the present study has been obtained from Unilever Research, Vlaardingen, the Netherlands, and from the Health Research and Development Council, The Hague.

We thank Kattiinka Grashuis for scoring of aortic calcification on radiographic films.

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REFERENCES