

## PREPARATION, COMPOSITION AND CONSUMPTION PATTERNS OF TEA-BASED BEVERAGES IN ARIZONA

I.A. Hakim, M.D., Ph.D.<sup>1</sup>, U. M. Weisgerber, Ph.D.<sup>2</sup>, R. B. Harris, Ph.D.<sup>1</sup>, D. Balentine,  
Ph.D.<sup>3</sup>, C.A.J. van-Mierlo, M.Sc.<sup>2</sup>, and I. Paetau-Robinson, Ph.D.<sup>3</sup>

<sup>1</sup>University of Arizona College of Public Health and the Arizona Cancer, College of Medicine,  
University of Arizona, PO Box 245024, 1515 N Campbell Ave, Tucson, AZ 85724, <sup>2</sup>Unilever  
Research Vlaardingen, UNC, PO Box 114, 3130 AC Vlaardingen, The Netherlands, <sup>3</sup>Thomas J  
Lipton Company, 800 Sylvan Ave, Englewood Cliffs, NJ 07632, U.S.A.

### ABSTRACT

Flavonoids in black and green tea have been implicated in cancer chemoprevention. The concentration of flavonoids in tea is likely to vary by preparation techniques. Inconsistencies between epidemiological studies may arise from the lack of information on methods of preparation. The purpose of this study was to assess the pattern of tea consumption among an older Arizonan population and to determine tea polyphenol and flavonoid levels in the most commonly used tea preparation techniques for a Southwestern US population. A specific tea questionnaire was developed using focus groups and semi-structured interviews. The reliability of the tea questionnaire was very high even after 6 months ( $r=0.93$  for average tea intake/day). Forty samples, representing the most typical preparation techniques of hot, iced, and sun tea, were analyzed by HPLC for total flavonoids, catechins, theaflavins, thearubigins, caffeine and gallic acid. In black tea, the highest concentrations of flavonoids ( $\mu\text{g/ml}$ ) were found in brewed hot tea (range: 541-692) while the lowest concentrations were for instant tea preparations (range: 90-100). Results show that tea concentration, brewing time, and beverage temperature also have major influences on flavonoid concentrations. Use of specific questions focusing on tea preparation and availability of quantitative estimates of tea flavonoids should enhance epidemiological studies of the relationship between tea consumption and disease risk.

© 2000 Elsevier Science Inc.

Key words: tea flavonoids, questionnaires, preparation techniques

### INTRODUCTION

Tea is a beverage made from the leaves of *Camellia sinensis* species of the Theaceae family. This beverage is one of the most ancient and, next to water, the most widely consumed liquid in the world. Tea leaves are primarily manufactured as green or black or oolong, with black tea representing approximately 80% of the tea products consumed. EGCG is the major green tea polyphenol (>40% dry weight). The major components of black tea are theaflavins (1-2% dry weight) and thearubigins (10-20% dry weight) (1). Tea has been known to have various clinical merits, such as antiviral, antibacterial, antipyretic, and anticarcinogenic effects (2). Tea polyphenols scavenge active oxygen radicals (3) and they inhibit DNA biosynthesis of the tumor cells (4) and chemocarcinogen-induced carcinogenesis (5). They also block the inhibition effect of carcinogen in intercellular communication (6) and induce apoptosis (7). Tea-derived polyphenols exhibit antimutagenic and genotoxic activities probably associated with anticarcinogenic activity (8).

Since the concentration of polyphenols and flavonoids in tea is likely to vary by preparation

techniques, the current study was designed to determine the usual tea consumption patterns of an older Southwestern United States population and to then evaluate tea polyphenol and flavonoid levels in the most commonly used tea preparation techniques. A detailed tea questionnaire (TQ) was developed to assess specific tea preparation methods and patterns of drinking.

## SUBJECTS & METHODS

### Study Population

A population-based case-control study (1993-1996) was conducted with cases of squamous cell carcinoma (SCC) of the skin randomly selected from persons identified through the Southeastern Arizona Skin Cancer Registry. Controls subjects were recruited through a random-digit dialing process to reflect the age and gender distribution of the cases. All subjects completed an extensive interview for demographic, behavioral, and past ultraviolet exposure information. A total of 404 cases and 391 control were initially interviewed. Sixty percent of these interviewed subjects (n=566) provided complete dietary data (four 24-hour dietary recalls) and constitute the population for this current study of tea consumption. These individuals were re-contacted by telephone between February 1998 – November 1998 and were asked to complete the tea consumption questionnaire.

### Tea Questionnaire

A detailed tea questionnaire (TQ) was developed after a series of focus groups identified usual tea drinking preparations and behaviours. This questionnaire asked about average tea intake over the past year, as well as a lifetime consumption pattern and any changes made. Detailed information was sought for the past year's tea intake during winter and summer by type of tea consumed (black, green or herbal and hot or iced). Information was also sought for use of regular or decaffeinated tea products and the usual brewing strength (weak, medium or strong). Usual or typical recipes for tea preparation were obtained, e.g. number of tea bags/cup and brewing time. This questionnaire was then evaluated for short (1 week) and long term (6 month) reliability within a randomly selected sample of men and women from the original case-control study who had not completed the 24-hour dietary recalls (n=40). The correlation coefficients between baseline and 6 month interviews were as follows: (1)  $r=0.93$  for tea consumption pattern (e.g. self-defined as non-tea drinker, occasional, or regular drinker); (2)  $r=0.95$  for usual frequency of iced black tea for summer consumption and  $r=0.98$  for winter consumption; and (3)  $r=0.97$  for frequency of hot black tea during summer and  $r=0.90$  for winter consumption

### Other Variables

All participants completed a structured interview detailing personal, behavioural, and demographic characteristics. The interview instrument sought information on: skin characteristics, sunburns and tanning history, use of suntan lotions and sunscreens, residential history, UV exposure during past year, family history of skin cancer, past medical history, tobacco and alcohol use, physical characteristics, and demographic information. All subjects also completed four 24-hour dietary recalls, which included 4 randomly selected days within two weeks of the clinic visit. Daily mean nutrient intakes were calculated with the use of the Minnesota Nutrition Data System (9).

### Quality Control

A trained, experienced interviewer conducted all interviews. After each interview, questionnaires were reviewed for completeness and coded. Data entry was through screen-based entry programs that included range checks.

### Tea analysis

Forty samples, representing various preparation techniques of hot, iced, and sun tea, were sent to Thomas J.Lipton (NJ) to be analysed by HPLC for catechins, theaflavins and gallic acid. Total polyphenols were measured using Folin Ciocalteu reagent. Thearubigins represent a fraction of complex catechin polymers and cannot be measured directly. Based on green tea analyses where 65% of total polyphenols are flavonoids, this proportion was used to determine thearubigin concentration in black tea by subtraction of catechins and theaflavins from total flavonoids. When tea cools, complexes form between caffeine and polyphenols, to precipitate as so called 'tea cream'. These complexes are measured in the total flavonoid fraction but not in the HPLC measurement of individual compounds. The tea samples were prepared and coded in Arizona and then sent frozen to Lipton Laboratory for analysis.

### Data Analyses

Tea consumption was defined by various methods. It was first assessed by asking participants to self-define themselves as non-tea drinkers, occasional drinkers, or regular tea drinkers. All occasional and regular tea drinkers were then asked to report their usual consumption of black tea, green tea, and herbal tea for summer and winter and for hot and iced tea products. Frequency of hot and iced black tea consumption and frequency of hot and iced green tea consumption were asked separately for summer and winter, from which combined consumption frequencies were calculated. Initial analyses compared occasional and regular tea drinkers to non-tea drinkers. Separate analyses were then conducted comparing drinkers of only hot tea with drinkers of only iced tea and with drinkers of both teas. Preparation techniques for hot and iced tea were reported and compared between males and females. Tea composition data were calculated based on standard low (0.48 g tea leaves per 100 ml water) and high (0.96g tea leaves per 100 ml water). Tea composition data for the Arizona tea-based beverages were calculated based on the mean level of each component in the different tea samples analysed in each category. All statistical analyses were performed by using STATA computer software (10).

## **RESULTS**

The study population is composed of an older educated Southwestern United States population with 64.4% of cases and 65.3% of controls having some college education. In this Arizona population, 66.4% reported drinking tea during the past year. Black tea was the predominant variety of tea consumed, with 51.8% of all subjects reporting iced black tea drinking and 30.7% reporting hot black tea use. Only 8.7% of this population reported drinking green tea. Overall, about 35% of women compared to 29% of men reported drinking non-herbal tea regularly (here defined as drinking tea at least once a week) and 27% of women and 38% of men reported no tea drinking in the past year. Only 6% of the study population reported drinking only decaffeinated tea. Frequency of tea consumption was not associated with smoking history or average daily alcohol intake. Exclusive iced tea consumption was slightly higher in men and current smokers (Table 1).

### Hot Black Tea

Strength of tea was defined by the question "How do you usually drink your tea", with the categories presented as "strong", "medium", or "weak". On average, 82% of tea drinkers defined their tea as of medium strength. Although women (11%) reported more strong hot tea consumption than men (4%), this difference was not statistically significant. Strength of the usual tea product was also evaluated using reported usual brewing time. The majority of subjects (68.5%) reported brewing tea for 2-3 minutes while 19.4% reported brewing their hot tea for more than 3 minutes.

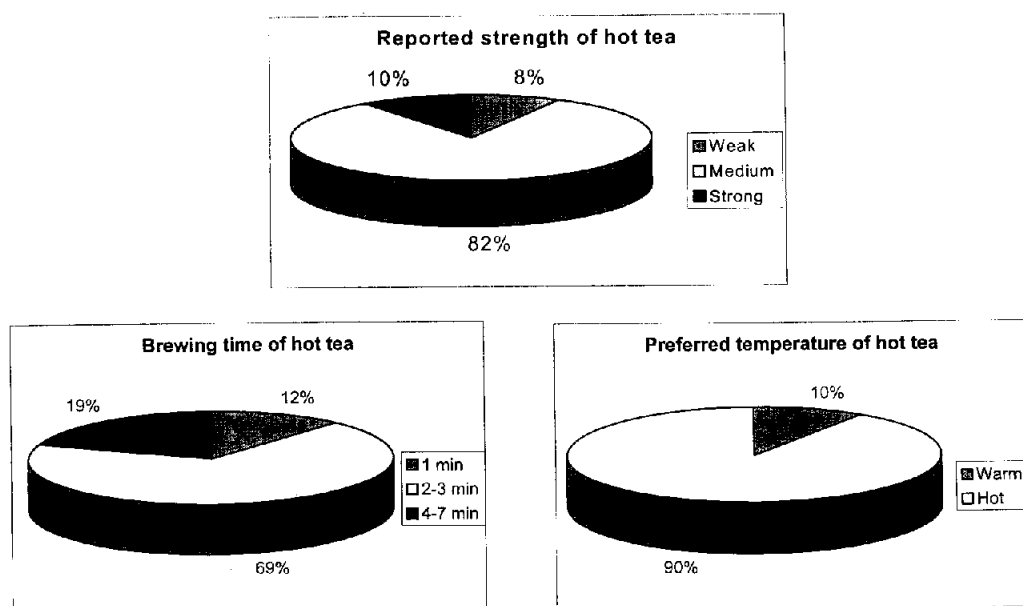
TABLE 1  
 Characteristics of the Population According to Pattern and Type of Tea Consumption.

Variable	Total n=450	Usual Tea Consumption <sup>a</sup>			Type of Black Tea Consumption		
		None n=151	Occasional n=158	Regular n=141	Exclusive hot tea n=66	Exclusive iced tea n=132	Both hot & iced tea n=87
Age (years) <sup>b</sup>	66.4	65.4	66.7	66.9	69.5	67.3	65.9
Male (%)	58.4	66.9	54.4	53.9	53.0	65.9	40.2
Smoking (%)							
Never	37.1	37.1	37.3	36.9	40.9	28.8	47.1
Former	49.3	48.3	50.0	49.6	50.0	56.1	41.4
Current	13.6	14.6	12.7	13.5	9.1	15.1	11.5
Alcohol (g/day) <sup>b</sup>	6.9	7.7	6.3	6.6	7.0	6.6	6.5
Energy (Kcal/day) <sup>b</sup>	1514.6	1576.5	1473.3	1494.4	1459.9	1481.1	1478.2
Calories from fat <sup>b</sup>	30.0	29.9	29.8	30.3	28.9	30.6	29.9

a Includes black and/or green tea

b Mean values

FIGURE 1  
 Characteristics of Hot Black Tea Beverages in Arizona

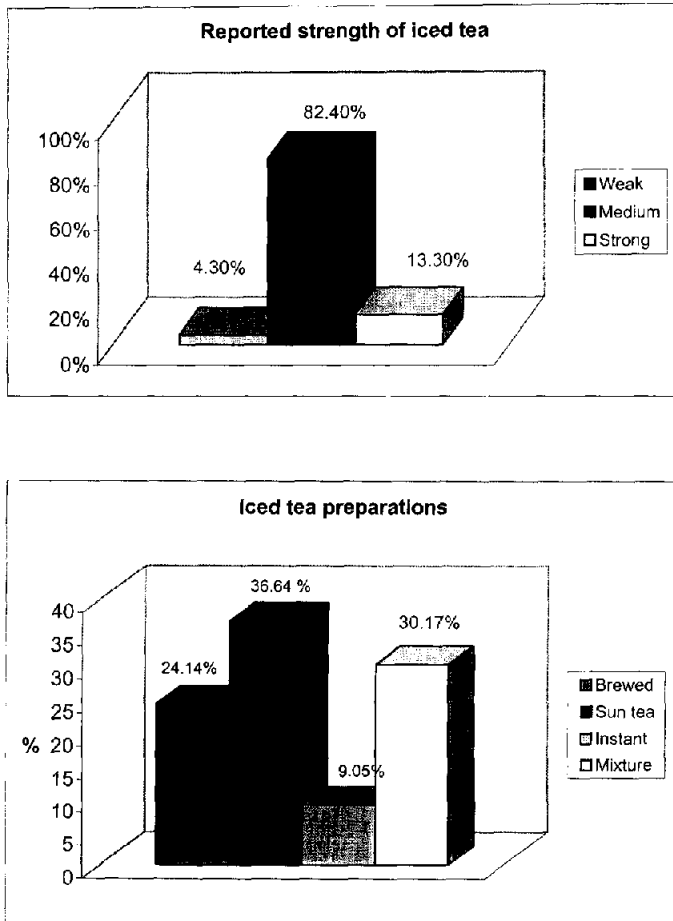


Again, we found no significant difference between males and females. Subjects were also asked to identify the preferred temperature of the hot tea consumed, with the categories as room temperature, warm, hot, or very hot. Almost all subjects reported drinking either hot (90.4%) or warm (9.6%) tea. There was a highly significant correlation between reported usual temperature of hot tea consumed and reported strength of hot tea ( $r = 0.94, p < 0.001$ ) as well as reported brewing time of hot tea ( $r = 0.45, p < 0.001$ ) (Figure 1).

Iced Black Tea

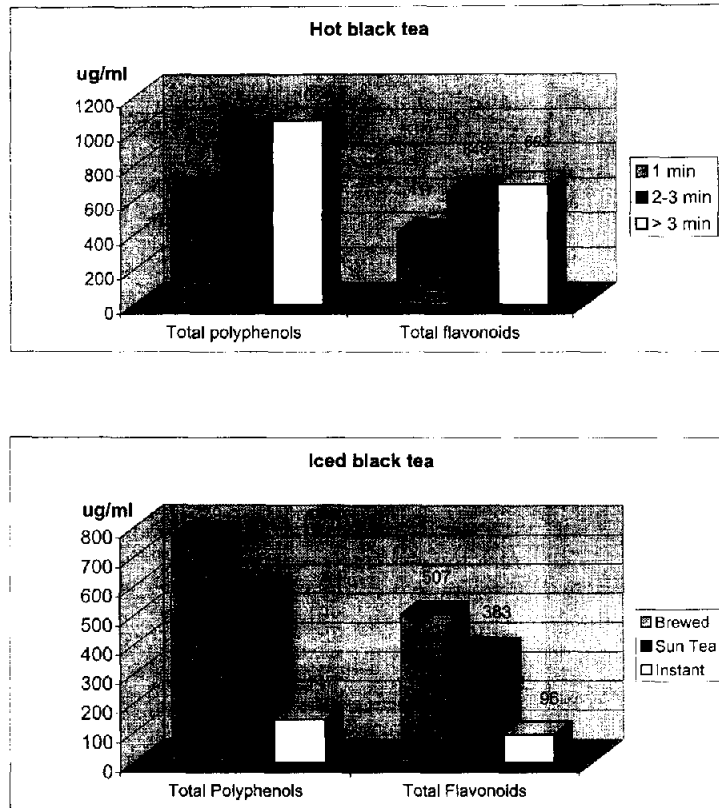
In Figure 2 we presented the reported strength of the iced tea consumed, as well as, the pattern of iced tea consumption by various preparation techniques (brewed iced tea only, sun tea only, instant iced tea only and a mixture of the above). Again, on average 83% of iced tea drinkers defined their tea as of medium strength. Iced tea was prepared in relatively larger amounts (e.g. 1/2 to 1 gallon) by either brewing in hot water, or using instant tea or as sun tea. Sun tea was

FIGURE 2  
Characteristics of Iced Black Tea Beverages in Arizona



prepared in large containers (e.g. 6 tea bags of 2.26-g each/ gallon water), and then leaving the tea bags to brew in the sun for approximately 6 hours. Our data show that more than third of our study population drank iced tea exclusively as sun tea (36.64%), while only 24.14% reported consuming only brewed iced tea. In Arizona, a third of the population reported consuming iced tea as brewed, or instant or as sun tea (mixture). We found a statistically significant difference ( $\chi^2=10.42$ ;  $P=0.015$ ) between males and females. Females (33.96%) tended to drink more brewed iced tea than males (15.87%).

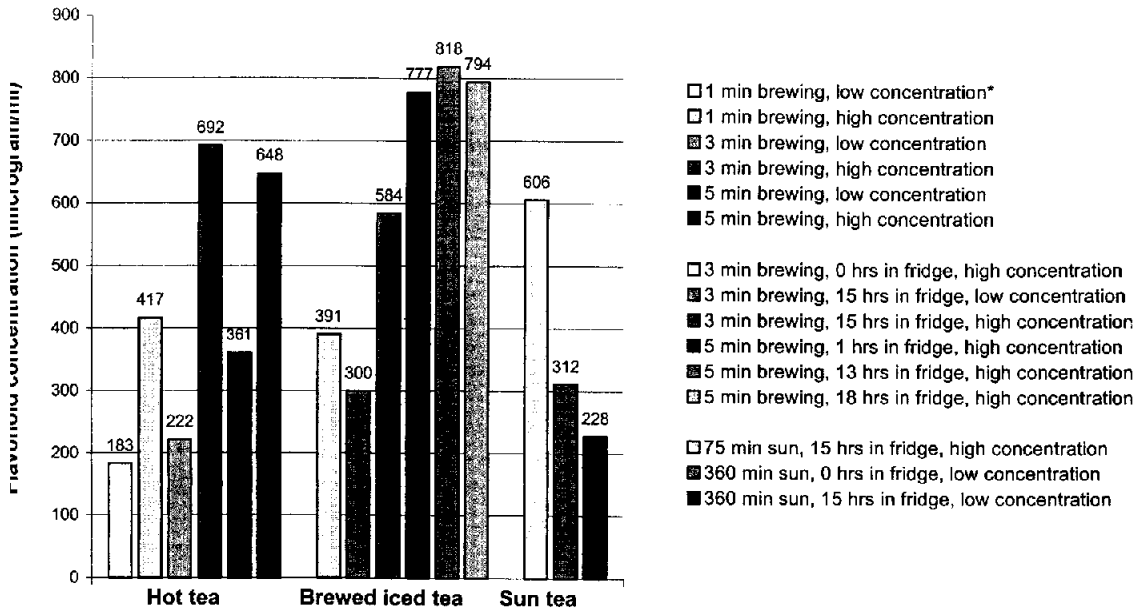
FIGURE 3  
Concentrations of Flavonoids and Polyphenols in Hot and Iced Black Tea Beverages



### Tea composition

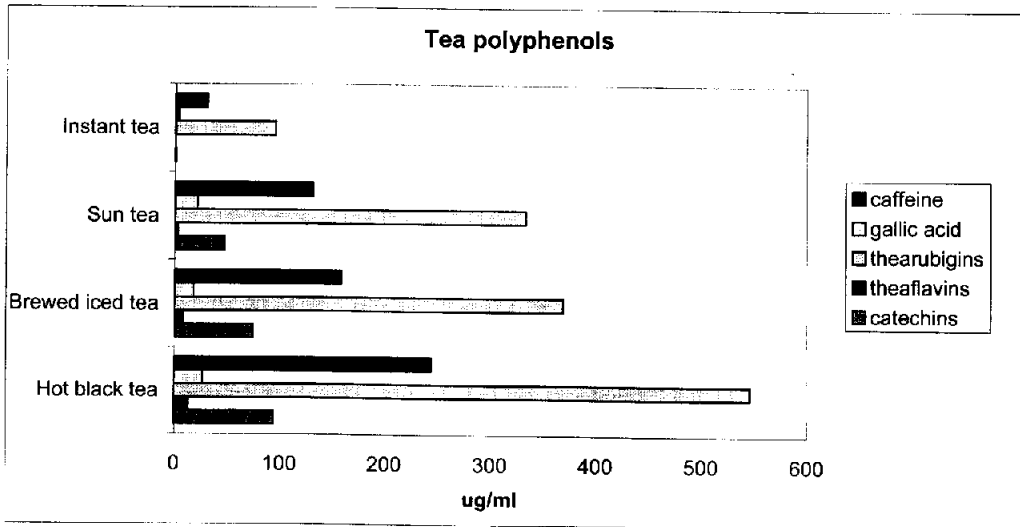
Tea leaf concentration, brewing time, and beverage temperature have major influences on flavonoid and polyphenols concentrations. Instant tea is considerably lower in flavonoids (91-100  $\mu\text{g/ml}$ ) (Figure 3). However, hot tea, brewed iced tea and sun tea at comparable leaf concentrations resulted in very similar flavonoid concentrations in the infusion (Figure 4). Theaflavins constituted 2% and < 1% of tea flavonoids in hot and iced black tea beverages respectively. We were not able to detect theaflavins in any of the instant black tea samples (Figure 5).

FIGURE 4  
Flavonoid Concentrations in Black Tea Infusions Prepared with Similar Tea Leaf Concentrations (ug/ml)



Low concentrations are 0.48 g tea leaves / 100 ml for hot tea and 0.36 g tea leaves / 100 ml for iced tea (brewed and sun).  
High concentrations are 0.96 g tea leaves / 100 ml for both hot tea and iced tea.

FIGURE 5  
Composition of Commonly Consumed Black Tea Beverages



## DISCUSSION

Epidemiological studies suggest that tea may reduce the risk of cardiovascular diseases (11-15) and cancers (16-18). Differences in the types of tea consumed and tea drinking habits are likely to vary by populations and could contribute to the inconsistencies between studies. In this Arizona population, 66.4% reported drinking tea during the past year. Black tea was the predominant variety of tea consumed. Both black and green teas contain high levels of antioxidants, even more than most vegetables and fruits (19,20). The antioxidant activities of 2 cups of tea are equivalent to 4 apples or 7 glasses of orange juice or 20 glasses of apple juice (20). Since dry tea leaves are not consumed directly, brewing conditions may influence the final antioxidant capacity in the tea as consumed.

Almost all the subjects reported preparing their hot tea by brewing one tea bag per one cup (2.26 g /240 ml of hot water). Our data show that, while 82% of tea drinkers defined their tea as of medium strength, only 68.5% of them reported brewing tea for 2-3 minutes. On the contrary, while more than 19% of tea drinkers reported brewing their hot tea for more than 3 minutes, only 10% of them defined their tea as strong. We found that the correlation between the reported strength of the tea and actual brewing time was highly significant. Therefore, in epidemiological studies, it will be advisable to use brewing time as a surrogate for the strength of the tea beverage, and hence its flavonoid content.

It is difficult to state a definitive composition for iced tea beverage as, in Arizona, methods of beverage preparation varied greatly. This beverage was prepared by either cooling traditionally brewed tea or by prolonged steeping of tea (bags or leaves) in the sun (sun tea). Cold water-soluble instant tea was used exclusively by 9% of iced tea drinkers to prepare their iced tea. We found a statistically significant difference between males and females. Women reported drinking more brewed iced tea than men.

Tea can be an important source of antioxidant phytochemicals. However, the variation that exists in tea preparations will be translated to variation in antioxidant capacity. Hot tea, brewed iced tea and sun tea at comparable leaf concentrations result in very similar flavonoid concentrations in the infusion, while instant tea is considerably lower in flavonoid concentration. However, iced tea as a beverage is likely to be prepared more diluted than hot tea. The wide variability in preparation techniques of iced tea is translated into corresponding variability in flavonoid concentrations in the resulting tea infusions. Lack of specific information on the type of tea consumed (e.g., black or green), amount and duration of tea intake, and method of tea preparation (e.g. hot or iced, strong or weak) has limited all studies. Therefore, in future epidemiological studies, it is important to collect more specific information on the qualitative and quantitative aspects of tea consumption. The use of specific questions focusing on tea preparation and availability of quantitative estimates of tea flavonoids should enhance future epidemiological studies of the relationship between tea and chronic disease prevention

## ACKNOWLEDGEMENTS

This publication was made possible by Public Health Service Grant P01 CA27502. Its contents are solely the responsibility of the authors and do not necessarily represent the official views of the National Cancer Institute. This research was supported in part by a grant from Unilever Research Vlaardingen, UNC, PO Box 114, 3130 AC Vlaardingen, The Netherlands. The

authors would like to thank Dr. David Alberts, Director of the Division of Cancer Prevention and Control for his support, Steve Rodney for his assistance with data management, and Mary Lurie for her assistance with interviewing and data entry.

### REFERENCES

1. NCI, Chemoprevention Branch and Agent development Committee. Clinical development Plan: Tea Extracts. *J. Cell. Biochem* 1996; 26S:236-257.
2. Carper J. *The food Pharmacy*. Bantan Books, New York 1989; pp 289-296.
3. Cheng S, Ding L, Zhen Y, Lin P, Zhu Y, Chen Y, & Hu X. Progress in studies on the antimutagenicity and anticarcinogenicity of green tea epicatechins. [Review] *Chinese Medical Sciences Journal* 1991; 6(4):233-8.
4. Katiyar SK, Agarwal R and Wang ZY. (-)-Epigallocatechin-3-gallate in camellia sinensis leaves from Himalayan region of bikkim inhibitory effects against biochemical events and tumor initiation in sencar mouse skin. *Nutr. Cancer* 1992; 18(1):73-83.
5. Xu G, Song PJ and Reed PI. Effects of fruit juices, processed vegetable juice, orange peel and green tea on endogenous formation of N-nitrosoproline in subjects from a high-risk area for gastric cancer in Moping County, China. *European Journal of Cancer Prevention* 1993; 2(4):327-35.
6. Sigler K and Ruch RJ. Enhancement of gap junctional intercellular communication in tumor promoter-treated cells by components of green tea. *Cancer Lett* 1993. 69:15-19.
7. Zhao Y, Cao J, Ma H and Liu J. (1997). Apoptosis induced by tea polyphenols in HL-60 cells. *Cancer Lett.*; 121:163-167.
8. Okai Y and Okai KH. Potent suppressing activity of the non-polyphenolic fraction of green tea (*Camellia sinensis*) against genotoxin-induced umu C gene expression in *Salmonella typhimurium* (TA 1535/pSK 1002)-association with pheophytins a and b. *Cancer lett* 1997; 120:117-123
9. Nutrition Data System, (NDS) version 2.9; Nutrition Coordinating Center, University of Minnesota, 1997.
10. Stata Corp. Stata statistical software, intercooled stata, release 6.0, College Station, TX: Stata Corporation, 1999.
11. Sesso HD, Gaziano JM, Buring JE, and Hennekens CH: Coffee and tea intake and the risk of myocardial infarction. *Am J Epidemiol* 1999; 149: 162-7.
12. Hertog GLM, Feskens EJM, Hollman PCH, Katan MB, Kromhout D: Dietary antioxidant flavonoids and risk of coronary heart disease: the Zutphen Elderly Study. *Lancet* 1993; 342: 1007-11.
13. Keli SO, Hertog MGL, Feskens EJM, Kromhout D: Dietary flavonoids antioxidant vitamins, and incidence of stroke. *Arch Intern Med* 1996; 154:637-42.

14. Stensvold I, Tverdal A, Solvoll K, Foss OP: Tea consumption: Relationship to cholesterol, blood pressure and coronary and total mortality. *Prev. Med.* 1992; 21: 546-53.
15. Yochum L, Kushi LH, Meyer K, Folsom AR: Dietary flavonoid intake and risk of cardiovascular disease in postmenopausal women. *Am J Epidemiol* 1999; 149: 943-9.
16. Jain M.G., Hislop, G.T., Howe, G.R., Burch, J.D. and Ghadirian, P. Alcohol and other beverage use and prostate cancer risk among Canadian men. *Int J Cancer* 1998; 78:707-711.
17. Zheng W., Doyle, TJ, Kushi, L.H., Sellers, T.A., Hong, C.P, and Folsom, A.R. Tea consumption and cancer incidence in a prospective cohort study of postmenopausal women. *Am. J. Epidemiol* 1996; 144(2):175-82.
18. Mendilaharsu, M., De Stefani, E., Deneo-Pellegrini, H., Carzoglio, J.C., and Ronco, A. Consumption of tea and coffee and the risk of lung cancer in cigarette-smoking men: a case-control study in Uruguay. *Lung Cancer* 1998; 19(2):101-7.
19. Prior, R.L., and Cao, G. Antioxidant capacity and polyphenolic components of teas: implications for altering in vivo antioxidant status. *Proceedings of the Society for Experimental Biology and Medicine* 1999; 220(4):255-61.
20. Paganga, G., Miller, N., and Rice-Evans, C.A. The polyphenolic content of fruit and vegetables and their antioxidant activities. What does a serving constitute? *Free Radical Research* 1999; 30(2):153-62.

Accepted for publication August 24, 2000.