U.S. Department of Justice
Washington, DC 20530

Supplemental Statement
Pursuant to Section 2 of the Foreign Agents Registration Act of 1938, as amended

OMB NO. 1105-0002

For Six Month Period Ending 31 DEC 1996
(Insert date)

I - REGISTRANT

1. (a) Name of Registrant

Tea Council of the U.S.A., Inc.

(b) Registration No.

01853

(c) Business Address(es) of Registrant

230 Park Avenue
New York, NY 10169

2. Has there been a change in the information previously furnished in connection with the following:

(a)	If an individual:		
	(1) Residence address	Yes ☐	No ☐
	(2) Citizenship	Yes ☐	No ☐
	(3) Occupation	Yes ☐	No ☐
(b)	If an organization:		
	(1) Name	Yes ☐	No ☒
	(2) Ownership or control	Yes ☐	No ☒
	(3) Branch offices	Yes ☐	No ☒

(c) Explain fully all changes, if any, indicated in items (a) and (b) above.

010716

IF THE REGISTRANT IS AN INDIVIDUAL, OMIT RESPONSE TO ITEMS 3, 4, AND 5(a).

3. If you have previously filed Exhibit C[1], state whether any changes therein have occurred during this 6 month reporting period.

Yes ☐ No ☒

If yes, have you filed an amendment to the Exhibit C? Yes ☐ No ☐

If no, please attach the required amendment.

97 JAN 21 PM 3:54
CRM/ISA/REGISTRATION UNIT

1 The Exhibit C, for which no printed form is provided, consists of a true copy of the charter, articles of incorporation, association, and by laws of a registrant that is an organization. (a waiver of the requirement to file an Exhibit C may be obtained for good cause upon written application to the Assistant Attorney General, Criminal Division, Internal Security Section, U.S. Department of Justice, Washington, D.C. 20530.)

4. (a) Have any persons ceased acting as partners, officers, directors or similar officials of the registrant during this 6 month reporting period? Yes ☒ No ☐

If yes, furnish the following information:

Name	Position	Date Connection Ended
Ralph F. Genzano	Director	10-8-96
Barry Gettins	Director	10-8-96
John O'Shaughnessy	Director	10-8-96

(b) Have any persons become partners, officers, directors or similar officials during this 6 month reporting period? Yes ☒ No ☐

If yes, furnish the following information:

Name	Residence Address	Citizenship	Position	Date Assumed
David Gordon	82 Stoner Dr. West Hartford, CT 06107	U.S.A.	Director	10-8-96

5. (a) Has any person named in item 4(b) rendered services directly in furtherance of the interests of any foreign principal? Yes ☐ No ☒

If yes, identify each such person and describe his service.

(b) Have any employee or individuals, who have filed a short form registration statement, terminated their employment or connection with the registrant during this 6 month reporting? Yes ☐ No ☒

If yes, furnish the following information:

Name	Position or connection	Date terminated

(c) During this six month reporting period, has the registrant hired as employees or in any other capacity, any persons who rendered or will render services to the registrant directly in furtherance of the interests of any foreign principal(s) in other than a clerical or secretarial, or in a related or similar capacity? Yes ☐ No ☒

If yes, furnish the following information:

Name	Residence Address	Citizenship	Position	Date Assumed

6. Have short form registration statements been filed by all of the persons named in Items 5(a) and 5(c) of the supplemental statement? Yes ☐ No ☐

Not Applicable

If no, list names of persons who have not filed the required statement.

II - FOREIGN PRINCIPAL

7. Has your connection with any foreign principal ended during this 6 month reporting period?
Yes ☐ No ☒

If yes, furnish the following information:

Name of foreign principal *Date of termination*

8. Have you acquired any new foreign principal[2] during this 6 month reporting period?
Yes ☐ No ☒

If yes, furnish following information:

Name and address of foreign principal *Date acquired*

9. In addition to those named in Items 7 and 8, if any, list foreign principals[2] whom you continued to represent during the 6 month reporting period.

Sri Lanka Tea Board
Indonesian Tea Association
Tea Board of India

10. **EXHIBITS A AND B**

(a) Have you filed for each of the newly acquired foreign principals in Item 8 the following:

Exhibit A[3]	Yes ☐	No ☐	Not Applicable
Exhibit B[4]	Yes ☐	No ☐	

If no, please attach the required exhibit.

(b) Have there been any changes in the Exhibits A and B previously filed for any foreign principal whom you represented during this six month period? Yes ☐ No ☒

If yes, have you filed an amendment to these exhibits? Yes ☐ No ☐

If no, please attach the required amendment.

2 The term "foreign principal" includes, in addition to those defined in section 1(b) of the Act, an individual organization any of whose activities are directly or indirectly supervised, directed, controlled, financed, or subsidized in whole or in major part by a foreign government, foreign political party, foreign organization or foreign individual. (See Rule 100(a) (9)). A registrant who represents more than one foreign principal is required to list in the statements he files under the Act only those principals for whom he is not entitled to claim exemption under Section 3 of the Act. (See Rule 208.)

3 The Exhibit A, which is filed on form CRM-157 (Formerly OBD-67) sets forth the information required to be disclosed concerning each foreign principal.

4 The Exhibit B, which is filed on Form CRM-155 (Formerly OBD-65) sets fourth the information concerning the agreement or understanding between the registrant and the foreign principal.

III - ACTIVITIES

11. During this 6 month reporting period, have you engaged in any activities for or rendered any services to any foreign principal named in Items 7, 8, and 9 of this statement? Yes ☒ No ☐

If yes, identify each such foreign principal and describe in full detail your activities and services:

Sri Lanka Tea Board, Indonesian Tea Association, Tea Board of India

The Tea Council's sole objective is to try to increase the consumption of tea in the U.S.A. All of its publicity/promotion programs, consisting of consumer booklets, radio interviews and press releases are aimed toward that stated objective.

A. Tea and Health Campaign as outlined in Item 11A

12. During this 6 month reporting period, have you on behalf of any foreign principal engaged in political activity[5] as defined below?

Yes ☐ No ☒

If yes, identify each such foreign principal and describe in full detail all such political activity, indicating, among other things, the relations, interests and policies sought to be influenced and the means employed to achieve this purpose. If the registrant arranged, sponsored or delivered speeches, lectures or radio and TV broadcasts, give details as to dates, places, of delivery, names of speakers and subject matter.

13. In addition to the above described activities, if any, have you engaged in activity on your own behalf which benefits any or all of your foreign principals? Yes ☒ No ☐

If yes, describe fully.

Please see Item 11A

5 The term "political activities" means any activity that the person engaging in believes will, or that the person intends to, in any way influence any agency or official of the Government of the United States or any section of the public within the United States with reference to formulating, adopting or changing the domestic or foreign policies of the United States or with reference to political or public interests, policies, or relations of a government aforeign country or a foreign political party.

IV - FINANCIAL INFORMATION

14. (a) **RECEIPTS-MONIES**

During this 6 month reporting period, have you received from any foreign principal named in Items 7, 8, and 9 of this statement, or from any other source, for or in the interests of any such foreign principal, any contributions, income or money either as compensation or otherwise? Yes ☒ No ☐

If no, explain why.

If yes, set forth below in the required detail and separately for each foreign principal an account of such monies[6]

Date	From Whom	Purpose	Amount
12/30/96	Sri Lanka Tea Board	Full Member Dues	$17,625.00

Total $17,625.00

(b) **RECEIPTS - FUND RASING CAMPAIGN**

During this 6 month reporting period, have you received, as part of a fund raising campaign[7], any money on behalf of any foreign principal named in items 7, 8, and 9 of this statement? Yes ☐ No ☒

If yes, have you filed an Exhibit D to your registration? Yes ☐ No ☐

If yes, indicate the date the Exhibit D was filed. Date _____.

(c) **RECEIPTS-THINGS OF VALUE**

During this 6 month reporting period, have you received any thing of value[9] other than money from any foreign principal named in Items 7, 8, and 9 of this statement, or from any other source, for or in the interests of any such foreign principal? Yes ☐ No ☒

If yes, furnish the following information:

Name of foreign principal	Date received	Description of thing of value	Purpose

6, 7 A registrant is required to file an Exhibit D if he collects or receives contributions, loans, money, or other things of value for a foreign principal, as part of a fund raising campaign. (See Rule 201(e).)
8 An Exhibit D, for which no printed form is provided, sets forth an account of money collected or received as a result of a fund raising campaign and transmitted for a foreign principal.
9 Things of value include but are not limited to gifts, interest free loans, expense free travel, favored stock purchases, exclusive rights, favored treatment over competitors, "kickbacks," and the like.

15. (a) **DISBURSEMENTS-MONIES**

During this 6 month reporting period, have you

(1) disbursed or expended monies in connection with activity on behalf of any foreign named in Items 7, 8, and 9 of this statement? Yes ☒ No ☐

(2) transmitted monies to any such foreign principal? Yes ☐ No ☒

If no, explain in full detail why there were no disbursements made on behalf of any foreign principal.

If yes, set forth below in the required detail and separately for each foreign principal an account of such monies, including monies transmitted, if any, to each foreign principal.

Date	To Whom	Purpose	Amount

See Item # 15 - "Proforma Report of the Treasurer for the 12 Months Ending December 31, 1996"

a) Types of expenses included in Account #199 - Association Fee (Administrative Costs for shared office expenses).

- postage, UPS Delivery/Messengers, Federal Express
- Printing of letterhead, stationary, envelopes, memo pads, etc.
- Copier paper, machine maintenance, toner
- All other office supply paper
- Typewriter/Computer supplies
- Bathroom/kitchen supplies
- Pens/pencils/highlighters, etc.
- Envelopes, folders, labels
- Telephone/fax charges
- Maintenance on Telephone system

f) Under Tea and Health Campaing - Account numbers 301-399, disbursements are made to:

Aronow & Pollock Communications, Inc.
524 Broadway, 3rd Floor
New York, NY 10013

Total

(b) **DISBURSEMENTS-THINGS OF VALUE**

During this 6 month reporting period, have you disposed of anything of value[10] other than money in furtherance of or in connection with activities on behalf of any foreign principal named in Items 7, 8, and 9 of this statement?

Yes ☐ No ☒

If yes, furnish the following information:

Date disposed	Name of person to whom given	On behalf of what foreign principal	Description of thing of value	Purpose

(c) **DISBURSEMENTS-POLITICAL CONTRIBUTIONS**

During this 6 month reporting period, have you from your own funds and on your own behalf either directly or through any other person, made any contributions of money or other things of value[11] in connection with an election to any political office, or in connection with any primary election, convention, or caucus held to select candidates for political office?

Yes ☐ No ☒

If yes, furnish the following information:

Date	Amount or thing of value	Name of political organization	Name of candidate

10, 11 Things of value include but are not limited to gifts, interest free loans, expense free travel, favored stock purchases, exclusive rights, favored treatment over competitors, "kickbacks" and the like.

V - INFORMATIONAL MATERIALS

16. During this 6 month reporting period, did you prepare, disseminate or cause to be disseminated any informational materials[12]?
Yes ☐ No ☒

IF YES, RESPOND TO THE REMAINING ITEMS IN SECTION V.

17. Identify each such foreign principal.

Not applicable

18. During this 6 month reporting period, has any foreign principal established a budget or allocated a specified sum of money to finance your activities in preparing or disseminating informational materials?
Yes ☐ No ☒

If yes, identify each such foreign principal, specify amount, and indicate for what period of time.

19. During this 6 month reporting period, did your activities in preparing, disseminating or causing the dissemination of informational materials include the use of any of the following:

☐ Radio or TV broadcasts
☐ Magazine or newspaper articles
☐ Motion picture films
☐ Letters or telegrams
☐ Advertising campaigns
☐ Press releases
☐ Pamphlets or other publications
☐ Lectures or speeches
☐ Other (specify) None

20. During this 6 month reporting period, did you disseminate or cause to be disseminated informational materials among any of the following groups:

☐ Public Officials
☐ Legislators
☐ Government agencies
☐ Newspapers
☐ Editors
☐ Civic groups or associations
☐ Libraries
☐ Educational institutions
☐ Nationality groups
☐ Other (specify) None

21. What language was used in the informational materials:
☐ English ☐ Other (specify) None

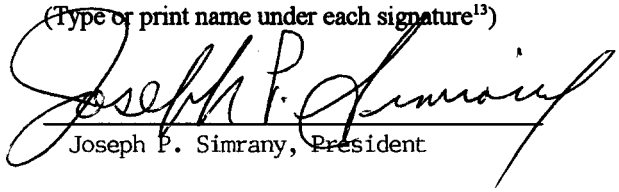
22. Did you file with the Registration Unit, U.S. Department of Justice a copy of each item of such informational materials disseminated or caused to be disseminated during this 6 month reporting period? Yes ☐ No ☒

23. Did you label each item of such informational materials with the statement required by Section 4(b) of the Act?
Yes ☐ No ☒

12 The term informational materials includes any oral, visual, graphic, written, or pictorial information or matter of any kind, including that published by means of advertising, books, periodicals, newspapers, lectures, broadcasts, motion pictures, or any means or instrumentality of interstate or foreign commerce or otherwise. Informational materials disseminated by an agent of a foreign principal as part of an activity in itself exempt from registration, or an activity which by itself would not require registration, need not be filed pursuant to Section 4 (b) of the Act.

VI--EXECUTION

In accordance with 28 U.S.C. § 1746, the undersigned swear(s) or affirm(s) under penalty of perjury that he/she has (they have) read the information set forth in this registration statement and the attached exhibits and that he/she is (they are) familiar with the contents thereof and that such contents are in their entirety true and accurate to the best of his/her (their) knowledge and belief, except that the undersigned make(s) no representation as to truth or accuracy of the information contained in the attached Short Form Registration Statement(s), if any, insofar as such information is not within his/her (their) personal knowledge.

(Date of signature)	(Type or print name under each signature[13])
January 15, 1997	 Joseph P. Simrany, President

13 This statement shall be signed by the individual agent, if the registrant is an individual, or by a majority of those partners, officers, directors or persons performing similar functions, if the registrant is an organization, except that the organization can, by power of attorney, authorize one or more individuals to execute this statement on its behalf.

UNITED STATES DEPARTMENT OF JUSTICE
REGISTRATION UNIT
CRIMINAL DIVISION
WASHINGTON, D.C. 20530

NOTICE

Please answer the following questions and return this sheet in triplicate with your supplemental statement:

1. Is your answer to Item 16 of Section V (Political Propaganda - page 7 of Form CRM-154, formerly Form OBD-64 - Supplemental Statement):

   Yes _____ *or No* X

(If your answer to question 1 is "yes" do not answer question 2 of this form.)

2. Do you disseminate any material in connection with your registration:

   Yes X *or No* _____

(If your answer to question 2 is "yes" please forward for our review copies of all such material including: films, film catalogs, posters, brochures, press releases, etc. which you have disseminated during the past six months.)

Joseph P. Simrany
Signature

1/10/97
Date

Joseph P. Simrany, President
Please type or print name of signatory on the line above

_____
Title

0715

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CRM/ISS/REGISTRATION UNIT

ARONI & POLLOCK COMMUNICATIONS, INC.

Post-it® Fax Note 7671 | Date 1/6/97 | # of pages ▶ 2
To Jean Singer | From Diana Boniface-Hatton
Co./Dept. Tea Council | Co.
Phone # | Phone #
Fax # | Fax #

ITEM # 11-A

MEMORANDUM

TO: Jean Singer DATE: January 6, 1997

FROM: Diana Boniface-Hatton

SUBJECT: Tea & Health Program Update for U.S. Justice Department

This memo details the Tea & Health public relations activities conducted by ARONOW & POLLOCK COMMUNICATIONS from June through December, 1996.

Scientific Information Exchange

The information exchange provides current scientific information to health professionals and professional health communicators. This program generates articles and discussions about the health benefits of tea.

In recent months, these efforts include:

- disseminating black tea information kits on an ongoing basis to health and nutrition media representatives;
- mailing the updated *Your Cup of Tea* white paper to an additional list of health communicators along with recent tea studies
- revising information contained in the *Totally Tea* brochure, and
- planning editorial tea tastings to discuss tea's health benefits with key media.

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Broadcast Publicity

To promote the health benefits of tea during the month January -- Hot Tea Month -- the Tea Council made preparations to distribute via satellite a video news release on the health benefits of tea.

Crisis Communications

Due to a number of sensationalized reports in the news media questioning the safety of iced tea served in restaurants, the Tea Council implemented a crisis communications plan. in recent months, the plan has consisted of:

- presenting the "Tempest in a Teapot" case history to attendees at the International Milk and Food Engineer Sanitarians (IAMFES) conference;
- sending accurate information to television stations planning to broadcast iced tea contamination stories;
- monitoring coverage of tea contamination stories and sending follow-up correspondence to stations as necessary.

Ongoing Publicity

Health editors, writers and broadcasters need materials to use as background information for their articles, columns and broadcasts. The Tea Council responds to ongoing media inquiries by providing resource materials on tea's health benefits and when appropriate, arranging interviews with members of the scientific advisory panel.

Scientific Advisory Panel

The Tea Council convened their scientific advisory panel in December to discuss progress on current tea research and to discuss the planning of a scientific symposium for early 1998.

Tea Council VNR
Draft #4
Final version with quotes
12/30/96

Medical science now suggests that the benefits of drinking tea may be far greater than just relaxation or a quick pick-me-up.

Some laboratory and observational studies suggest that tea may play a role in fighting cancer and possibly heart disease.

One recent observational study in older women found a correlation between drinking two cups of tea per day and a reduced risk of digestive tract cancer. Another study in men indicated that heavy tea drinkers may have a decreased risk of stroke.

Dr. Jeff Blumberg: "Tea is one of those components of our diet that people are really beginning to look at. There are many research studies that are underway today that very soon will tell us the kinds of health benefits that we anticipate from tea."

Dr. Carol Greenwood: "I would say that the main thrust right now is looking in terms of cancer and heart disease, and it's just because they're the huge killers in North America and the huge healthcare dollars that go into it."

Excited by preliminary findings, researchers are continuing to look at tea's potential to reduce the risk of certain chronic diseases, such as heart disease and cancers.

Dr. Jeff Blumberg: "Tea contains a lot of antioxidant nutrients that can reduce the risk of heart disease by interfering with those mechanisms that oxidize cholesterol and begin to lead to atherosclerosis."

It's no wonder tea consumption is on the rise. More and more Americans are turning to tea, as both a warm-up and a refresher, in the never-ending quest for the perfect way to relax.

Between 1990 and 1995, US tea consumption doubled from 2 billion to 4 billion dollars. Each year, we sip 50 billion servings, 40 billion of them over ice.

The most frequently consumed beverage in the world next to water is becoming the increasingly popular alternative here in the U.S. -- both hot and over ice, at work. at meals and on the go.

And we're talking about good old, regular tea, the kind of tea you find in your supermarket.

Americans are waking up to the pleasure of this 5,000 year old beverage as a way to enjoy peace and calm in the middle of a hectic schedule.

In fact, a growing number of fine hotels and even hip restaurants are now featuring afternoon tea services where shoppers and business types can relax and feel the stress melt away.

More and more of us are also turning to tea to warm and relax,

Because research shows we have nothing to lose and *possibly* a good deal to gain from sipping tea.

ARONOW & POLLOCK COMMUNICATIONS, INC

November 11, 1996

Dear Editor,

When formatting your upcoming stories for the cooler weather, I hope you will consider the health benefits of tea a hot topic. Enclosed are three recently published studies that may be of interest to you for possible coverage, including:

"Tea Consumption and Cancer Incidence in a Prospective Cohort Study of Postmenopausal Women," published in the *American Journal of Epidemiology,* 1996, vol 144, No. 2, 175-82. This study suggests that tea, one of the most popular beverages consumed worldwide, may protect against some cancers in postmenopausal women.

"Prospective Study of Beverage Use and the Risk of Kidney Stones," published in the *American Journal of Epidemiology,* 1966, vol 143, 240-7. This study demonstrated that tea may have a protective effect on stone formation that involves more than additional fluid intake alone.

"In vivo antioxidant effect of green and black tea in man," published in the *European Journal of Clinical Nutrition* (January, 1996) 50, 28-32. This study's findings demonstrate that tea possesses a strong antioxidant activity *in vitro.* It provides compelling evidence that tea also has a potent *in vivo* activity in man and suggests that the absorption of the bioactive components of tea take place in the upper part of the gastrointestinal system.

Please call if you have questions or need additional information about these materials. I'd be happy to help. Thanks for your consideration.

Sincerely,

Harriet Shelare

Harriet Shelare

Diana Boniface

Diana Boniface

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YOUR CUP OF Tea

Dating back almost 5,000 years, tea is one of the world's oldest beverages. It is also one of the world's most widely consumed beverages with the people of some countries drinking as many as 20 cups a day.

Over the course of history, a wide range of health benefits has been attributed to tea. According to legend, the Chinese emperor Shen Nung, known as the "Divine Healer," was the first to discover its comforts when some tea leaves blew into a pot of boiling water. The first written record of the effects of tea on health dates to the second century A.D., in the textbook of Chinese herbal medicine named for Shen Nung and known as the "Shen Nung Ben Cao Jing." The beverage made its way to Japan about 593 A.D., and later to other parts of the Far East and West, entering many cultures and having a role in folk medicine around the world.

With the advancement of modern biomedical research, an increasing number of studies has suggested that tea may have health benefits unforeseen by folk medicine. Its benefits may extend to a reduction in tooth decay, the lowering of high blood pressure, and a protective effect against heart disease and some forms of cancer.

Tea is a rich source of the plant substances known as *flavonoids*, which are believed to be partially responsible for the health benefits attributed to oranges, broccoli, wines and a number of other food plants in the human diet. The most significant flavonoids in tea are known as catechins and flavonols. An important characteristic of these compounds is their ability to prevent oxidation caused by two kinds of chemical agents — known as *peroxides* and *free radicals* — from damaging membranes, genetic material, and other components of living tissues. Although it is not clear how they arise within the body, both peroxides and free radicals exert their damaging effects because they are highly energetic and able to attack and combine with a wide variety of other molecules.

What enables the catechins and flavonols to counteract peroxides and free radicals is the chemical structure of their molecules, which contain constituents known as *phenolic groups*. These phenolic groups, each of which consist of an atom of oxygen linked to an atom of hydrogen, can "snare" peroxides and free radicals by combining with them chemically, thus neutralizing their destructive potential before they can cause damage.

THE TYPES OF TEA

All of the more than 3,000 different types of tea consumed throughout the world come from the tea plant, *Camellia sinensis*. It is grown today in more than 30 countries. There are three basic types of manufacture, which result in *black tea, green tea* and *oolong tea*. Today, black tea accounts for 77% of the world's tea manufacture, while green tea constitutes approximately 21% and oolong tea about 2% of tea production.

The difference between green tea, black tea, and oolong tea is in the way each is processed from the fresh leaves of the tea plant. Tea leaves, besides containing the catechins that appear to be responsible for many of the potential beneficial effects of tea, also contain an enzyme known as *polyphenol oxidase*. After the leaves are harvested, this enzyme works within them to transform catechins into substances known as catechin *quinones*.

One of the outcomes of manufacturing green tea is preserving the catechins originally present in tea leaves. The result is that polyphenol oxidase is stopped from transforming the catechins into quinones. This is done by steaming or pan firing the harvested leaves, which inactivates the oxidase enzyme. As a result of this inactivation of polyphenol oxidase, green tea has a composition closely similar to that of fresh tea leaves, and is rich in simple catechins. The principal catechin in green tea is known as epigallocatechin gallate (EGCG). A single cup of green tea contains from 40 to 90 milligrams of EGCG, which has shown a capacity to inhibit oxidation that can damage cells. This antioxidant effect of EGCG is believed to derive from the large number of phenolic groups in its molecule, which is shown in Figure 1.

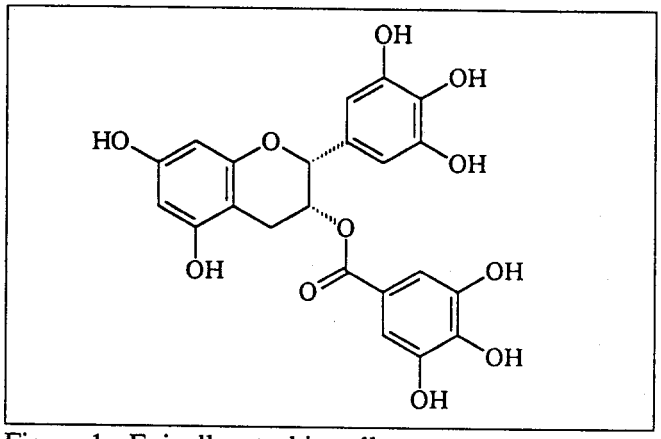

Figure 1 - Epigallocatechin gallate

In being able to prevent the damaging oxidation of substances in living cells, the flavonoids of tea work similarly to other antioxidants that occur naturally in edible plants. Among these other antioxidants are beta-carotene, a substance widely present in vegetables and fruits, and vitamins C and E. Vitamin E, known scientifically as alpha-tocopherol, is abundant in wheat germ and vegetable oils, and plays an important role in preventing oxidative damage to vitamin A, carotenoids, and unsaturated fatty acids in the human diet. Vitamin E contains a phenolic group like that of the catechins, as can be seen in Figure 2.

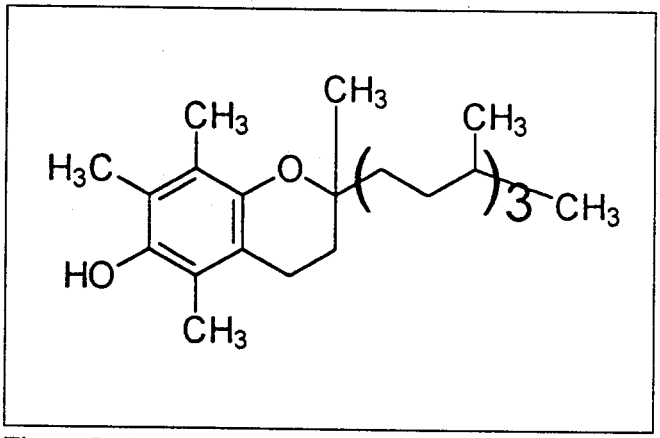

Figure 2 - Vitamin E (alpha-tocopherol)

Contrasting with the production of green tea, the manufacture of black tea allows polyphenol oxidase to transform catechins into quinones. The various catechin quinones produced by this enzymatic reaction combine with one another to yield more complex polyphenols that give the various kinds of black tea their unique taste and color. Among these color- and flavor-producing substances are those known as *thearubigins*, which black tea contains in high concentrations, and a group of reddish-orange substances known as *theaflavins*, which are present only in small quantities but contribute significantly to its red color.

Falling between green tea and black tea is oolong tea, which is manufactured in such a way that only some of the catechins are transformed into quinones.

Like EGCG and the other catechins prevalent in green tea, the theaflavins in black tea show antioxidant and antimutagenic activity in model laboratory studies. As in the case of EGCG, the theaflavins' antioxidant activity stems from their high content of phenolic groups. This can be seen in Figure 3, which depicts the chemical structure of theaflavin.

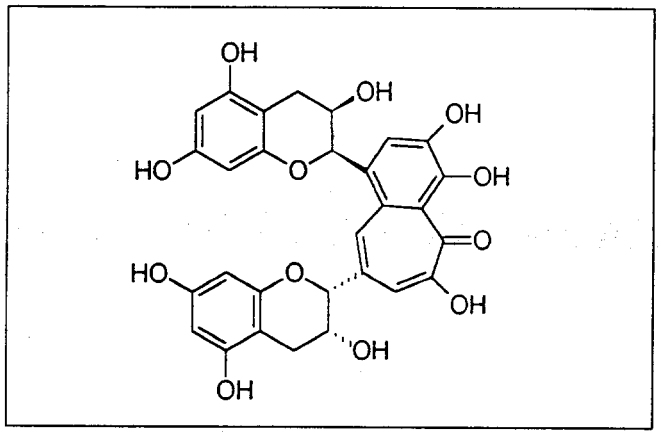

Figure 3 - Theaflavin

HOW TEA MAY PREVENT DISEASE

A growing number of studies have examined the effects of tea and its component substances in laboratory experiments and animal "models" of cancer, heart disease, and other human illnesses.

TEA AND CANCER

A very recent study at Rutgers University[1] (Wang et al.: *Cancer Res.* 54:3428-3435, 1994) showed that both green and black teas exerted a protective effect against skin tumors in mice. The tumors were generated by treating the animals' skin with dimethylbenzanthracene, a cancer-causing agent,

and exposing them to ultraviolet-B light, which is also present in the sunlight. Mice given solutions of black or green tea, or decaffeinated preparations of both kinds of tea, had from 65 to 93% fewer epithelial cancers known as carcinomas, and from 60 to 90% fewer skin tumors known as keratoacanthomas, which are pre-cancerous lesions.

In an earlier study at Rutgers University[2] (Ho et al.: *Prev. Med.* 21:520-525, 1992), EGCG and other catechins extracted from green tea, as well as gallic acid, a substance more abundant in black tea, strongly limited the oxidation of lard. In vitro tests showed that EGCG had an inhibitory effect on lipoxygenase, an enzyme that can transform fat-related substances into peroxides and other oxidative products.

More recent studies have confirmed the antioxidative activity of catechins and theaflavins. In a Japanese study reported in 1994[3] (Shiraki et al.: *Mutation Res.* 323:29-34, 1994), theaflavins sharply curtailed peroxide-induced damage to fatty substances in the membranes of rabbit red blood cells, and limited the damaging effect of hydrogen peroxide on deoxyribonucleic acid (DNA), the molecule that contains the essential genetic information in living cells. In another Japanese study[4] (Yoshino et al.: *Biol. Pharm. Bull.* 17(1):146-149, 1994), theaflavins and a "thearubigin" fraction derived from black tea, as well as EGCG and several other catechins, were more effective than either vitamin C or vitamin E, in limiting the oxidative attack of tertiary butyl hydroperoxide on lipids in rat livers.

Black and green teas also appear to influence the family of liver enzymes called the cytochrome P450 system, which are responsible for metabolism of foreign chemicals. Some of these enzymes act to transform procarcinogens like benzopyrene into active carcinogens that attack and oxidize the DNA of living cells[5] (Sohn, O.S.: *Xenobiotica* 24(2): 119-127, 1994).

Other studies have found that tea can inhibit the formation of nitrosamines, a group of highly active molecules that can be formed within the body and cause mutations by attacking the DNA of living cells. Nitrosamines have been implicated as one factor involved with development of gastric, esophageal, and other cancers. A 1992 clinical study[6] (Stich: *Prev. Med.* 21:377-384, 1992; [7]Yang and Wang: *J. Natl. Cancer Inst.* 85(13):1038-1049, 1993 [p. 1044]) found that catechins and other flavonoids present in green and black tea can inhibit the formation of a nitrosamine called nitrosoproline, in human volunteers. Black, green, and oolong teas have also been found to reduce the numbers of nitrosamine-induced tumors of stomach and intestine in rats[8] (Chen: *Prev. Med.* 21:385-391, 1992 [p.389]).

Although no direct studies of the effects of tea on humans have been done, its association with various disease states has been examined in a number of indirect or *epidemiology* studies. In one such study, conducted in Japan, the frequency of stomach cancer proved to be far lower among persons who drank 10 or more cups of green tea daily than among those who consumed less than this. In a more recent Polish study[9] (Shibata, A.: *International Journal of Cancer* 58: 46-49, 1994), the frequency of pancreatic cancer declined in relation to an increasing lifetime consumption of black tea. And in a Chinese study[10] (Gao, Y.T., et al.: *Journal of the National Cancer Inst.* 86(11):855, 1994), the consumption of green tea appeared to cut the risk of esophageal cancer by as much as 60%, in women who did not smoke or drink alcohol.

TEA AND HEART DISEASE

Human epidemiology and animal studies both suggest that tea drinking may reduce the risk of cardiovascular disease. Most studies have focused on the relationship between tea and the concentrations of blood cholesterol and other fatty substances that are risk factors of heart disease. In a Japanese study, rats were fed a diet that creates high levels of cholesterol in the blood. Dietary supplementation with EGCG[11] (Matsuda, H., et al.: *Journal of Ethnopharmacology* 17: 213-224, 1986), and a green tea flavonoid fraction[12] (Muramatsu, K., et al.: *J. of Nutr. Sci. Vitaminol.* 32: 613-622, 1986) reduced the animals' blood cholesterol as well as the concentrations of low-density lipoprotein (LDL). In a study involving rats that spontaneously exhibit high blood pressure, or hypertension, a diet containing green-tea catechins prevented the blood-pressure increase.

Epidemiology studies corroborate a protective effect of tea against cardiovascular disease. One such

study, conducted from 1986 to 1988 and involving 1,306 Japanese men[13] (Kono, S., et al.: *Preventive Medicine*. 21: 526-531, 1992), found that a greater consumption of green tea was linked to a lower total concentration of cholesterol in the blood. On the other hand, consumption of tea did not appear to reduce the blood concentration of the fatty substances known as triglycerides, which are positively associated with heart disease.

A Dutch study of 805 men aged 65 to 84 years[14] (Hertog et al.: *Lancet* 342:1007-1011, 1993) began in 1985 and was completed in 1990. The investigation focused on the subjects' dietary intake of flavonoids, a group of substances present in tea as well as vegetables, fruits, and wine. The study found that an increasing intake of foods containing flavonoids—61% of which came from black tea, 13% from onions, and 10% from apples—was strongly linked to a lower rate of death from coronary disease.

LOOKING AHEAD

The past quarter century has witnessed a growing recognition that a diet rich in vegetables, fruits, and other edible plants may play an important role in preventing some types of cancer, coronary disease, and other human illnesses. Ongoing scientific research on the role of tea as a part of a healthy diet, like fruits and vegetables, in prevention of chronic diseases is exciting. While knowledge expands about the role of tea and tea flavonoids in prevention of cardiovascular disease, cancer, and other human illnesses, a cup of tea will continue to provide the pleasure and comfort that it has for nearly 5,000 years.

REFERENCES

1. Wang, Z.Y., et al. Inhibitory effects of black tea, green tea, decaffeinated black tea, and decaffeinated green tea on ultraviolet B light-induced skin carcinogenesis in 7, 12-dimethylbenz{a}anthracene-initiated SKH-1 mice. *Cancer Research*, 1994; 54: 3428-3435.
2. Ho, C.T., et al. Antioxidative effect of polyphenol extract prepared from various Chinese teas. *Preventive Medicine*, 1992; 21(4): 520-525.
3. Shiraki, M., et al. Antioxidative and antimutagenic effects of theaflavins from black tea. *Mutation Research*, 1994; 323: 29-34.
4. Yoshino, K., et al. Antioxidative effects of black tea theaflavins and thearubigin on lipid peroxidation of rat liver homogenates induced by *tert*-butyl hydroperoxide. *Biological Pharmacological Bulletin*, 1994; 17(1): 146-149.
5. Sohn, O. S., et al. Effects of green and black tea on hepatic xenobiotic metabolizing systems in the male F344 rat. *Xenobiotica*, 1994; 24(2): 119-127.
6. Stich, H. Teas and tea components as inhibitors of carcinogen formation in model systems and man. *Preventive Medicine*, 1992; 21(3): 377.
7. Yang, C.S., Wang, Z.Y. Tea and cancer. *Journal of the National Cancer Institute*, 1993; 85(13): 1044.
8. Chen, J. The effects of Chinese tea on the occurrence of esophageal tumors induced by N-nitrosomethyl-benzylamine in rats. *Preventive Medicine*, 1992; 21(3): 389.
9. Shibata, A., et al. A prospective study of pancreatic cancer in the elderly. *International Journal of Cancer*, 1994; 58: 46-49.
10. Gao, Y.T., et al. Reduced risk of esophageal cancer associated with green tea consumption. *Journal of the National Cancer Institute*, 1994; 86(11):855.
11. Matsuda, H., et al. Effects of crude drugs on experimental hypercholesterolemia. I. tea and its active principles. *Journal of Ethnopharmacology*, 1986; 17: 213-224.
12. Muramatsu, K., et al. Effects of green tea catechins on plasma cholesterol level in cholesterol-fed rats. *Journal of Nutrition Science Vitaminology*, 1986; 32: 613-622.
13. Kono, S., et al. Green tea consumption and serum lipid profiles: a cross-sectional study in Northern Kyushu, Japan. *Preventive Medicine*, 1992; 21: 526-531.
14. Hertog, Michael G.L., et al. Dietary antioxidant flavonoids and risk of coronary heart disease: The Zutphen Elderly Study. *The Lancet*, 1993; 342: 1007-11.

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Tea Consumption and Cancer Incidence in a Prospective Cohort Study of Postmenopausal Women

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Tea has consistently been shown to inhibit the occurrence of tumors in experimental animals. The evidence for such a beneficial effect in humans, however, is limited. The authors examined the association between non-herbal tea consumption and cancer incidence in a prospective cohort study of 35,369 postmenopausal Iowa women. In this cohort, information on the frequency of tea drinking and other dietary and lifestyle factors was collected by mailed survey in 1986. After 8 years of follow-up, 2,936 incident non-skin cancer cases were ascertained in this cohort through the State Health Registry of Iowa. Proportional hazards regressions were used to derive adjusted relative risks and 95% confidence intervals for the association between tea consumption and cancer incidence. After controlling for confounding factors, the authors found that regular tea consumption was related to a slight, but not statistically significant, reduced incidence of all cancers combined. Inverse associations with increasing frequency of tea drinking were seen for cancers of the digestive tract (p for trend, 0.04) and the urinary tract (p for trend, 0.02). For women who reported drinking ≥2 cups (474 ml) of tea per day, compared with those who never or occasionally drank tea, the relative risk for digestive tract cancers was 0.68 (95% confidence interval (CI) 0.47–0.98) and for urinary tract cancers, 0.40 (95% CI 0.16–0.98). Similar inverse associations were seen for specific digestive and urinary tract cancers, although site-specific analyses were not statistically significant. No appreciable association of tea drinking was found with melanoma, non-Hodgkin's lymphoma, or cancers of the pancreas, lung, breast, uterine corpus, or ovary. This study suggests that tea, one of the most popular beverages consumed worldwide, may protect against some cancers in postmenopausal women. *Am J Epidemiol* 1996;144:175–82.

bladder neoplasms; colonic neoplasms; diet; health promotion; neoplasms; prospective studies; tea

The potential tumor-inhibitory effect of tea has been demonstrated in numerous in vivo and in vitro studies (1, 2). Administration of tea infusion or tea polyphenols to experimental animals has been shown to reduce the occurrence of tumors, including those of the digestive tract, and to regress the growth of experimental tumors (2). Epidemiologic studies on the relation between tea consumption and cancer risk have been inconsistent (1–8). Some early studies reported an elevated risk of esophageal cancer associated with consumption of high-temperature tea (1, 2). This association, however, may be caused by thermal injury of hot tea on the esophageal mucosa. Both positive and inverse associations with tea have also been reported for other cancers, including those of the oropharynx, stomach, pancreas, colon, rectum, breast, kidney, and bladder (1–8). Such inconsistent associations may be explained partially by differences in tea-drinking habits and the types of tea consumed in various study populations or by failure to control for potential confounding factors.

Interest in the potential cancer-inhibiting effect of tea has been heightened recently by a large case-control study in China, in which a dose-response relation between green tea consumption and reduced risk of esophageal cancer was observed in both men and women (3). Most tea consumed in Western societies, however, is black tea; while it is made from the same plant, many of its constituents differ from those in green tea (1, 2). In addition, most previous epidemiologic investigations on tea and cancer were case-control studies (1–7); they were unable to assess the effect of tea on total cancer incidence. In this report, we examined the relation of tea consumption with the incidence of major cancers in the Iowa Women's Health Study, a prospective cohort study of postmenopausal women.

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Abbreviation: CI, confidence interval.

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MATERIALS AND METHODS

Details on the methods used in this cohort study have been published elsewhere (9, 10). Briefly, in January 1986, a questionnaire was sent to 98,826 randomly selected women aged 55–69 years whose names appeared on the 1985 Iowa State driver's license list. A total of 41,837 women (42.3 percent) completed and returned the questionnaire and were followed for cancer incidence and total mortality. With the exception of lung cancer, the incidence rates of other major cancers among cohort members were similar to the rates observed among women who did not respond to the 1986 baseline mailed questionnaire (11).

The vital status for cohort members was determined through computer linkage of participant identifiers with Iowa death certificates and the National Death Index and through mailed follow-up questionnaires in 1987, 1989, and 1992. Information on cancer diagnosis was ascertained through the State Health Registry of Iowa, part of the National Cancer Institute's Surveillance, Epidemiology, and End Results (SEER) program. Based on our follow-up surveys, it is estimated that the outmigration rate among cohort members is less than 1 percent annually. Through December 31, 1993, after 8 years of follow-up, 3,567 women had developed at least one new primary cancer.

The self-administered questionnaire used in the 1986 baseline survey included information on demographic characteristics, menstrual and reproductive history, weight, personal habits, usual diet, medication use, and medical conditions. A paper tape measure and written instructions were enclosed for having a friend measure the circumferences of the waist and hip. The waist/hip circumference ratio was calculated (10). Diet was assessed using a semiquantitative food frequency questionnaire almost identical to that used in the 1984 Nurses' Health Study (9). Usual intakes of 127 food items were ascertained. Tea consumption was assessed in the food questionnaire by asking study participants to record their usual consumption level of non-herbal tea in one of the following nine categories: never or less than once per month, 1–3 cups per month, 1 cup per week, 2–4 cups per week, 5–6 cups per week, 1 cup per day, 2–3 cups per day, 4–5 cups per day, and ≥6 cups per day (1 cup = 237 ml). These categories were then combined into four consumption levels for data analysis (never or monthly (0–3 cups per month), weekly (1–6 cups per week), 1 cup per day, and 2 or more cups per day) to ensure stable estimates of cancer risk in relation to tea consumption. Those who reported never or occasionally (monthly) drinking tea were combined into one category, since very low intake of tea (≤3 cups per month) is unlikely to confer any beneficial effect (2).

For the present analyses, we excluded women who reported a history of cancer other than skin cancer at baseline (n = 3,896), those who had missing information on tea consumption level (n = 2,264), and those who were not postmenopausal (n = 569). A total of 261 women met two or more of the exclusion criteria. After the above exclusions, a total of 35,369 cohort members remained for analysis, including 2,936 women who were diagnosed with cancers during the 8-year follow-up period. Additional exclusions were made for analysis of breast cancer (excluding 1,884 women with prior mastectomy), ovarian cancer (8,064 women with prior bilateral oophorectomy), and cancers of the cervix and uterine corpus (14,350 women with prior hysterectomy).

Relative risks were used to measure the strength of associations between tea consumption and cancer incidence. Proportional hazards regression (Cox model) was used to control for potential confounders and to derive adjusted relative risks and 95 percent confidence intervals (12). Person-years were accumulated up to the date of cancer diagnosis for cancer cases and to the date of loss to follow-up, death, or December 31, 1993, for noncases. All relative risks were adjusted for the following common risk or protective factors shared by most major cancers: age, education, smoking status, pack-years of cigarette smoking, physical activity, all fruit and vegetable intake, waist/hip circumference ratio, and family history of cancer among female relatives (9, 13–18). Relative risks were also adjusted for total energy intake to evaluate the potential independent association of tea consumption and to reduce potential variation in tea intake that may arise from over- or underreporting of the overall food consumption level (19). Additional adjustment for alcohol consumption did not alter the results appreciably. With the exception of age and pack-years of smoking, the strata for adjusting variables included in multiple Cox regression models are specified in table 1. Age and pack-years of smoking were adjusted as continuous variables, given their linear relations with the risks of most major cancers. Additional adjustments were made in the analyses of pancreatic cancer (prior history of diabetes), lymphoma and kidney cancers (prior history of blood transfusion), and cancers of the breast, ovary, and uterine corpus (age at menarche, age at menopause, and age at first pregnancy). These additional adjusting variables were found to be risk factors for the corresponding cancer sites in this study population (13, 16, 17). A trend test for a dose-response relation across levels of tea consumption was performed by treating an ordinal-score variable (i.e., 1, 2,

TABLE 1. Relation of tea consumption with selected demographic and risk factors among postmenopausal women, the Iowa Women's Health Study, 1985–1993

	No. of cohort members*	Tea consumption frequency (%)*			
		Never/ monthly	Weekly	Daily	
				1 cup†	≥2 cups
Cohort members	35,369	58.3	24.3	8.7	8.6
Baseline age (years)					
55–59	12,648	59.4	23.5	8.2	8.9
60–64	12,530	58.9	24.2	8.5	8.5
65–69	9,977	56.1	26.0	9.6	8.3
Education					
<High school	6,593	60.8	22.1	8.6	8.5
High school	14,789	59.6	23.8	8.3	8.3
>High school	13,907	55.6	26.2	9.3	9.0
Cigarette smoking					
Never smoked	22,932	56.1	25.9	9.6	8.4
Exsmoker	6,750	59.6	23.4	8.0	9.0
Current smoker					
1–19 pack-years	958	66.2	20.7	5.7	7.4
20–39 pack-years	2,123	65.0	19.6	6.7	8.8
≥40 pack-years	1,992	67.5	18.4	5.1	9.0
Physical activity					
Low	16,480	60.1	22.5	8.7	8.7
Moderate	9,578	56.1	26.4	9.2	8.4
Vigorous	8,706	57.5	25.7	8.3	8.5
Fruit/vegetable intake (servings/month)					
Quartile 1 (<103.4)	8,842	66.0	20.0	7.0	7.4
Quartile 2 (103.4–146.1)	8,842	60.0	23.4	8.4	8.3
Quartile 3 (146.2–198.9)	8,843	55.3	24.8	9.3	8.6
Quartile 4 (>198.9)	8,841	51.8	26.0	10.2	10.1
Waist/hip ratio					
Quartile 1 (<0.78)	8,814	58.4	24.3	8.9	8.3
Quartile 2 (0.78–0.83)	8,507	58.3	25.1	8.1	8.5
Quartile 3 (0.84–0.89)	8,769	57.9	24.2	9.1	8.8
Quartile 4 (>0.89)	8,824	58.4	24.1	8.8	8.8
Family history of cancer in female relatives					
No	22,030	58.2	24.7	8.8	8.5
Yes	13,340	58.7	24.0	8.6	8.7

* Percentages were calculated after excluding women with missing data and may not add up to 100% in each row because of rounding.
† Metric equivalent: 1 cup = 237 ml.

3, and 4) as a continuous variable in proportional hazards regression after adjustment for potential confounders.

RESULTS

The distribution of tea consumption level by common risk factors for major cancers is presented in table 1. Overall, about 41.7 percent of women reported drinking non-herbal tea at least once a week, and 17.3 percent drank tea daily. Only 8.6 percent of women reported drinking 2 or more cups of tea a day. These frequencies did not vary appreciably with age, physical activity level, body fat distribution (measured by waist/hip circumference ratio), or a family history of cancer in female relatives. Current smokers tended to drink tea less frequently than nonsmokers or exsmokers, but further reduction in consumption frequency was not evident with increasing pack-years of smoking. There were clear positive correlations of tea drinking with education level and total fruit and vegetable intake. Coffee consumption was found not to be

correlated with tea drinking; the percentages of daily tea drinkers were 18.5 percent, 14.2 percent, and 16.8 percent from the lowest to the highest coffee consumption groups (data not shown in table).

Table 2 presents adjusted relative risks for major cancers in relation to the tea consumption level at baseline. There was a statistically significant inverse association between tea consumption and cancers of the digestive tract, including cancers of the upper digestive tract, colon, and rectum ($p = 0.04$). Women who reported drinking 2 or more cups of tea per day had an incidence rate of these cancers 68 percent of that in those who never or occasionally drank tea. The risk was further reduced among women who drank 4 or more cups of tea per day (relative risk = 0.37, 95 percent confidence interval (CI) 0.14–0.98). This decreased risk among frequent tea drinkers was seen for all specific cancer sites of the digestive tract, with a more pronounced effect for cancers of the upper digestive tract (month, pharynx, esophagus, and stomach). None of the statistical tests for a linear trend in these site-specific analyses, however, was significant. Another notable inverse association with tea consumption was seen for urinary tract cancer, with the incidence 40 percent as high in women who drank 2 or more cups of tea per day. Again, the inverse association was seen for each specific cancer site, although the trend tests for site-specific analyses were not statistically significant. An apparent inverse association was also observed for leukemia with tea intake, but the dose-response relation was not statistically significant, perhaps because [illegible] preciable association of tea drinking was found for other major cancers, including melanoma, non-Hodgkin's lymphoma, and cancers of the lung, breast, uterine corpus, and ovary. Overall, drinking 2 or more cups of tea a day was related to a 10 percent reduction in incidence of total cancer, but neither the point estimate of the relative risk nor the trend test for linear association was statistically significant.

TABLE 2. Relative risks (RRs)* and 95% confidence intervals (CIs) for the associations of tea with major concerns among postmenopausal women, the Iowa Women's Health Study, 1986–1993

Cancer sites	Tea consumption frequency at baseline											p value for trend test
	Never/monthly		Weekly			1 cup/day†			≥2 cups/day			
	No. of cases	RR	No. of cases	RR	95% CI	No. of cases	RR	95% CI	No. of cases	RR	95% CI	
Digestive tract	327	1.00	135	1.01	0.83–1.24	46	0.96	0.71–1.31	32	0.68	0.47–0.98	0.04
Upper digestive organs	47	1.00	16	0.87	0.49–1.54	5	0.75	0.30–1.90	3	0.44	0.14–1.40	0.16
Colon	206	1.00	90	1.07	0.83–1.37	33	1.09	0.75–1.58	21	0.71	0.45–1.11	0.16
Rectum and anus	78	1.00	29	0.89	0.58–1.36	9	0.77	0.39–1.55	8	0.70	0.34–1.46	0.31
Pancreas‡	34	1.00	17	1.23	0.68–2.21	8	1.62	0.74–3.51	4	0.80	0.28–2.24	0.60
Lung, trachea, and bronchus	200	1.00	58	0.91	0.68–1.23	25	1.19	0.78–1.81	29	1.05	0.71–1.55	0.54
Melanoma	41	1.00	22	1.20	0.71–2.01	6	0.90	0.38–2.13	7	1.11	0.50–2.47	0.99
Breast §,‖	567	1.00	259	1.06	0.92–1.23	92	1.06	0.85–1.32	97	1.14	0.92–1.41	0.28
Corpus uteri §,‖	141	1.00	63	1.02	0.75–1.37	29	1.29	0.87–1.94	16	0.76	0.45–1.27	0.47
Ovary §,‖	71	1.00	16	0.53	0.31–0.91	10	0.95	0.49–1.64	10	0.98	0.50–1.90	0.64
Cervix ‖	32	1.00	11	0.87	0.44–1.74	7	1.60	0.70–3.66	2	0.44	0.10–1.82	0.40
Urinary tract¶	85	1.00	36	1.06	0.72–1.57	8	0.65	0.31–1.35	5	0.40	0.16–0.98	0.02
Renal cell¶	42	1.00	13	0.73	0.39–1.37	3	0.45	0.14–1.47	3	0.47	0.15–1.53	0.15
Bladder and other urinary organs	43	1.00	23	1.42	0.85–2.38	5	0.87	0.34–2.22	2	0.33	0.08–1.35	0.08
Non-Hodgkin's lymphoma	71	1.00	29	0.95	0.61–1.46	14	1.23	0.69–2.18	10	0.92	0.47–1.79	0.99
Leukemia	41	1.00	17	1.00	0.57–1.77	5	0.80	0.32–2.04	2	0.33	0.08–1.35	0.11
Any cancers combined ‖	1,132	1.00	449	0.97	0.87–1.09	160	0.98	0.83–1.16	148	0.90	0.76–1.07	0.27

* Basic multiple regression model in which all relative risks were adjusted for age, education, smoking status, pack-years of smoking, physical activity, all fruit and vegetable intake, waist/hip ratio, and family history of cancer.

† Metric equivalent: 1 cup = 237 ml.

‡ Adjusted for diabetes and other confounding variables listed in the basic model.

§ Adjusted for age at menarche, age at menopause, age at first pregnancy, and other confounding variables listed in the basic model.

‖ Excluding women with baseline mastectomy for breast cancer analyses, hysterectomy for endometrial and cervical cancer analyses, oophorectomy for ovarian cancer analyses, or any of the above conditions for analyses of any cancers combined.

¶ Adjusted for a history of blood transfusion and other confounding variables listed in the basic model.

Additional analyses were performed by the year of cancer diagnosis following the baseline survey (0–4 years vs. 5–8 years) to examine the consistency of the observed associations between tea drinking and digestive and urinary tract cancers over the 8-year follow-up period. Inverse associations with tea drinking were seen for both cancer sites in both the earlier and the later years of follow-up, although the trend tests were no longer statistically significant because of a smaller number of cases (data not shown in table). For example, compared with never or occasionally drinking tea, drinking 2 or more cups of tea a day was associated with relative risks of 0.75 (95 percent CI 0.48–1.20) for digestive tract cancers and 0.31 (95 percent CI 0.08–1.29) for urinary tract cancer in years 0–4 of follow-up and with relative risks of 0.71 (95 percent CI 0.39–1.29) for digestive tract cancer and 0.57 (95 percent CI 0.17–1.84) for urinary tract cancer in years 5–8 of follow-up.

Table 3 presents the relation of intake levels of regular coffee with cancers of the digestive and urinary tracts and with all sites combined. No apparent association was observed for coffee drinking with these cancer groups, suggesting that our observed tea-cancer associations may be due to compounds other than caffeine in tea.

DISCUSSION

Although tea has been shown to inhibit cancer occurrence in most animal experiments, the association of tea with human cancers has been less consistent. Previous epidemiologic studies reported negative, null, or even positive associations of tea consumption with a variety of cancers, including cancers of the oropharynx, esophagus, stomach, colorectum, pancreas, breast, bladder, and kidney (1–8). Our prospective cohort study among postmenopausal women found that regular tea drinking may protect against some, but not all, cancers. Women who reported drinking 2 or more cups of tea per day had 40–70 percent of the incidence rates of cancers of the digestive and urinary tracts compared with women who never or infrequently drank tea. In contrast, coffee drinking was not found to be related to the risk of these cancers.

Our finding of an inverse association of tea consumption with digestive tract cancers, particularly those of the upper digestive tract, is consistent with previous epidemiologic studies (1–4, 20–27), including a recently published large case-control study of esophageal cancer in China (3). In that study, a 50 percent reduction in the risk of esophageal cancer among women who drank green tea regularly was reported. Stomach cancer is the cancer site most consistently related to tea intake in epidemiologic studies (1, 2, 4, 20–23). The inverse association of tea consumption with this malignancy has been demonstrated in studies conducted in China (20), Japan (21, 22), Sweden (4), and Turkey (23). Studies on the associations of tea with cancers of the oropharynx and colorectum have been less consistent (1, 2, 24–27). An early cohort study in the United Kingdom reported reduced risks of both colon and rectal cancers with heavy tea consumption, but a dose-response relation was not evident (24). An inverse association was also suggested for adenomatous polyps of the sigmoid colon in a Japanese case-control study (25). In contrast, a cohort study among Japanese men in Hawaii found that the daily intake of black tea was associated with a statistically significant increased risk of rectal cancer. The relative risks in that study, however, were adjusted only for age and alcohol intake (28). A substantially reduced risk of cancer of the nasopharynx but not other pharyngeal cancers was linked to tea drinking in an early case-control study (27). Recently, Franceschi et al. (26) in an Italian study found that tea

TABLE 3. Relative risks (RRs)* and 95% confidence intervals (CIs) for the associations of caffeinated coffee drinking with cancers of the digestive tract, urinary tract, and all sites combined, the Iowa Women's Health Study, 1986–1993

Coffee consumption level	Cancer sites								
	Digestive tract			Urinary tract			All sites combined		
	No.	RR*	95% CI	No.	RR†	95% CI	No.‡	RR*	95% CI
Never/monthly	186	1.00		52	1.00		629	1.00	
Weekly	66	0.95	0.72–1.23	13	0.69	0.38–1.27	225	0.92	0.80–1.08
1 cup/day§	54	1.01	0.74–1.37	9	0.60	0.30–1.22	195	1.04	0.89–1.22
2–3 cups/day	130	0.97	0.78–1.22	38	1.03	0.67–1.56	453	0.93	0.82–1.05
≥4 cups/day	110	1.03	0.81–1.32	36	0.79	0.48–1.29	397	0.97	0.85–1.11

* Basic multiple regression model in which all relative risks were adjusted for age, education, smoking status, pack-years of smoking, physical activity, all fruit and vegetable intake, total energy intake, waist/hip ratio, and family history of cancer.

† Additionally adjusted for prior history of blood transfusion.

‡ Excluding women with baseline mastectomy, hysterectomy, and oophorectomy.

§ Metric equivalent: 1 cup = 237 ml.

drinking may protect against oral cancers. In another Italian case-control study, tea was found to be inversely related to the risk of oral and pharyngeal cancer but not to cancers of the other digestive tract organs (29). No inverse association of tea was found for digestive tract cancers in several previous investigations (1, 2), including a few recently completed studies (5, 6, 30). Some early studies even reported an increased risk of digestive tract cancers (particularly esophageal cancer) associated with tea consumption (1, 2). The positive association with esophageal cancer, however, may be largely attributed to thermal injury of hot tea to the mucosa of the upper digestive tract rather than the chemicals in tea per se, since only hot tea, not normal-temperature tea, has been found to be associated with an elevated risk of esophageal cancer (1–3).

There have been many studies that investigated the relation between tea consumption and urinary tract cancer over the past two decades (1, 2). With a few exceptions, most of these studies reported no association between tea drinking and cancer risk. In a cohort study among British men, Kinlen et al. (24) found a statistically significant positive association of tea with kidney cancer but not with cancer of the bladder. Positive associations for bladder cancer (31) and renal cell carcinoma (32) were also reported in two early case-control studies, but trends in risk were inconsistent. In contrast, Yu et al. (33) reported a significantly reduced risk of renal cell carcinoma among women who drank tea daily. Inverse associations with bladder cancer were also reported from several recent studies, although none of the dose-response relations was statistically significant (34–37). In agreement with these more recent studies, our results showed that regular tea consumption was related to a significantly reduced risk of urinary tract cancers in a dose-response manner.

Tea intake has also been inversely linked with some other cancers. Of note is cancer of the pancreas, for which both positive and negative associations were reported in some earlier studies (1, 2). Two recent investigations, however, found that tea drinking may protect against this fatal malignancy (7, 8). In addition, inverse associations of tea drinking with cancers of the breast and ovary have also been reported (1, 2, 38), but results from previous studies have been very inconsistent. We did not find appreciable associations of tea with these common cancers among postmenopausal women in this cohort study.

The inverse association of tea drinking and digestive tract cancers in the present study may be explained in part by an anticancer effect on the digestive tract via direct contact. This association is also supported by the inhibitory effects of tea on the endogenous formation of *N*-nitroso compounds (2), potential carcinogens particularly for upper digestive tract cancers, and the formation of heterocyclic aromatic amines (39), potential carcinogens for cancer of the large bowel. Regarding urinary tract cancers, some anticancer tea constituents may exist in urine and act directly on the renal and transitional cells during the secretion and storage process of urine. It has been shown recently that (−)-epigallocatechin and (−)-epicatechin, two major tea polyphenols, can be detected in urine among volunteers who ingest tea preparations (40).

Differences in the types of tea consumed and tea drinking habits in various study populations are likely to have contributed to inconsistencies in the relation between tea and cancer across previous studies. In some populations, tea drinking is positively correlated with cigarette smoking and alcohol drinking (3, 21, 22). Residual confounding effects of these factors could favor a positive association between tea and cancer. In addition, it has been shown from experimental studies that tea may counteract the adverse effect of some, but not all, carcinogens and that the tumor-inhibitory effect of tea may depend on its intake level (1, 2). For example, at high concentrations, tea can effectively block endogenous formation of *N*-nitroso compounds, while at low levels it may facilitate nitrosation reactions (2). Therefore, differences in exposure levels of both tea and various cancer etiologic factors in various study populations may result in apparently contradictory findings in epidemiologic studies.

Some limitations of this study need to be considered when interpreting the study results. Although tea drinking, like many other personal habits, is likely to be recalled with reasonable accuracy, misclassification of exposure to tea may still exist. The impact of misclassification errors is likely to be compounded with longer follow-up, since no additional assessment of tea consumption was made during the follow-up period, and some cohort members may have changed their tea-drinking habits during this time period. These random errors likely attenuate the true association between tea consumption and cancer incidence. On the other hand, some statistically significant findings could be due to chance alone, given the multiple comparisons made in this paper. Although there are some rationales for analyzing the relations of tea consumption with the combined cancer groups of all digestive tract organs or all urinary tract organs, the etiologic factors for some specific cancer sites in each cancer group are somewhat different. These two cancer groups were formed, however, on the basis of not only the consistency of the inverse association for each

cancer site but also the possible anticancer effect of tea via direct contact. Another limitation of this study is the small number of women (only 8.6 percent of the total cohort) who reported drinking tea regularly (2 or more cups per day); this not only resulted in an unstable estimate of the relative risks of cancer for this heavy tea-drinking group but also precluded detailed analyses of the tea-cancer associations for certain cancer sites. In addition, regular tea drinkers may be at a low risk of developing cancer because of reasons other than tea drinking. The inverse tea-cancer association, however, persisted after careful adjustment for all measured confounders. Furthermore, the specific inverse associations with digestive and urinary tract cancers, the biologic plausibility of these associations, and the observed dose-response relations for urinary tract cancers also indicate that the observed associations are unlikely due to chance alone.

In summary, this prospective cohort study among postmenopausal women suggests that regular tea consumption may protect against cancers of digestive and urinary tract organs. These associations are supported by the potential tumor-inhibitory effects of tea and tea polyphenols seen in most animal studies and by similar findings in some previous epidemiologic studies. Further studies are warranted to assess whether our observed associations are etiologically meaningful.

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REFERENCES

1. Coffee, tea, mate, methylxanthines and methylglyoxal. IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. Lyon, 27 February to 6 March 1990. IARC Monogr Eval Carcinog Risks Hum 1991;51:1–513.
2. Yang CS, Wang ZY. Tea and cancer. J Natl Cancer Inst 1993;85:1038–49.
3. Gao YT, McLaughlin JK, Blot WJ, et al. Reduced risk of esophageal cancer associated with green tea consumption. J Natl Cancer Inst 1994;86:855–8.
4. Hansson LE, Nyren O, Bergstrom R, et al. Diet and risk of gastric cancer. A population-based case-control study in Sweden. Int J Cancer 1993;55:181–9.
5. Hu J, Nyren O, Wolk A, et al. Risk factors for oesophageal cancer in northeast China. Int J Cancer 1994;57:38–46.
6. Olsen J, Kronborg O. Coffee, tobacco and alcohol as risk factors for cancer and adenoma of the large intestine. Int J Epidemiol 1993;22:398–402.
7. Zatonski WA, Boyle P, Przewozniak K, et al. Cigarette smoking, alcohol, tea and coffee consumption and pancreas cancer risk: a case-control study from Opole, Poland. Int J Cancer 1993;53:601–7.
8. Shibata A, Mack TM, Paganini-Hill A, et al. A prospective study of pancreatic cancer in the elderly. Int J Cancer 1994; 58:46–9.
9. Kushi LH, Sellers TA, Potter JD, et al. Dietary fat and postmenopausal breast cancer. J Natl Cancer Inst 1992;84:1092–9.
10. Folsom AR, Kaye SA, Sellers TA, et al. Body fat distribution and 5-year risk of death in older women. JAMA 1993;269: 483–7.
11. Bisgard KM, Folsom AR, Hong C-P, et al. Mortality and cancer rates in nonrespondents to a prospective study of older women: 5-year follow-up. Am J Epidemiol 1994;139: 990–1000.
12. Cox DR. Regression models and life tables (with discussion). J R Stat Soc (B) 1972;34:187–220.
13. Sellers TA, Kushi LH, Potter JD, et al. Effect of family history, body fat distribution, and reproductive factors on risk of postmenopausal breast cancer. N Engl J Med 1992;326: 1323–9.
14. Steinmetz KA, Potter JD, Folsom AR. Vegetables, fruit, and lung cancer in the Iowa Women's Health Study. Cancer Res 1993;53:536–43.
15. Steinmetz KA, Kushi LH, Bostick RM, et al. Vegetables, fruit, and colon cancer in the Iowa Women's Health Study. Am J Epidemiol 1994;139:1–15.
16. Cerhan JR, Wallace RB, Folsom AR, et al. Transfusion history and cancer risk in older women. Ann Intern Med 1993;119: 8–15.
17. Zheng W, Kushi LH, Potter JD, et al. Dietary intakes of energy and animal foods and endometrial cancer incidence. The Iowa Women's Health Study. Am J Epidemiol 1995;142: 388–94.
18. Zheng W, Sellers TA, Doyle TJ, et al. Retinol, antioxidant vitamins, and cancers of the upper digestive tract in a prospective cohort study of postmenopausal women. Am J Epidemiol 1995;142:955–60.
19. Willett WC, Stampfer MJ. Total energy intake: implications for epidemiologic analyses. Am J Epidemiol 1986;124:17–27.
20. Yu G-P, Hsieh C-C. Risk factors for stomach cancer: a population-based case-control study in Shanghai. Cancer Causes Control 1991;2:169–74.
21. Tajima K, Tominaga S. Dietary habits and gastro-intestinal cancers: a comparative case-control study of stomach and large intestinal cancers in Nagoya, Japan. Jpn J Cancer Res 1985;16:705–16.
22. Kono S, Ikeda M, Tokudome S, et al. A case-control study of gastric cancer and diet in northern Kyushu, Japan. Jpn J Cancer Res 1988;79:1067–74.
23. Memik F, Nak SG, Gulten M, et al. Gastric carcinoma in northwestern Turkey: epidemiologic characteristics. J Environ Pathol Toxicol Oncol 1992;11:335–8.
24. Kinlen LJ, Willows AN, Goldblatt P, et al. Tea consumption and cancer. Br J Cancer 1988;58:397–401.
25. Kono S, Shinchi K, Ikeda N, et al. Physical activity, dietary habits and adenomatous polyps of the sigmoid colon: a study of self-defense officials in Japan. J Clin Epidemiol 1991;44: 1255–61.
26. Franceschi S, Barra S, LaVecchia C, et al. Risk factors for cancer of the tongue and the mouth. A case-control study from northern Italy. Cancer 1992;70:2227–33.
27. Henderson BE, Louie E, Jing JS, et al. Risk factors associated with nasopharyngeal carcinoma. N Engl J Med 1976;295: 1101–6.
28. Heilburn LK, Nomura A, Stemmermann GN. Black tea consumption and cancer risk: a prospective study. Br J Cancer 1986;54:677–83.
29. La Vecchia C, Negri E, Franceschi S, et al. Tea consumption and cancer risk. Nutr Cancer 1992;17:27–31.
30. Agudo A, González CA, Marcos G, et al. Consumption of

alcohol, coffee, and tobacco, and gastric cancer in Spain. Cancer Causes Control 1992;3:137–43.
31. Jensen OM, Wahrendorf J, Knudsen JB, et al. The Copenhagen case-control study of bladder cancer. II. Effect of coffee and other beverages. Int J Cancer 1986;37:651–7.
32. McLaughlin JK, Mandel JS, Blot WJ, et al. A population-based case-control study of renal cell carcinoma. J Natl Cancer Inst 1984;72:275–84.
33. Yu MC, Mack TM, Hanisch R, et al. Cigarette smoking, obesity, diuretic use, and coffee consumption as risk factors for renal cell carcinoma. J Natl Cancer Inst 1986;77:351–6.
34. La Vecchia C, Negri E, Decarli A, et al. Dietary factors in the risk of bladder cancer. Nutr Cancer 1989;12:93–101.
35. Slattery ML, West DW, Robison LM. Fluid intake and bladder cancer in Utah. Int J Cancer 1988;42:17–22.
36. Kunze E, Chang-Claude J, Frentzel-Beyme R. Life style and occupational risk factors for bladder cancer in Germany. Cancer 1992;69:1776–90.
37. Nomura AMY, Kolonel LN, Hankin JH, et al. Dietary factors in cancer of the lower urinary tract. Int J Cancer 1991;48: 199–205.
38. Hunter DJ, Manson JE, Stampfer MJ, et al. A prospective study of caffeine, coffee, tea, and breast cancer. (Abstract). Am J Epidemiol 1992;136:1000–1.
39. Weisburger JH, Nagao M, Wakabayashi K, et al. Prevention of heterocyclic amine formation by tea and tea polyphenols. Cancer Lett 1994;83:143–7.
40. Yang CS, Lee MJ, Chen LS, et al. Analysis of plasma and urinary tea polyphenols in animals and humans. Cancer Epidemiol Biomarkers Prev (in press).

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Prospective Study of Beverage Use and the Risk of Kidney Stones

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Patients with kidney stones are routinely advised to increase their fluid intake to decrease the risk of stone recurrence. However, there has been no detailed examination to determine whether the effect on recurrence varies by the type of beverage consumed. The authors conducted a prospective study of the relation between the intake of 21 different beverages and the risk of symptomatic kidney stones in a cohort of 45,289 men, 40–75 years of age, who had no history of kidney stones. Beverage use and other dietary information was measured by means of a semiquantitative food frequency questionnaire in 1986. During 6 years of follow-up (242,100 person-years), 753 incident cases of kidney stones were documented. After adjusting simultaneously for age, dietary intake of calcium, animal protein and potassium, thiazide use, geographic region, profession, and total fluid intake, consumption of specific beverages significantly added to the prediction of kidney stone risk ($p < 0.001$). After mutually adjusting for the intake of other beverages, the risk of stone formation decreased by the following amount for each 240-ml (8-oz) serving consumed daily: caffeinated coffee, 10% (95% confidence interval 4–15%); decaffeinated coffee, 10% (3–16%); tea, 14% (5–22%); beer, 21% (12–30%); and wine, 39% (10–58%). For each 240-ml serving consumed daily, the risk of stone formation increased by 35% (4–75%) for apple juice and 37% (1–85%) for grapefruit juice. The authors conclude that beverage type may have an effect on stone formation that involves more than additional fluid intake alone. *Am J Epidemiol* 1996;143:240–7.

beverages; epidemiologic factors; kidney calculi; prospective studies

Kidney stones are a common, painful, and costly medical condition. Approximately 12 percent of the US population will form a stone at some time (1, 2). Recurrences are common with 30–50 percent of men forming another stone within 5 years of the incident stone (2–4). To decrease the likelihood of stone recurrence, patients are routinely advised to increase their urine volume by increasing their fluid intake. Increasing fluid intake is not a proven remedy; however, most authors (5–8) support this recommendation, even though some (9) do not.

Although the effects of particular beverages on changes in urine composition have been studied, little information is available on changes in stone formation rates. Using a retrospective case-control design to examine the relation between the intake of six beverages and a history of kidney stones, Shuster et al. (10) observed an inverse association for beer and coffee consumption and a direct association for carbonated beverage (soda) consumption. No associations were found for milk, tea, or water. Other risk factors for kidney stones, such as other dietary variables (11), were not controlled for in the analysis. This study was followed by a randomized trial of decreasing soft drink use on the risk of stone recurrence (12). A significant decrease of 6.4 percent in the stone recurrence rate was observed in the group advised to avoid soda consumption.

We have previously shown an inverse association between total fluid intake and the risk of stone formation in a prospective cohort study of 45,619 US men (11). To investigate whether the type of fluid ingested is important, we examined the relation between the use of 21 specific beverages and the risk of symptomatic kidney stones.

METHODS

Population

The Health Professionals Follow-up Study is a longitudinal study of cancer and cardiovascular and other

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diseases among 51,529 male dentists, optometrists, osteopaths, pharmacists, podiatrists, and veterinarians who were 40–75 years old in 1986. The participants returned a mailed questionnaire in 1986 concerning diet, medical history, and medications. Of the 49,999 participants who provided complete information on diet, we excluded 3,965 men (7.9 percent) who reported a history of kidney stones prior to 1986 because they might have changed their fluid intake as a consequence of the kidney stone. The prevalence at baseline ranged from 5.3 percent in the men younger than 45 years to 10.0 percent in those 70 years and older.

Assessment of diet

We assessed the men's diets using a semiquantitative food frequency questionnaire that contained inquiries about the average use of 131 food items, including 21 beverages, during the previous year. We computed nutrient intakes from the reported frequency of consumption of each food or beverage using published data on the nutrient content of the specified portions (13). We also collected information on the amount of supplemental calcium (e.g., calcium carbonate) intake, either alone or in multivitamin preparations. We previously reported on the reproducibility and validity of this dietary questionnaire in this cohort (14).

Study participants reported specific beverage use as the number of times a standard serving size was consumed, with nine frequency categories ranging from less than once per month to six or more times per day. The beverages included in the questionnaire are listed in table 1. The average daily intake of each beverage was calculated by multiplying the reported frequency of use by the serving size for the beverage. Red and white wine were combined into a single variable (wine) because there was no a priori hypothesis that the individual types of wine would have a differential effect on the risk of stone formation. Reasonable levels of reproducibility and validity were observed for

TABLE 1. Distribution (%) of beverage use among 45,289 men with no history of kidney stones at baseline in 1986,* Health Professionals Follow-up Study

Beverage (serving size†)	Frequency of use (%)									
	<1/ month	1–3/ months	1/ week	2–4/ weeks	5–6/ weeks	1/day	2–3/ days	4–5/ days	≥6/ days	Missing
Water (1 glass)	2.7	2.3	2.5	5.7	4.8	17.7	33.7	19.9	10.3	0.5
Milk (8-oz glass)										
Skim/low fat	24.3	7.9	5.3	17.3	9.1	17.5	13.2	1.3	0.5	3.5
Whole	65.5	8.7	3.4	5.5	1.8	3.3	2.0	0.3	0.1	9.4
Juices (small glass)										
Apple	47.2	24.7	10.8	8.0	1.7	1.7	0.5	0.1	0.03	5.3
Orange	16.1	17.1	11.7	18.6	8.8	21.1	2.7	0.3	0.3	3.3
Grapefruit	64.0	15.8	5.6	4.6	1.1	1.5	0.4	0.1	0.02	7.1
Other fruit	49.2	22.6	9.4	7.5	1.7	2.4	0.4	0.04	0.04	6.6
Tomato	52.9	26.9	10.0	4.7	1.0	1.1	0.1	0.01	0.01	3.3
Punch/lemonade (1 glass)	64.6	16.8	7.4	4.4	1.2	1.2	0.4	0.1	0.01	3.9
Sodas (1 can)										
Cola, with caffeine	54.5	16.6	9.9	9.6	2.7	2.7	1.2	0.1	0.04	2.6
Cola, without caffeine	76.7	9.8	4.8	2.6	0.5	0.5	0.2	0.01	0.004	4.9
Cola, diet, with caffeine	61.4	10.8	6.4	8.3	2.9	3.9	2.6	0.4	0.1	3.2
Cola, diet, without caffeine	60.1	12.1	7.6	8.6	2.5	3.5	1.8	0.2	0.1	3.4
Noncola	62.1	18.6	9.3	5.2	0.8	0.8	0.3	0.01	0.002	2.9
Noncola, diet	58.9	15.0	8.8	7.6	1.9	2.1	0.8	0.1	0.04	4.7
Coffee/tea (1 cup)										
Coffee, caffeinated	28.9	5.7	4.3	7.2	4.7	13.1	22.9	8.1	2.8	2.4
Coffee, decaffeinated	44.9	9.3	5.5	8.5	4.7	9.3	11.1	2.8	0.8	3.1
Tea	40.7	15.2	9.1	10.5	4.6	9.0	6.3	1.1	0.3	3.2
Alcoholic beverages										
Beer (1 can)	42.4	17.9	11.4	14.1	4.1	4.3	3.2	0.6	0.2	1.8
Wine (4-oz glass)	39.1	11.6	20.4	12.3	8.4	2.7	2.3	0.4	0.4	2.3
Liquor (1 shot)	46.1	15.7	8.4	12.2	4.7	5.1	5.6	0.7	0.2	1.2

* Values are percentage of cohort reporting the particular category of use. Total percentage may not add up to exactly 100 due to rounding.
† Serving sizes are consistent with the exact wording of the food frequency questionnaire.

reported intake of individual beverages using the semiquantitative food frequency questionnaire (15). Pearson correlations between the food records and the questionnaire ranged from 0.52 for plain water to 0.93 for coffee, with a mean correlation of 0.77.

We calculated daily total fluid intake from beverages by using data on frequency of use and serving size of the individual beverages. The data consist of reported fluid intake but not urine volumes. However, as part of the diet validation study (14), we collected 24-hour urine samples and found a high correlation (r = 0.59) between reported fluid intake and 24-hour urine volume (unpublished data).

Nutrient values were adjusted for total energy intake using a regression model with total caloric intake as the independent variable and absolute nutrient intake as the dependent variable (16, 17). As total energy intake for an individual tends to be fixed in a very narrow range, changes in nutrient intake must be made primarily by altering the composition of the diet, not the total amount of food consumed. Energy-adjusted values reflect the nutrient composition of the diet independent of the total amount of food consumed. In addition, energy adjustment reduces any variation introduced by underreporting or overreporting of intake on the food frequency questionnaire, thus improving the accuracy of nutrient measurement (14). Beverage intake was not adjusted for total energy intake.

Assessment of nondietary factors

In 1986, participants provided information on their state of residence, weight, height, and use of thiazide diuretics. The level of physical activity in metabolic equivalents per week was computed from the reported frequency and duration of various forms of mild to vigorous exercise.

Follow-up and case ascertainment

We sent follow-up questionnaires in 1988, 1990, and 1992, asking the participants whether they had had a kidney stone diagnosed since January 1986. After up to six mailings for each follow-up period (18), the response rate was greater than 94 percent.

When a kidney stone was reported on a follow-up questionnaire, we mailed a supplementary form to confirm the self-report and to ascertain the date of occurrence, symptoms, and family history of kidney stones. The rate of response to the supplementary questionnaire was 93 percent. The primary end point was an incident kidney stone accompanied by pain or hematuria. To confirm the validity of the self-report, we obtained the medical records from a random sample of 60 cases. All were confirmed except two, which were bladder stones.

We considered only cases that occurred during the first 6 years of follow-up—between the return of the 1986 baseline questionnaire and January 31, 1992. After excluding men who lived outside the US and men for whom the stone could not be confirmed or the occurrence was outside the study period, 45,289 men with no history of kidney stones at baseline remained.

Statistical analysis

For each participant, the person-months of follow-up were counted from the return date of the 1986 questionnaire to the date of a kidney stone or death, or January 31, 1992, whichever came first. We allocated person-months of follow-up according to the 1986 exposure status and calculated incidence as the number of events divided by the person-time of follow-up. The proportion of subjects with missing information for any individual beverage was less than 10 percent (table 1). These subjects were assigned to the lowest category of intake for that beverage because in the validation study, we found that a missing item was not consumed at all in the majority of instances. The categories for all items on the food frequency questionnaire, not just the beverages, were selected in 1988 based on the frequency response for each individual item. The goal was to have a sufficient number of participants in each category, particularly the extreme categories, to provide sufficient power to examine associations in subjects with extreme intake. For the beverage analyses, we did look at the nine individual response categories after examining the grouped categories to determine whether substantial variation was being masked. Because the results were very similar, the a priori selected grouped categories were used to provide more stable estimates of the associations. The relative risk—the incidence rate in a particular category of beverage use divided by the corresponding rate in the comparison category—was used as the measure of association (19).

We formally tested the null hypothesis that beverage type did not influence risk of kidney stones by adding intake of all specific beverages (except for water) to a multiple logistic model containing total fluid intake and evaluating the change in total model deviance (likelihood ratio test) with 20 df. Clinically, patients with kidney stones are frequently advised to drink a certain number of 240-ml (8-oz) glasses of liquid each day to produce at least 2 liters of urine. Therefore, for the multivariate model, we converted the daily use of each beverage into the number (or fraction) of 240-ml

servings consumed per day. This provides an estimation of the effect on the risk of stone formation of increasing the consumption by one unit (240 ml) of an individual beverage. Relative risks for specific beverages were adjusted simultaneously for other stone risk factors (11) using similar multiple logistic models (20). Variables considered in the multivariate models were specific beverage use (240-ml portions per day), age (5-year categories), quintiles of body mass index (weight in kilograms divided by the square of the height in meters), quartiles of physical activity level, geographic region (seven categories), subject's specific health profession, use of thiazide diuretics (yes/no), and quintiles of dietary intake of calcium, animal protein, and potassium. We previously showed that these dietary variables are related to risk of kidney stones (11). For all relative risks, we calculated 95 percent confidence intervals; all p values are two tailed.

RESULTS

The frequency of use of the individual beverages by the cohort members as reported on the baseline questionnaire is shown in table 1. During 242,100 person-years of follow-up over a 6-year period, 753 cases of incident symptomatic kidney stones were documented. After controlling for potential confounding by other risk factors, the relative risk of stone formation for men in the highest quintile of total fluid intake (≥2,538 ml/day) compared with the lowest quintile (<1,275 ml/day) was 0.65 (95 percent confidence interval 0.51–0.84).

After we controlled simultaneously for potential confounding by other risk factors including age, profession, geographic region, thiazide use, intake of dietary calcium, potassium, and animal protein, and total fluid, we found that the addition of specific beverages contributed significantly to the prediction of kidney stones (change in −2 log likelihood = 72.9 with 20 df, $p < 0.001$). In an additional model including all specific beverages but not total fluid intake, significant inverse associations were observed for caffeinated coffee, decaffeinated coffee, tea, beer, and wine (table 2). Apple juice and grapefruit juice were directly associated with risk (table 2).

For each 240-ml serving consumed daily, the risk of stone formation decreased by the following amount for the stated beverage when analyzed as a continuous variable: caffeinated coffee, 10 percent (95 percent confidence interval 4–15 percent); decaffeinated coffee, 10 percent (3–16 percent); tea, 14 percent (5–22 percent); beer, 21 percent (12–30 percent); and wine, 39 percent (10–58 percent). For each 240-ml portion consumed daily, the risk of stone formation increased by 35 percent (95 percent confidence interval 4–75 percent) for apple juice and 37 percent (1–85 percent) for grapefruit juice.

TABLE 2. Age-adjusted and multivariate relative risk of incident kidney stones for individual beverages per 240-ml (8-oz) serving size per day, Health Professionals Follow-up Study, 1986

Beverage	Age-adjusted		Multivariate*	
	RR†	95% CI†	RR	95% CI
Water	0.96	0.92–1.00	0.97	0.93–1.02
Milk				
Skim/low fat	0.89	0.82–0.96	0.97	0.85–1.10
Whole	0.86	0.72–1.02	0.87	0.71–1.05
Juices				
Appie	1.23	0.95–1.60	1.35	1.04–1.75
Orange	0.82	0.67–1.01	0.94	0.76–1.16
Grapefruit	1.19	0.87–1.61	1.37	1.01–1.85
Other fruit	0.80	0.49–1.32	0.83	0.50–1.36
Tomato	1.06	0.57–1.97	1.41	0.76–2.63
Punch/lemonade	1.14	1.01–1.30	1.11	0.97–1.27
Sodas				
Cola, with caffeine	1.11	1.01–1.22	1.06	0.96–1.18
Cola, without caffeine	0.98	0.75–1.29	0.93	0.70–1.23
Cola, diet, with caffeine	0.93	0.85–1.02	0.93	0.85–1.02
Cola, diet, without caffeine	1.01	0.92–1.12	1.02	0.92–1.12
Noncola	1.09	0.88–1.35	1.02	0.81–1.28
Noncola, diet	1.09	0.97–1.23	1.11	0.98–1.26
Coffee/tea				
Coffee, caffeinated	0.87	0.83–0.92	0.90	0.85–0.96
Coffee, decaffeinated	0.90	0.84–0.97	0.90	0.84–0.97
Tea	0.85	0.77–0.94	0.86	0.78–0.95
Alcoholic beverages				
Beer	0.80	0.72–0.90	0.79	0.70–0.88
Wine	0.60	0.40–0.88	0.61	0.42–0.90
Liquor	0.94	0.52–1.70	0.72	0.39–1.33

* The multivariate model included age (in 5-year categories), profession, geographic region (seven categories), use of thiazide diuretics (yes/no), dietary intake of calcium, animal protein, and potassium (quintile groups), and all 21 beverages (continuous variables with each unit representing 240 ml (8 oz) per day of that beverage).

† RR, relative risk; CI, confidence interval.

The risk of stone formation was examined according to category of consumption of the beverages for which we observed an overall association. In table 3, the multivariate relative risks are presented by frequency of beverage consumption using the standard serving size for each beverage (see table 1). The risk of stone formation decreased with increasing frequency of use of caffeinated and decaffeinated coffee, tea, beer, and

TABLE 3. Multivariate relative risks for beverages associated with the incidence of symptomatic kidney stones,* Health Professionals Follow-up Study

Beverage	Frequency of use								
	<1/ month	1–3/ month	1/ week	2–4/ week	5–6/ week	1/day	2–3/ day	4–5/ day	≥6/day
Caffeinated coffee									
Cases	281			270			156	46	
Person-years	75,477			84,607			55,539	26,477	
Multivariate RR†	1.0			0.89			0.80	0.54	
95% CI†				0.74–1.06			0.64–0.99	0.38–0.75	
Decaffeinated coffee									
Cases	397			269			64	23	
Person-years	116,236			90,366			26,927	8,570	
Multivariate RR	1.0			0.88			0.68	0.77	
95% CI				0.74–1.04			0.51–0.89	0.50–1.19	
Tea									
Cases	328			385			34	6	
Person-years	105,884			117,377			15,293	3,546	
Multivariate RR	1.0			1.01			0.65	0.53	
95% CI				0.87–1.18			0.45–0.93	0.23–1.19	
Beer									
Cases	355	129	202		60		7		
Person-years	106,589	43,343	61,866		20,445		9,857		
Multivariate RR	1.0	0.91	1.01		0.91		0.22		
95% CI		0.73–1.14	0.83–1.25		0.68–1.22		0.10–0.46		
Wine									
Cases	332	95	156	85	74		11		
Person-years	99,609	28,129	49,630	29,956	27,216		7,559		
Multivariate RR	1.0	0.99	0.90	0.86	0.86		0.50		
95% CI		0.77–1.26	0.73–1.12	0.66–1.12	0.65–1.13		0.27–0.92		
Apple juice									
Cases	374	175	88	79			37		
Person-years	126,965	60,154	26,286	19,320			9,375		
Multivariate RR	1.0	0.83	0.93	1.18			1.29		
95% CI		0.68–1.01	0.72–1.20	0.91–1.54			0.90–1.84		
Grapefruit juice									
Cases	504	136	48	40			25		
Person-years	171,996	38,175	13,500	10,997			7,431		
Multivariate RR	1.0	1.20	1.26	1.30			1.40		
95% CI		0.98–1.48	0.92–1.73	0.93–1.82			0.93–2.12		

* The multivariate model included age (in 5-year age categories), profession, geographic region (seven categories), use of thiazide diuretics (yes/no), dietary intake of calcium, animal protein, and potassium (quintile groups), and all 21 beverages (continuous variables with each unit representing 240 ml (8 oz) per day of that beverage).

† RR, relative risk; CI, confidence interval.

wine. The risk increased with increasing use of apple juice and grapefruit juice.

We also compared the risk of kidney stones associated with specific beverages with that of water, a presumably neutral fluid, to control for a similar volume of intake. Caffeinated coffee ($p = 0.03$), tea ($p = 0.02$), beer ($p = 0.001$), and wine ($p = 0.02$) were inversely associated with risk and were significantly different from water; decaffeinated coffee was marginally significant ($p = 0.07$). Apple juice ($p = 0.02$) and grapefruit juice ($p = 0.03$) were directly associated with risk and significantly different from water.

DISCUSSION

These prospective data confirm that greater fluid intake is associated with reduced risk of kidney stones, further suggesting that greater consumption of caffeinated and decaffeinated coffee, tea, beer, and wine decreases the risk of symptomatic kidney stones whereas greater consumption of apple and grapefruit juice increases the risk. The overall risk from beverages depends on the risk of consuming the specific beverage. Although we do not know the composition of the stones formed, the vast majority of stones in this group of men will contain calcium oxalate (2, 21).

The urinary concentration of lithogenic factors and risk of crystal formation can be lowered simply by increasing urine volume (6). In the steady state in healthy individuals, fluid intake is the main determinant of urine volume. Thus, patients who have formed kidney stones are routinely advised to increase their fluid intake. However, information on the effect of different beverages on risk of stone occurrence is sparse. The effects of specific beverages, such as orange juice (22) and sugared cola (23), have been examined in relation to changes in the urine composition but not to stone formation. The effects on stone formation of water hardness (24, 25) and primary fluid intake (10) have been studied using retrospective designs; however, these studies failed to address the important role of other dietary factors (11).

The mechanism for the protective effect of caffeinated coffee and tea may be mediated through caffeine. Caffeine interferes with the action of antidiuretic hormone on the distal nephron (26) resulting in increased urine flow and a more dilute urine, which would lower the risk of crystal formation. However, this beneficial effect may be partly offset by the increase in urinary calcium excretion caused by caffeine (27). The protective effect of beer and wine may be due to the inhibitory effect of alcohol on antidiuretic hormone secretion (26) with increased urine flow and decreased urinary concentration. A similar magnitude of association was seen for liquor; however, the confidence interval was wide.

The inverse association for tea and coffee may seem surprising given the common belief that these beverages, particularly tea, have significant amounts of oxalate. Very little information on the oxalate content of foods and beverages is available. Kasidas and Rose (28), using foods bought in England and an enzymatic method to measure oxalate content, found that a 240-ml portion of tea contained 17 mg of oxalate and a 240-ml serving of instant coffee contained 8 mg of oxalate. Thus, it appears that the amount of oxalate contributed to the diet by these beverages, although not trivial, is probably small.

It is unclear why decaffeinated coffee was associated with a decreased risk. It is possible that for those beverages inversely associated with risk, it was simply the fluid intake that was protective; however, we did not observe similar protection from all beverages at a similar volume of intake. Because we examined a large number of beverages, the possibility exists that some associations may have occurred by chance. Although the data are internally consistent for major sources of caffeine and alcohol, the findings for decaffeinated coffee clearly require confirmation.

The mechanism for the increased risk associated with apple and grapefruit juice consumption is also not obvious. Although the pH of these beverages is acidic (~3.5) (29), the actual total acid load is small. The oxalate content of these beverages has been reported to be less than 1 mg/liter (28). These beverages do contain a substantial amount of sugar, which can increase calcium excretion (30). However, other acidic beverages containing considerably more sugar, such as punch and lemonade and soda, were not associated with increased risk. Additional data from extended follow-up and other populations are required to confirm or refute the associations with these fruit juices.

Shuster et al. (12) studied the relation between soft drink consumption and the risk of kidney stone recurrence. The study population consisted of 1,009 male subjects with incident or recurrent stone disease who drank at least one-half can of soda per day. Of the randomly selected subjects advised to avoid soda consumption, 170/504 (64.6 percent) remained free of recurrence at 3 years. Of the other half of subjects who were given no special instructions regarding soda use, 205/505 (58.2 percent) were free of recurrence at 3 years ($p = 0.05$). In a subanalysis, the beneficial effect of abstaining from soda was limited to those whose most consumed soda was acidified by phosphoric but not citric acid; no clear mechanism was proposed. The patients were managed by their primary urologist; however, information on therapeutic interventions and beverages consumed instead of soda was not available. Thus, the reduction in recurrence among the participants given special dietary instructions may have been due to the increase in a beverage type other than soda.

After we adjusted for age alone, it appeared that the consumption of caffeinated sugared cola was associated with increased risk (table 2), which is consistent with previous reports (10, 12). However, after controlling for other potential risk factors, the association was no longer significant for sugared cola or for the other types of soda (table 2). The intake of caffeinated

sugared cola was inversely correlated with dietary calcium (correlation coefficient = −0.14, $p < 0.001$) and dietary potassium (correlation coefficient = −0.28, $p < 0.001$). We have previously reported that the risk of stone formation decreases with increased intake of dietary calcium and potassium (11). Thus, it appears that the results of these previous studies on soda consumption may have been confounded by other dietary factors. Furthermore, the previously mentioned randomized trial evaluating the role of soda on stone recurrence failed to account for other beverages that were consumed in the test group in place of soda. If the test subjects changed from caffeinated sugared cola to caffeinated coffee, for example, a decrease in the recurrence rate may have occurred due to the protective effect of coffee rather than to a stone-promoting effect of sugared colas.

The presence or lack of association between a beverage and risk of stone formation could be a result of substantial correlation between the use of specific beverages or total volume. Although correlations between beverages in the multivariate model would not on average affect the relative risks, they could widen the confidence intervals. The greatest correlation (r = 0.43) was between sugared colas and sugared noncolas; most other correlations were less than 0.20 (data not shown). Although beer (r = 0.32) and caffeinated coffee (r = 0.40) were moderately correlated with total fluid intake, other beverages such as water (r = 0.48) with similar or larger correlation coefficients were not significantly associated with a reduced risk of stone formation.

Biased recall of diet was avoided in this study because the intake data were collected before the diagnosis of kidney stones was made. However, nondietary risk factors for kidney stones could have influenced our results if they were strongly associated with the use of certain beverages.

These findings were observed among men ages 40 years and older with no history of kidney stones. In our cohort, more than 50 percent of the men were younger than age 60 at baseline; only 8 percent had a history of kidney stones and were therefore excluded. With the information currently available in the literature, we have no reason to believe that the relations we observed would be different in women, younger men, or men with a history of kidney stones containing calcium oxalate.

Our findings suggest that the effect of beverages on stone formation involves more than increasing fluid intake. In this study, greater intakes of caffeinated and decaffeinated coffee, tea, beer, and wine were associated with a decreased risk of stone formation, whereas greater intakes of apple and grapefruit juice were related to increased risk. Before definitive recommendations can be given to patients who have had kidney stones, additional studies are needed.

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REFERENCES

1. Sierakowski R, Finlayson B, Landes RR, et al. The frequency of urolithiasis in hospital discharge diagnoses in the United States. Invest Urol 1978;15:438–41.
2. Johnson CM, Wilson DM, O'Fallon WM, et al. Renal stone epidemiology: a 25-year study in Rochester, Minnesota. Kidney Int 1979;16:624–31.
3. Ljunghall S, Danielson BG. A prospective study of renal stone recurrences. Br J Urol 1984;56:122–4.
4. Coe FL, Keck J, Norton ER. The natural history of calcium urolithiasis. JAMA 1977;238:1519–23.
5. Strauss AL, Coe FL, Deutsch L, et al. Factors that predict relapse of calcium nephrolithiasis during treatment. Am J Med 1982;72:17–24.
6. Pak CYC, Sakhaee K, Crowther C, et al. Evidence justifying a high fluid intake in treatment of nephrolithiasis. Ann Int Med 1980;93:36–9.
7. Smith LH, van den Berg CJ, Wilson DM. Current concepts in nutrition: nutrition and urolithiasis. N Engl J Med 1978;298:87–9.
8. Broadus AE, Thier SO. Metabolic basis of renal-stone disease. N Engl J Med 1979;300:839–45.
9. Ljunghall S, Fellstrom B, Johansson G. Prevention of renal stones by a high fluid intake? Eur Urol 1988;14:381–5.
10. Shuster J, Finlayson B, Scheaffer RL, et al. Primary liquid intake and urinary stone disease. J Chron Dis 1985;38:907–14.
11. Curhan GC, Willett WC, Rimm EB, et al. A prospective study of dietary calcium and other nutrients and the risk of symptomatic kidney stones. N Engl J Med 1993;328:833–8.
12. Shuster J, Jenkins A, Logan C, et al. Soft drink consumption and urinary stone recurrence: a randomized prevention trial. J Clin Epidemiol 1992;45:911–16.
13. Willett WC, Sampson L, Stampfer MJ, et al. Reproducibility and validity of a semiquantitative food frequency questionnaire. Am J Epidemiol 1985;122:51–65.
14. Rimm EB, Giovannucci EL, Stampfer MJ, et al. Reproducibility and validity of an expanded self-administered semiquantitative food frequency questionnaire among male health professionals. Am J Epidemiol 1992;135:1114–26.
15. Feskanich D, Rimm EB, Giovannucci EL, et al. Reproducibility and validity of food intake measurements from a semiquantitative food frequency questionnaire. J Am Diet Assoc 1993;93:790–6.
16. Willett WC. Nutritional epidemiology. New York: Oxford University Press, 1990.
17. Willett W, Stampfer MJ. Total energy intake: implications for epidemiologic analyses. Am J Epidemiol 1986;124:17–27.
18. Rimm EB, Stampfer MJ, Colditz GA, et al. Effectiveness of

various mailing strategies among nonrespondents in a prospective cohort study. Am J Epidemiol 1990;131:1068–71.
19. Rothman KJ. Modern epidemiology. Boston: Little, Brown, 1986.
20. Kleinbaum DG, Kupper LL, Muller KE. Applied regression analysis and other multivariable methods. Boston: PWS-KENT Publishing Company, 1988.
21. Coe FL, Parks JH, eds. Nephrolithiasis: pathogenesis and treatment. Chicago: Year Book Medical, 1988.
22. Wabner CL, Pak CYC. Effect of orange juice consumption on urinary stone risk factors. J Urol 1993;149:1405–9.
23. Weiss GH, Sluss PM, Linke CA. Changes in urinary magnesium, citrate and oxalate levels due to cola consumption. Urology 1992;39:331–3.
24. Churchill DN, Maloney CM, Bear J, et al. Urolithiasis—a study of drinking water hardness and genetic factors. J Chron Dis 1980;33:727–31.
25. Shuster J, Finlayson B, Scheaffer R, et al. Water hardness and urinary stone disease. J Urol 1982;128:422–5.
26. Robertson GL. Regulation of vasopressin secretion. In: Seldin DW, Giebisch G, eds. The kidney: physiology and pathophysiology, 2nd ed. New York: Raven Press, 1992.
27. Hasling C, Sondergaard K, Charles P, et al. Calcium metabolism in postmenopausal osteoporotic women is determined by dietary calcium and coffee intake. J Nutr 1992;122:1119–26.
28. Kasidas GP, Rose GA. Oxalate content of some common foods: determination by an enzymatic method. J Hum Nutr 1980;34:255–66.
29. van der Horst G, Wesso I, Burger AP, et al. Chemical analysis of cool drinks and pure fruits juices—some clinical implications. S Afr Med J 1984;66:755–8.
30. Lemann J Jr, Adams ND, Gray RW. Urinary calcium excretion in human beings. N Engl J Med 1979;301:535–41.

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In vivo antioxidant effect of green and black tea in man

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Objective: Evaluation of the *in vitro* antioxidant activity of green and black tea, their *in vivo* effect on plasma antioxidant potential in man and the effect of milk addition.
Design: The antioxidant activity of the tea, with and without milk, was tested *in vitro* by measuring the length of the peroxyl radical induced lag-phase. The *in vivo* activity was tested on two groups of five healthy adults. Each group ingested 300 ml of either black or green tea, after overnight fast. The experiment was repeated on a separate day, adding 100 ml whole milk to the tea (ratio 1 : 4). Five subjects acted as controls. The human plasma antioxidant capacity (TRAP) was measured before and 30, 50 and 80 min from the ingestion of tea.
Results: Both teas inhibited the *in vitro* peroxidation in a dose-dependent manner. Green tea was sixfold more potent than black tea. The addition of milk to either tea did not appreciably modify their *in vitro* antioxidant potential. *In vivo*, the ingestion of tea produced a significant increase of TRAP ($P < 0.05$), similar in both teas, which peaked at 30–50 min. When tea was consumed with milk, their *in vivo* activity was totally inhibited.
Conclusions: The paper shows that tea possesses a strong antioxidant activity *in vitro* which is believed to be exerted by its polyphenols moiety. It also provides compelling evidence that tea has also a potent *in vivo* activity in man. The promptness of the *in vivo* response suggests that the absorption of the bioactive components of tea takes place in the upper part of the gastrointestinal system. The inhibition of this effect by milk is thought to be due to the complexation of tea polyphenols by milk proteins. These findings might help to clarify the putative role of dietary polyphenols in modulating oxidative stress *in vivo*.
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Descriptors: antioxidants, men, milk, plasma, polyphenols, tea

Introduction

Oxidative damage is thought to represent one of the mechanisms leading to chronic diseases such as atherosclerosis and cancer (Halliwell & Gutteridge, 1989). Therefore interest is growing in the defence afforded by antioxidant nutrients against free radical reactions. This presumptive role has prompted research which focused first on recognised antioxidant nutrients such as ascorbic acid, α-tocopherol and β-carotene (Halliwell & Gutteridge, 1989).

More recently, the attention has shifted to polyphenols. Polyphenols are secondary plant metabolites occurring widely in plant food (Harborne, 1989). They possess outstanding antioxidant and free radical scavenging properties, suggesting a possible protective role in man (Laughton, 1991; Scott *et al*, 1993). The *in vitro* antioxidant activity of polyphenols has been amply researched and there is a vast literature documenting their ability to act as primary as well as secondary antioxidants through sequestration of metal ions (Morel *et al*, 1994) and by scavenging reactive oxygen species (Rafat Husain *et al*, 1987; Robak & Gryglewski, 1988; Hanasaki *et al*, 1994). However, the mechanisms through which these compounds act *in vivo* are still incompletely understood and many uncertainties persist relative to their bioavailability and metabolic fate in man (Gugler, Leshik & Dengler, 1975; Kleiynen & Knipschild, 1992).

Recent papers have provided the first evidence of an epidemiological link between polyphenol intake and risk of cardiovascular disease in man (Hertog *et al*, 1993*a*). Evidence of an *in vivo* antioxidant effect in man after ingestion of polyphenol-rich beverages, tea (Serafini *et al*, 1994) and wine (Maxwell *et al*, 1994) has also been recently published.

Polyphenols are a wide family of compounds, characterised by the presence of one or more aromatic rings and hydroxyl groups. Their antioxidant potential is closely related to the number of hydroxyls, the higher the number the more potent the chain-breaking antioxidant action of the compound (Rafat Husain *et al*, 1987).

Tea (*Camellia sinensis*) is a widely consumed beverage throughout the world. It has been calculated that the average consumption is slightly in excess of 100 ml per day (Balentine, 1992), but it can reach up to 20 cups or more per day (Graham, 1992). Tea was the main dietary source of five selected flavonoids consumed by a Dutch population group (Hertog *et al*, 1993*b*). Tea leaves contain more than 35% of their dry weight in polyphenols whose nature differs depending on the manufacturing procedure (Balentine, 1992). There are three

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main classes of commercial tea. Green tea, a non-fermented type of tea preferred in China, is rich in flavanols (mainly catechin, epicatechin and epigallocatechin), flavandiols and simple phenolic acids. Black tea, mostly consumed in the Western world but also in South and South East Asian countries and in the whole African continent, is obtained by fermentation of the green tea. During fermentation, simple polyphenols undergo an enzymatic polymerisation that leads to the formation of complex compounds of condensation. The principal condensation compounds present in black tea are theaflavins and thearubigens with a molecular weight of about 500–3000 (Balentine, 1992). These polyhenols are responsible for the characteristic reddish colour and the astringency of the black tea (Rider *et al*, 1992). The sensation of astringency is produced by the precipitation in the mouth of the glycoproteins present in the mucous secretion of the salivary glands (Rider *et al*, 1992). The mitigation of this astringency when milk is added to tea is due the formation of a complex between black tea polyphenols and alfa and beta casein of milk (Brown & Wright, 1963). The third class of tea is the Oolong tea which, being semifermented, has intermediate characteristics.

In this paper we have investigated the *in vitro* antioxidant action of green and black tea, with and without milk. We have also studied their *in vivo* effect by measuring the total plasma antioxidative capacity (TRAP) in man.

Methods

Chemical and reagents

All chemicals were purchased from Sigma Chemical Co., St Louis, MO, USA, unless otherwise stated. 6-Hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid (Trolox) was purchased from Aldrich Chemical Co. (Milwaukee, MI, USA); 2,2'-diazobis(2-amidinopropane) dihydrochloride (ABAP) from Polyscience (Warrington, PA, USA).

Solutions and reagents for TRAP assays were made using Milli-Q (Millipore, Bedford, MA, USA) double-distilled water (resistance > 18 mΩ/cm^2) and passed through Chelex 100 resin Na^+ form.

Measurement of antioxidant potential

The method employed to assess the antioxidant activity of tea was recently developed in our laboratory (Ghiselli *et al*, 1995). It is based on the protection afforded by plasma against the decay of a fluorescent target, R-Phycoerytrhin (R-PE) during a controlled peroxidation reaction. The reaction mixture is 1.5×10^{-8} M R-PE in 75 mM phosphate buffer, pH 7.0. Full details on the method are published elsewhere (Ghiselli *et al*, 1995). In the present study, tea or human plasma were added to the reaction mixture up to a 2.0 ml final volume and pre-incubated at 37°C for 5 min in 10 mm quartz fluorometer cells. The oxidation reaction was started by adding ABAP to a final concentration of 4.0 mM. The decay of R-PE fluorescence was monitored every 5 min for 90 min on a Perkin–Elmer (Norwalk, CT USA) LS-5 Luminescence Spectrometer equipped with a thermostatically controlled cell-holder. The monochromators operated at an excitation wavelength of 495 nm/5 nm slit width and at an emission wavelength 575 nm/5 nm slit width. Each assay was done in duplicated or triplicate. The reproducibility of the method is high (c.v. % < 3).

Results have been standardised using Trolox, a water-soluble analogue of α-tocopherol, and expressed as TRAP (μM), which represents the μmoles of peroxyl radicals trapped by one litre of plasma.

In vitro *study*

Tea infusions were prepared using two commercially available brands of tea (Twinings Earl Grey and Birko Chinese green tea, KI). 100 ml boiling tap water was poured over 2.0 g tea leaves. The mixture was allowed to stand for 1.5 min and then filtered. Pasteurised whole milk was used in the tea-with-milk experiments. The *in vitro* antioxidant activity was assessed by measuring the inhibition produced by the addition of increasing amounts of tea to the reaction mixture described above.

The effect of the presence of milk was tested by adding 2.0 μl of the tea + milk mixture and the results were corrected for the dilution. Tea + doubly distilled water was used as control.

In vivo *study*

The study protocol was approved by the Human Ethics Committee of the National Institute of Nutrition. Ten healthy and consenting volunteers were randomly divided in two equal groups. Each group was asked to ingest, after overnight fast, a bolus quantity (300 ml) of either black or green tea, unsweetened. The experiment was repeated on the same subjects, on a separate day, with the same type and quantity of tea added with 100 ml whole milk (ratio 1 : 4). Five subjects acted also as controls and drank 300 ml tap water.

Venous blood samples were collected in pre-chilled BD Vacutainer EDTA-tubes before and 30, 50 and 80 min after tea ingestion. The blood was immediately centrifuged at 12 000 × g for 3 min, plasma was separated, placed at −80°C and assayed for TRAP within 3 h.

Statistics

Data are expressed as mean ± s.e.m. The area under the curve (AUC) has ben calculated using Kaleidagraph 3.02 (Synergy Software, Reading, PA, USA) software for Macintosh. Linear regressions, one way ANOVA and *t*-test for independent samples were calculated using Statview II (Abacus Concepts Inc, Berkeley, CA, USA) software for Macintosh.

Results

In vitro *antioxidant effect of tea*

The results of the *in vitro* experiments are illustrated in Figure 1. The progressive lengthening of the lag-phase of the ABAP-induced peroxidation, elicited by increasing amounts (2, 4 and 8 μl) of tea is clearly visible. Plotting the lag-phases against the amount of tea, regressions lines have been obtained, one for green tea and one for the black tea, both highly significant with correlation coefficients close to 1. The slope of the green tea was steeper ($b = 31.6$) than that of black tea, ($b = 5.11$) disclosing a remarkably inferior potency of the latter. The antioxidant capacities of the two types of tea were

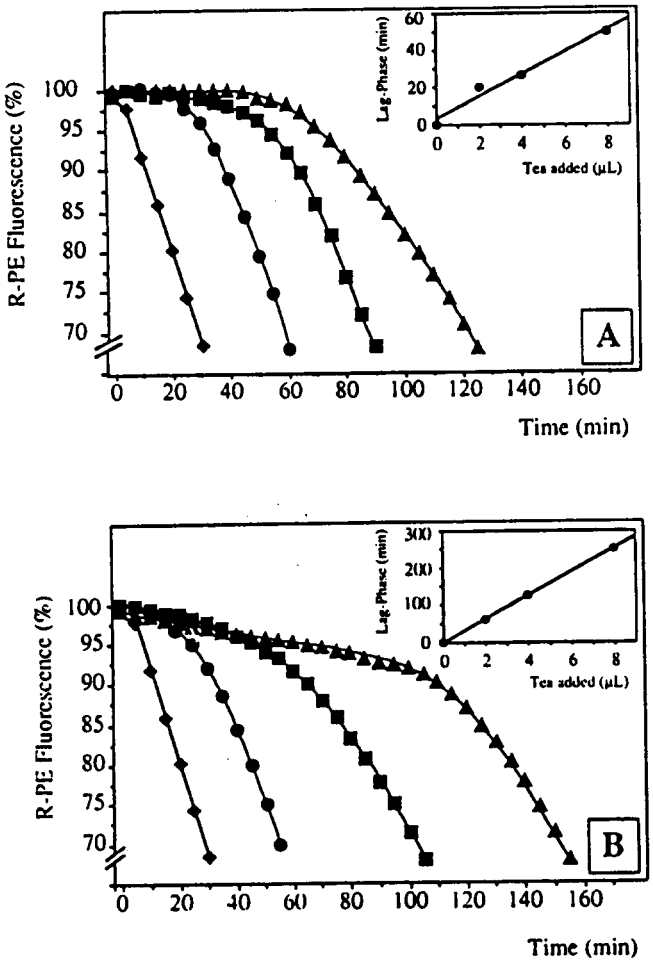

Figure 1 Effect of the addition of 0 (◆), 2 (●), 4 (■) and 8 (▲) μl of undiluted black (A) and 1.2 diluted green (B) tea to 2.0 ml reaction mixture on the delay (lag-phase) of the peroxidation reaction. Inset: linear regression of lag-phase *vs* the amount of tea added.

3542 and 17850 μM respectively for black and green tea.

Adding milk did not significantly modify the *in vitro* behaviour of either type of tea.

Table 1 Mean ± s.e.m. of plasma TRAP values[a] before and after drinking black or green tea

Time (min)	*Controls (n = 5)*	*Black tea (n = 5)*	*Green tea (n = 5)*
T0	1478 ± 160	1298 ± 187	1330 ± 170
T30	1468 ± 144	1505 ± 209	1786 ± 206
T50	1502 ± 172	1675 ± 128	1630 ± 156
T80	1470 ± 161	1428 ± 42	1488 ± 125

[a] TRAP values are expressed as μmol/l.

In vivo *antioxidant effect of tea*

The results of the *in vivo* tests are presented in Table 1. Mean basal TRAP values of the three groups were not significantly different (*t*-test for independent samples): 1478 ± 160, 1298 ± 187 and 1330 ± 170 μM respectively for controls, black tea and green tea. The TRAP values of the control subjects who consumed only tap water remained unchanged over the entire observation period. The increase of TRAP values induced by green tea (Figure 2) peaked at 30 min (+ 40%, calculated as average of the individual increments over basal values) and declined thereafter returning at T80, close to basal values. The peak response to black tea was similar (48% over baseline), but occurred slightly later (50 min) and fell at T80 to near basal values.

The overall antioxidant effect was assessed by measuring the AUC of the individual responses over the

Table 2 Mean ± s.e.m. and statistical significance of the area under the curve (AUC)[a] of all subjects

Controls (n = 5)	0.2 ± 0.8	
	Without milk	*With milk*
Black tea (*n* = 5)	14.1 ± 5.8[b]	−2.6 ± 1.6[c]
Green tea (*n* = 5)	21.3 ± 4.3[b]	0.6 ± 6.8[c]

[a] AUC values are expressed as mmol/l × 80 min.
[b] $P < 0.05$ *vs* controls.
[c] $P < 0.05$ *vs* tea without milk.

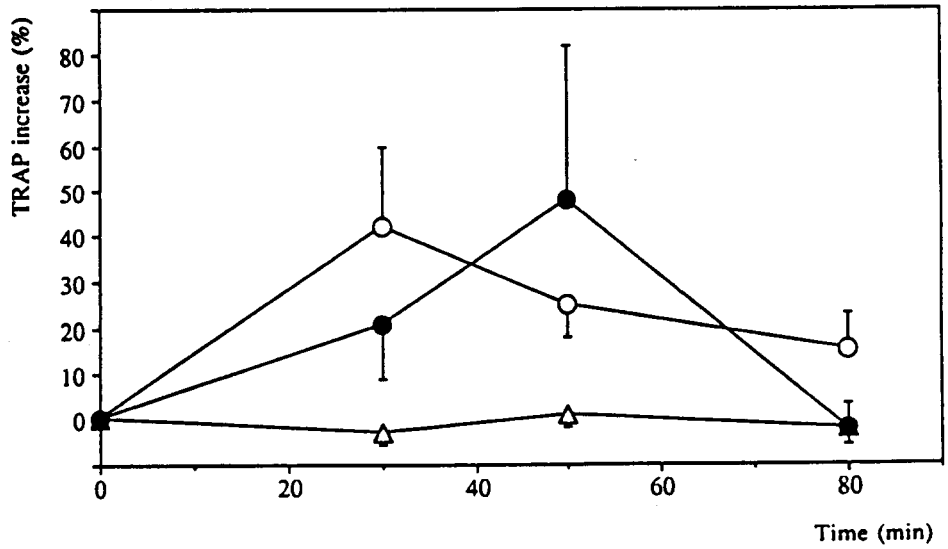

Figure 2 Effect of black (●) and green (○) tea ingestion on plasma TRAP values. Control subjects are represented by Δ symbol. Values are expressed as mean ± s.e.m. of the individual percent increments over basal values.

entire period of observation. The results are presented in Table 2. The AUC values for the green tea (21.3 ± 4.3) and the black tea (14.3 ± 5.8) were similar to each other and both differed significantly from the control group (0.2 ± 0.8).

The TRAP response when tea was drunk with milk was compared to that elicited by tea alone (Table 2). The AUCs of both tea- plus milk-mixtures were close to zero and significantly different (Fisher test $P < 0.05$) from those obtained with tea alone. These results suggest that milk had totally or nearly totally inhibited the *in vivo* effect exerted by tea alone.

Discussion

Ample experimental and epidemiological evidence supports the involvement of oxidative stress in the pathogenesis and in the progression of several chronic diseases (Halliwell *et al*, 1992). Dietary factors are capable of modulating the oxidative stress through several mechanisms, and might therefore play a crucial role in health protection.

Polyphenols are endowed with powerful antioxidative properties and are now considered to be potentially important for the prevention of chronic diseases in man. Polyphenols are present in all plant food and may thus be regarded as normal constituents of the human diet. The presumptive consumption of polyphenols in the USA has been calculated to be about 1.0 g/day (Kuhnau, 1976). The diets of the Mediterranean and of the Third World are likely to have a much higher content given their richness in plant food. Definitive evidence of polyphenol antioxidant and protective effects *in vivo* are still lacking, while ample evidence has firmly established their potent *in vitro* effect.

Recently published letters to the Lancet have reported the first *in vivo* effect of polyphenol-rich beverages, tea and wine in man (Serafini *et al*, 1994; Maxwell *et al*, 1994). Since then, Whitehead *et al* (1995) have published their full results, confirming that the consumption of moderate amounts of red wine elicits a prompt though temporary rise of plasma antioxidative defences.

In this paper we have first established that both green and black tea represent an excellent source of antioxidant polyphenols, green tea being about five times more potent than black tea. The higher concentration of simple, hydroxyl-rich polyphenols in green tea fully justifies its higher antioxidant potency. Lunder (1992) has shown that the antioxidant activity of tea is directly proportional to its polyphenol content. We can therefore reasonably assume that the antioxidant effect observed in our *in vitro* and *in vivo* experiments is caused by the polyphenols contained in the tea.

We have then established that, for what concerns the *in vivo* effect, the bolus ingestion of one large cup of tea produces an appreciable increase in plasma TRAP values in man. We were surprised to find that black tea produced a response of the same intensity of green tea. This finding was unexpected, given the profound difference observed in the *in vitro* activity of the two teas.

The mechanisms and the sites of absorption of polyphenols in humans, and their bioavailability in general, have not yet been elucidated. We can therefore only speculate that modifications must have taken place, after ingestion, in the molecular structure of black tea polyphenols, restituting their pristine antioxidative capacity. The promptness of the TRAP response (30–50 min) suggests that these modifications and the subsequent absorption of the modified polyphenols must occur in the higher tract of the gastrointestinal system, probably starting from the stomach. We postulate that the condensed polyphenols of black tea are rapidly broken down in the stomach by the acid gastric secretion, similarly to what happens when lemon juice is added into a cup of black tea. The simpler polyphenols released by the gastric hydrolysis of theaflavines and thearubigens would thus become available for absorption and for exerting their antioxidant activity.

Milk proteins are known to complex polyphenols (Brown & Wright 1963), but while this reaction has not affected their *in vitro* antioxidant activity, it totally inhibited their capacity to act *in vivo*. This discrepancy might be attributed to the fact that the milk protein–polyphenol complex, while retaining intact its antioxidative potential, becomes resistant to gastric hydrolysis. This resistance would render the complex unavailable for absorption in the upper gastrointestinal tract.

Another possible interpretation is the interference with the absorption in the stomach of simple phenolics due to the change in gastric pH following the introduction of milk. Simple phenolics are weak acid compounds and as such easily absorbable in their non-ionised form (Fingl & Woodbury, 1970). Even a small rise in gastric pH, such as that induced by the milk added to tea, would increase the phenols' ionisation, thereby reducing their passage through the gastric mucosa.

The findings presented in this paper are relevant to the much debated question of health-protective dietary habits and open the way to a better understanding of the inverse relationship between plant-rich diets and mortality for chronic degenerative disease (Block *et al*, 1992).

The demonstration that polyphenols are bio-active dietary compounds capable of raising plasma antioxidant defences in man, has far-reaching implications. More and better information on the type and quantity of individual polyphenols normally present in the diet becomes of higher importance, but also there is the need to gain a clear picture of their capacity of truly acting *in vivo*.

Notable is the fact that condensed polyphenols appear to develop an appreciable *in vivo* antioxidant action, following their gastric digestion, even though their *in vitro* activity might be modest. But even more importantly, our findings provide compelling insights on the potential for interferences with the *in vivo* antioxidant role of polyphenols by compounds which are normal constituents of human diets.

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References

Balentine AD (1992): Manufacturing and chemistry of tea. In *Phenolic compounds in food and their effects of health 1*, eds H Chi-Tang, YL Chang & H Mou-Tuan, pp 103–117. Washington DC: ACS.

Block G, Patterson B & Subar A (1992): Fruit vegetables and cancer

prevention: a review of the epidemiologic evidence. *Nutr. Cancer* 18, 1–29.

Brown PJ & Wright WB (1963): An investigation of the interactions between milk proteins and tea polyphenols. *J. Chromatog.* 11, 504–514.

Fingl E & Woodbury DM (1970): Section I General principles In *The pharmacological basis of therapeutics*, 4th edn, eds LS Goodman & Gilman, pp 1–42. New York: Macmillan.

Ghiselli A, Serafini M, Maiani G, Azzini E & Ferro-Luzzi A (1995): A fluorescence based method for measuring total plasma antioxidant capability. *Free Rad. Biol. Med.* 18(1), 29–36.

Graham HN (1992): Green tea composition, consumption, and polyphenol chemistry. *Prev. Med.* 21, 334–350.

Gugler R, Leschik M & Dengler HJ (1975): Disposition of quercetin in man after single oral and intravenous doses. *Eur. J. Pharmacol.* 9, 229–234.

Halliwell B & Gutteridge JMC (1989): Free radicals, ageing and disease. In *Free radicals biology and medicine*, eds B Halliwell & JMC Gutteridge, pp 416–508. Oxford: Clarendon Press.

Halliwell B, Gutteridge JMC & Cross CE (1992): Free radicals, antioxidants, and human disease: where are we now? *J. Lab. Clin. Med.* 119(6), 598–620.

Hanasaki Y, Ogawa S & Fukui S (1994): The correlation between active oxygens scavenging and antioxidative effects of flavonoids. *Free Rad. Biol. Med.* 16(6), 845–850.

Harborne JB (1989): General procedures and measurement of total phenolics. In *Methods in plant biochemistry* 1, *Plant phenolics*, ed JB Harborne, pp 1–28. Academic Press: New York.

Hertog MGL, Feskens EJM, Hollman PCH, Katan MB & Kromhout D (1993*a*): Dietary antioxidant flavonoids and risk of coronary heart disease: the Zutphen Elderly Study. *Lancet* 342, 1007–1011.

Hertog MGL, Hollman PCH, Katan MB & Krombout D (1993*b*): Estimation of daily intake of potentially anticarcinogenic flavonoids and their determinants in adults in The Netherlands. *Nutr. Cancer* 20, 21–29.

Kleiynen J & Knipschild P (1992): Ginkgo Biloba. *Lancet* 340, 1136–1139.

Kuhnau J (1976): The flavonoids. A class of semi-essential food components: their role in human nutrition. *World Rev. Nutr. Diet.* 24, 117–191.

Laughton MJ, Evans PJ, Moroney MA, Hoult JRS & Halliwell B (1991): Inhibition of mammalian 5-lipoxygenase and cyclooxygenase by flavonoids and phenolic dietary additives: relationship to antioxidant activity and to iron-reducing ability. *Biochem. Pharmacol.* 42, 1673–1681.

Lauder LT (1992): Catechins of green tea. In *Phenolic compounds in food and their effects on health II*, eds H Chi-Tang, YL Chang & H Mou-Tuan, pp 114–120. Washington DC: ACS.

Maxwell S, Cruickshank A & Thorpe G (1994): Red wine an antioxidant activity in serum. *Lancet* 344, 193–194.

Morel I, Lescoat G, Cillard P & Cillard J (1994): Role of flavonoids and iron chelation in antioxidant action. *Methods Enzymol.* 234, 437–443.

Rafat Husain S, Cillard J & Cillard P (1987): Hydroxyl radical scavenging activity of flavonoids. *Phytochemistry* 26(9), 2489–2491.

Rider PJ, Der Marderosian A & Porter JR (1992): Evaluation of total tannins and relative astringency in teas. In *Phenolic compounds in food and their effects on health I*, eds H Chi-Tang, YL Chang & H Mou-Tuan, pp 103–117. Washington DC: ACS.

Robak J & Gryglewski RJ (1988): Flavonoids are scavengers of superoxide anions. *Biochem. Pharmacol.* 37(5), 837–841.

Scott BC, Butler J, Halliwell B & Aruoma OI (1993): Evaluation of the antioxidant actions of ferulic acid and catechins. *Free Rad. Res. Commun.* 19(4), 241–253.

Serafini M, Ghiselli A & Ferro-Luzzi A (1994): Red wine, tea, and antioxidants. *Lancet* 344, 626.

Whitehead TP, Robinson D, Allaway S, Syms J & Hale A (1995): Effect of red wine ingestion on the antioxidant capacity of serum. *Clin. Chem.* 41, 32–35.

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DRAFT

Health Benefits of Tea
Revised Brochure Copy: Inside Section
Draft 1/6/97

In the Orient, tea has been considered central to good health for nearly 5,000 years. The Chinese drink tea to prevent sickness and employ medicines derived from tea to treat disease. Modern science has strengthened the connection between tea drinking and good health. Recent studies show that tea may protect against stroke, and tea drinking has previously been shown to reduce the risk for heart disease and certain cancers. The antioxidants in tea are thought to be responsible for these protective effects.

ANTIOXIDANTS: *Oxidants* are reactive oxygen molecules produced as by-products of normal body functions. Two kinds of oxidants, *peroxides* and *free radicals*, damage the human body because their high energy causes them to attack and damage molecules in cell membranes and genetic material. *Antioxidants* work to neutralize these oxidants and prevent cell damage that is thought to contribute to aging and chronic diseases, such as cancer and heart disease.

Beta carotene, vitamin E and vitamin C are well-known antioxidants, but scientists have recently identified antioxidant compounds in plants that may also lessen the risk of chronic disease. These plant compounds, called phytochemicals, have been found in certain fruits and vegetables and all types of tea: black, green and oolong.

While the exact source of tea's protective effects are unknown, scientists say that in all probability tea's polyphenols are the protective phytochemicals. Two of the most important groups of polyphenols in tea which have antioxidant properties are the catechins and flavonoids. Green tea is the richest source of disease-fighting catechins, and black and oolong teas contain significant levels of flavonoids.

TEA & STROKE: Current research suggests that flavonoid consumption may reduce the risk of stroke by inhibiting LDL oxidation and reducing platelet aggregation. In one study of Dutch men, drinking more than 4-5 cups of tea per day was associated with more than a 50% reduced risk of stroke.

TEA & HEART DISEASE: Animal studies have suggested that tea may help lower blood pressure and blood cholesterol. In a recent study of elderly men, tea drinking was associated with a lower number of deaths from coronary heart disease. Researchers attributed this reduced risk to tea polyphenols' interference with LDL oxidation activity.

TEA & CANCER: In recent animal studies, green and black teas have been shown to have a protective effect against skin cancer, and to reduce the number of tumors in the stomach and intestines. Epidemiological studies also show that a lifetime consumption of black tea is associated with a reduced risk some cancers.

TEA & FLUORIDE: Tea is also a good source of fluoride, a mineral that strengthens tooth enamel. While green tea contains more fluoride than black, sipping as little as one cup a day of either type may be a powerful anti-cavity strategy. Tea drinking also appears to reduce plaque formation and bacterial infections in the mouth.

TEA & CAFFEINE: Caffeine is a natural component of coffee, tea, cocoa, and is used in some soft drinks and medications. Caffeine is generally considered safe when consumed in moderation. While a serving of tea usually contains less than half the caffeine of coffee (or 40 mg.), actual caffeine levels are dependent upon specific blends and strength of brew.

TEA & IRON: Iron is involved in many cellular functions and is needed to transport oxygen to cells. Although there is a relationship between tea drinking and reduced absorption of iron, research indicates that individuals consuming a typical Western diet are not likely to be at risk for iron-deficiency anemia. Iron is found in many foods, including red meat, dried fruits and legumes, and is added to foods such as breakfast cereals. Tea has no effect on iron absorption when consumed between meals, but may slightly decrease the uptake of iron from plant foods when consumed with a meal. To offset tea's minor effect on iron absorption, foods rich in vitamin C, such as orange juice, strawberries or tomatoes can be eaten in the same meal. Adding milk or lemon to tea, especially to iced tea, works as well.

TEA & FLUID BALANCE: For millions of people, drinking tea plays a significant role in maintaining fluid balance, which is crucial for normal body function. Water is a major component of all living matter requiring continual replenishment. Water keeps the body cool, transports nutrients, and cushions joints. Most adults need about two quarts of fluid daily which should come from beverages and some fruits and vegetables.

January 3, 1996

TEA COUNCIL OF THE U.S.A., INC.
PRO-FORMA BALANCE SHEET
December 31, 1996

ASSETS

Cash on Hand	$100
Cash in Banks:	
Checking Account	1,921
Money Market Account	2,596
Dean Witter Liquid Assets	650,143
Dreyfus Liquid Assets	47,417
T Bills	433,589
Accounts Receivable	0
Inventory	1,006
Prepaid Expenses	0
Deposits with Suppliers	100
Total Assets	**$1,136,873**

LIABILITIES AND OPERATING BALANCE

Accounts Payable	$0
Operating Balance	1,136,873
Total Liabilities & Operating Balance	**$1,136,873**

DISTRIBUTION OF THIS STATEMENT:

Monthly: Tea Council Executive Committee
Quarterly: Tea Council Board of Directors

TEA COUNCIL OF THE U.S.A., INC.
Report of the Treasurer
For the 12 Months Ended December 31, 1996

	Actual To 12/31/96	Budget To 12/31/96	1996 Preliminary Budget
OPERATING BALANCE AT BEGINNING:	**$747,958**	**$747,958**	**$747,958**
Receipts:			
U.S.A. Trade Contributions	$692,940	$750,000	$750,000
Producing Country Contributions	17,625	19,000	19,000
Producing Country Observer Contribution	10,381	15,000	15,000
Interest Income	41,621	30,000	30,000
Total Receipts	**$762,567**	**$814,000**	**$814,000**
Expenditures:			
Tea Council Activities-Approved/Projected			
Administrative	$251,685	$262,515	$262,515
Approved Programs			
Consumer Publicity	6,895	23,500	23,500
Health Aspects	115,071	334,332	334,332
Total Programs & Administration	373,652	620,347	620,347
Uncommitted			
Total Expenditures	**$373,652**	**$620,347**	**$620,347**
OPERATING BALANCE AT END:	**$1,136,873**	**$941,611**	**$941,611**

		Actual To 12/31/96	Budget To 12/31/96	1996 Preliminary Budget
Tea Council Administrative				
120	Trademark Renewal	$780	$600	$600
125	D&O Liability Insurance	2,655	2,655	2,655
143	Accounting Fees	5,500	6,800	6,800
144	Legal Fees	0	2,000	2,000
170	Tea Convention	27,516	30,000	30,000
175	Council Letterhead	0	1,000	1,000
180	FARA	1,525	2,500	2,500
190	Miscellaneous	(250)	3,000	3,000
199	Association Fee	213,960	213,960	213,960
	Total	**$251,685**	**$262,515**	**$262,515**
Internal Consumer Publicity				
210	Binding	$0	$2,000	$2,000
220	Printing	0	8,000	8,000
225	Photographs	0	2,500	2,500
230	Print Clippings	6,895	7,000	7,000
250	Trade Publicity	0	4,000	4,000
	Total	**$6,895**	**$23,500**	**$23,500**
Tea & Health Campaign				
301	Scientific Advisory Panel	$4,050	$22,225	$22,225
305	Scientific Information Exchange	14,017	39,651	39,651
320	Health & Nutrition Partnerships/ADA	897	34,347	34,347
325	Tea Crisis Management	0	119,354	119,354
330	Ongoing PR Activity	42,137	46,628	46,628
340	Special Publicity Activity (Broadcast)	10,936	27,260	27,260
350	Epidemiology Review	8,000	8,000	8,000
370	Out-Of-Pocket	7,337	6,195	6,195
399	Account Management	27,698	30,672	30,672
	Total	**$115,071**	**$334,332**	**$334,332**
Total Expenditures		**$373,652**	**$620,347**	**$620,347**