Intake of Flavonoids and Lung Cancer

Loïc Le Marchand, Suzanne P. Murphy, Jean H. Hankin, Lynne R. Wilkens, Laurence N. Kolonel

Background: **To investigate the possible relationship between intake of flavonoids—powerful dietary antioxidants that may also inhibit P450 enzymes—and lung cancer risk, we conducted a population-based, case–control study in Hawaii.** ***Methods:*** **An in-person interview assessed smoking history and usual intake of 242 food items for 582 patients with incident lung cancer and 582 age-, sex-, and ethnicity-matched control subjects. Subjects who donated a blood sample were genotyped for the P450 enzyme variant allele CYP1A1*2 by use of a polymerase chain reaction-based method. Logistic regression analysis was used to compute odds ratios (ORs) and 95% confidence intervals (CIs). All *P* values are two-sided.** ***Results:*** **After adjusting for smoking and intakes of saturated fat and β-carotene, we found statistically significant inverse associations between lung cancer risk and the main food sources of the flavonoids quercetin (onions and apples) and naringin (white grapefruit). The lung cancer OR for the highest compared with the lowest quartile of intake was 0.5 (95% CI = 0.3–0.9) for onions (*P* for trend = .001) and 0.6 (95% CI = 0.4–1.0) for apples (*P* for trend = .03). The OR for the highest compared with the lowest tertile of intake for white grapefruit was 0.5 (95% CI = 0.2–0.9) (*P* for trend = .02). No association was found for important food sources of other flavonoids. Using published food-composition data for flavonoids, we found an inverse association between intake of quercetin and risk of lung cancer (*P* for trend = .07) that appears consistent with associations for its food sources. The effect of onions was particularly strong against squamous cell carcinoma (a cell type specifically associated with CYP1A1*2 in our study) and was modified by the CYP1A1 genotype, suggesting that CYP1A1 may play a role in this association.** ***Conclusion:*** **If replicated, particularly in prospective studies, these findings would suggest that foods rich in certain flavonoids may protect against certain forms of lung cancer and that decreased bioactivation of carcinogens by inhibition of CYP1A1 should be explored as underlying mechanisms. [J Natl Cancer Inst 2000; 92:154–60]**

Intake of vegetables and fruits has consistently been associated with a reduced risk of lung cancer *(1).* Although many constituents of these foods have shown anticarcinogenic activity in the laboratory, only a few have been widely studied in humans. Because β-carotene had been inversely associated with lung cancer in various populations *(1),* this nutrient was selected early on for chemoprevention trials among individuals at high risk (smokers and asbestos-exposed workers) or low risk (U.S. physicians) for lung cancer *(2–5).* The results of these large trials have been disappointing because they showed no beneficial effect *(3),* and even—in high-risk individuals—a detrimental effect on lung cancer incidence *(4,5).* α-Tocopherol supplementation also failed to prevent lung cancer in the Alpha-Tocopherol, Beta-Carotene Cancer Prevention Trial *(4).*

It has been stressed for some time that the association with β-carotene in observational studies is difficult to distinguish from that of vegetables and fruits and that, indeed, similar associations could be demonstrated between lung cancer and other plant constituents. For example, in a case–control study in Hawaii, we found inverse associations with total intake of vegetables and subgroups of vegetables rich in other phytochemicals (dark-green vegetables, cruciferous vegetables, tomatoes, and carrots) that were stronger than those for β-carotene *(6)* and other specific carotenoids *(7).* Recent literature reviews and more recent studies of diet and lung cancer *(8–12)* have further emphasized the need to consider various components of fruits and vegetables as risk factors for this disease.

Flavonoids are polyphenolic compounds that occur ubiquitously in foods and beverages of plant origin *(13).* They are strong antioxidants by efficiently scavenging free radicals *(14–16)* that are thought to be involved in DNA damage and tumor promotion *(17).* Quercetin and other related flavonoids inhibit carcinogen-induced tumors in rodents *(18,19).* One of the possible mechanisms for this protective effect is the well-documented ability of some flavonoids, such as quercetin and naringenin (the aglycone derived from naringin), to inhibit certain cytochrome P450 enzymes (CYP1A1 and CYP3A4, respectively) involved in the bioactivation of chemical carcinogens *(20).*

We report here on the associations between intake of flavonoids and lung cancer risk in a population-based, case–control study conducted in Hawaii. Because only limited food-composition data are yet available for flavonoids, we made an *a priori* decision to give greater emphasis to food sources in the interpretation of the data. We also examined the modifying effect of a common polymorphism in the CYP1A1 gene on these associations in an effort to identify underlying mechanisms.

METHODS

Study Population

The human subjects protocol for this study was reviewed and approved by the Committee on Human Studies of the University of Hawaii and by the institutional review board of each participating hospital. Lung cancer patients were identified by the rapid-reporting system of the Hawaii Tumor Registry, a member of the Surveillance, Epidemiology, and End Results (SEER)[1] Program of the National Cancer Institute. Eligible case patients were all patients with histologically confirmed primary lung cancer who were newly diagnosed during the period from January 1, 1992, through March 31, 1997, in all main medical centers on the island of Oahu, HI. Other eligibility criteria included age between 26 and 79 years, Oahu residency, no history of lung cancer, and appropriate ethnicity (at least 75% ethnic Japanese, at least 75% Caucasian of European descent, or any percentage Hawaiian/part-Hawaiian heritage). Histologic information was abstracted directly from each patient's pathology report. An interview was completed for 64% (375 men and 207 women) of the eligible case patients. The main reasons for nonparticipation were patient refusal (17%), physician refusal (2%), and death with absence of a suitable surrogate for interview (17%). Interviewed case patients were more likely to be Hawaiian (25% versus 19%), were less likely to have a distant metastasis (37% versus 50%), and were younger by an average of 1 year compared with noninterviewed cases.

Affiliation of authors: Etiology Program, Cancer Research Center of Hawaii, University of Hawaii, Honolulu.

Correspondence to: Loïc Le Marchand, M.D., Ph.D., Etiology Program, Cancer Research Center of Hawaii, University of Hawaii, 1236 Lauhala St., Suite 407, Honolulu, HI 96813 (e-mail: loic@crch.hawaii.edu).

See "Notes" following "References."

Control subjects were selected randomly from a list of Oahu residents interviewed by the State of Hawaii Department of Health as part of a health survey of a 2% random sample of state households. This source was supplemented with control subjects from Health Care Finance Administration participants on Oahu. One control subject was matched to each case patient on the basis of sex, ethnicity (within the three categories defined above), and age (±2 years). The overall participation rate for the control subjects was 62%. Reasons for nonparticipation included refusal (25%), inability to locate (10%), serious illness (1%), and death (2%). Compared with noninterviewed control subjects, interviewed control subjects were similar in their sex and race distribution but were slightly younger (mean age: 65.5 years versus 66.5 years). When possible, a proxy interview was obtained from the next of kin for subjects who were deceased or too ill (28.5% of case patients and 2.9% of control subjects). A blood sample was collected from 76% of interviewed case patients and from 80% of interviewed control subjects. There were no differences in the age, sex, and race distributions of control subjects who gave blood compared with those who did not. However, case patients who gave blood were younger by an average of 1 year and were less likely to have a distant metastasis than those who declined the blood donation.

Trained interviewers conducted in-person interviews at the subjects' homes. Fifty percent of case patients were interviewed within 3.5 months, and 88% were interviewed within 6.0 months of diagnosis. Written informed consent was obtained from each participant. The questionnaire included detailed demographic information, such as ethnic origin of each grandparent, a lifetime history of tobacco and alcohol use, a quantitative food-frequency questionnaire, and a personal history of exposure to environmental tobacco smoke, various relevant medical conditions and occupational exposures, and a family history of lung diseases. Information was collected on the types (nonfiltered cigarettes, filtered cigarettes, cigars, and pipes) of tobacco products ever smoked daily for at least 6 months and, for each tobacco product, the usual amount smoked per day, the age when smoking started, the overall duration of use, and, for ex-smokers, the age when smoking stopped. We also inquired about any periods of smoking cessation for each tobacco product during the subject's life. Smokers were considered current smokers if they smoked up to 1 year before the date of diagnosis for case patients or up to the date of interview for control subjects. In the analysis, cigarettes, pipes, and cigars were treated equivalently.

Food-Frequency Analysis

The food-frequency questionnaire used in this study *(21)* has been validated previously in our population and was designed to capture total dietary intake *(22)*. Frequencies and amounts consumed were sought for 242 food items or categories. The reference period for the dietary questionnaire was the year before diagnosis for case patients or before interview for control subjects. If a change had occurred in the types and amounts of foods consumed in the 3 years preceding diagnosis (for case patients) or interview (for control subjects), the reference period was the year before the change. For seasonal foods, the reference period was limited to the time period during which each food was available. Colored photographs of most food items, showing three different portion sizes, as well as measuring cups and spoons, were used in the interview to facilitate quantification of intakes.

Daily intake in grams for each food item was computed for each individual. A food-composition nutrient database was applied to the items to assess nutrient intake. Individual nutrient intakes were calculated by summing across all food items. Gram amounts of particular food groups of interest were calculated by summing the grams for the foods or components of mixed dishes within that food category. The proportion of each mixed dish represented by a particular food was estimated from standard recipes. The food-composition data were based primarily on the U.S. Department of Agriculture's (Washington, DC) nutrient database *(23)* and were supplemented with data from other research and commercial publications. Values for three flavonols (quercetin, kaempferol, and myricetin) and two flavanone glycosides (hesperidin and naringin) for the foods on the diet questionnaire were obtained from the literature *(24–34)*. If analytic data were unavailable for a food item, values were imputed from similar foods.

CYP1A1 Genotyping

Subjects were genotyped for a T→C substitution at the 3′ end of the CYP1A1 gene (CYP1A1*2 allele) that creates a *Msp*I restriction site. This polymorphism is in linkage disequilibrium with a A→G transition in exon 7 that results in an Ile–Val change in the heme-binding domain of the protein and greater enzymatic activity *(35)*. Laboratory personnel were blinded to the case–control status of the subjects. DNA extraction from blood lymphocytes and genotyping of subjects for the CYP1A1*2 allele were performed as described elsewhere *(36)*.

Statistical Methods

In the statistical analysis, we used conditional logistic regression to compute odds ratios (ORs) and 95% confidence intervals (CIs) for quartiles or tertiles of daily nutrient and food intakes, with adjustment for relevant covariates *(37)*. All reported *P* values are two-sided and considered to be statistically significant when less than .05. Linear dose–response was tested by entering in the model a trend variable assigned the median intake level for the quantile. Because the ORs from sex-specific analyses were very comparable, only the results for both sexes combined are presented to save space. The matched-pair design was not followed in analyses that included genotyping results because often only one member of the pair donated a blood sample. For those analyses, unconditional logistic regression was used, with further adjustment for the matching variables (age, sex, and ethnicity). Nutrient intakes were adjusted for caloric intake by use of the method of residuals *(38)*. Several ways of modeling the smoking effect were explored and included separate categorization for duration and amount, use of a "pack-years" and an "age-started" term, logarithmic transformations of the variables, and addition of higher polynomial terms. The best fitting model was one that included an indicator variable for smoking status (ever smoked or never smoked) and separate continuous terms for duration, amount, and (duration)^2. The log-likelihood ratio test was used to test the statistical significance of modeled effects. We also used this test to determine interactions among certain variables with respect to lung cancer risk. The test compared main effects, no interaction model with a fully parameterized model containing all possible interaction terms for the variables of interest.

RESULTS

Thirty-eight percent of the subjects were Caucasians, 37% were ethnic Japanese, and 25% were Hawaiians or part-Hawaiians. Table 1 presents relevant background characteristics of the lung cancer case patients and population control subjects. As expected, smoking was strongly associated with lung cancer risk. In addition and in agreement with past studies in this population *(6,39)*, case patients were less educated and consumed more saturated fat and less β-carotene and vegetables.

Table 2, A, shows the ORs and 95%

Table 1. Distribution of lung cancer case patients and control subjects by selected characteristics

	Male		Female	
Characteristic	Case patient (n = 375)	Control subject (n = 375)	Case patient (n = 207)	Control subject (n = 207)
Age, mean, y	65.5	65.4	66.0	65.6
Education, mean, y	12.3	13.6	12.2	13.3
Smoking status, %				
Never smoker	2.4	30.4	14.5	52.7
Ex-smoker	51.2	54.9	39.1	32.8
Current smoker	46.4	14.7	46.4	14.5
Pack-years, mean	64.9	26.9	42.7	13.9
Calories, mean, kcal/day	2967	2723	2221	2064
% calories from fat, mean	32.8	31.4	31.8	29.9
β-Carotene, mean, mg/day	3.8	5.7	5.4	6.3
Total vegetables, mean, g/day	277	346	270	311

Table 2. Odds ratios* (95% confidence intervals) for lung cancer by quantile

	Odds ratio (95% confidence interval) by				Two-sided *P* for trend†
	Q_1 (low)	Q_2	Q_3	Q_4 (high)	
A) Intake of flavonoid-rich foods‡					
Onions	1.0 (referent)	1.4 (0.9–2.3)	0.9 (0.5–1.4)	0.5 (0.3–0.9)	.001
Broccoli	1.0 (referent)	1.0 (0.6–1.6)	0.8 (0.5–1.3)	0.9 (0.5–1.4)	.48
Celery	1.0 (referent)	0.7 (0.4–1.1)	0.8 (0.5–1.4)	0.8 (0.5–1.3)	.70
Soy products	1.0 (referent)	1.6 (1.0–2.7)	1.2 (0.7–2.2)	1.0 (0.5–1.8)	.28
Apples	1.0 (referent)	0.9 (0.6–1.4)	1.0 (0.6–1.6)	0.6 (0.4–1.0)	.03
White grapefruit	1.0 (referent)	0.8 (0.4–1.6)	0.5 (0.2–0.9)		.02
Pink grapefruit	1.0 (referent)	0.7 (0.5–1.2)	0.9 (0.6–1.6)		.91
Other citrus fruits	1.0 (referent)	0.9 (0.5–1.4)	0.9 (0.5–1.4)	0.9 (0.5–1.4)	.74
Red wine	1.0 (referent)	0.8 (0.4–1.8)	0.7 (0.4–1.2)		.20
White wine	1.0 (referent)	0.8 (0.4–1.4)	1.0 (0.6–1.7)		.96
Black tea	1.0 (referent)	1.5 (0.8–2.6)	1.1 (0.7–1.8)	1.1 (0.7–1.8)	.83
Green tea	1.0 (referent)	1.0 (0.6–1.7)	0.7 (0.4–1.3)	0.9 (0.5–1.6)	.62
B) Flavonoid intake§					
Quercetin	1.0 (referent)	0.9 (0.6–1.5)	0.7 (0.4–1.1)	0.7 (0.4–1.1)	.07
Kaempferol	1.0 (referent)	1.1 (0.6–1.7)	0.9 (0.6–1.5)	0.9 (0.5–1.4)	.41
Myricetin	1.0 (referent)	1.7 (1.0–2.7)	1.5 (0.9–2.4)	1.0 (0.6–1.6)	.42
Hesperidin	1.0 (referent)	1.1 (0.7–1.8)	1.2 (0.7–1.9)	1.2 (0.7–2.0)	.54
Naringin	1.0 (referent)	0.7 (0.4–1.2)	0.7 (0.5–1.1)		.17
Total flavonoids‖	1.0 (referent)	0.8 (0.5–1.4)	1.3 (0.8–2.1)	0.8 (0.5–1.4)	.89

*Adjusted by matching for age, sex, and ethnicity and for the following covariates: smoking status, duration, $(\text{duration})^2$, number of cigarettes smoked per day, and intakes of β-carotene and saturated fat. These were 582 case–control pairs.

†*See* "Statistical Methods" section for further details.

‡Tertiles of intake were used for grapefruit, red wine, and white wine. The interquartile ranges (25th–75th percentile) for food intake (g/day) were as follows: onions, 7.5–20.1; broccoli, 5.6–32.0; celery, 2.4–8.0; soy products, 2.0–34.2; apples, 2.3–49.7; other citrus fruits, 18.4–50.0; black tea, 0.0–171.1; and green tea, 0.0–171.1. The intertertile ranges (33rd–67th percentile) for food intake (g/day) were as follows: white grapefruit, 0.0–9.9; pink grapefruit, 0.0–9.9; red wine, 0.0–1.0; and white wine, 0.0–1.0. Soy products include tofu and soybeans eaten alone or in soups and mixed dishes.

§The interquartile ranges (25th–75th percentile) for flavonoid intake (mg/day) were as follows: quercetin, 8.8–16.7; kaempferol, 1.7–5.9; myricetin, 0.4–1.8; and hesperidin, 3.7–38.7. The intertertile range (33rd–67th percentile) for naringin intake (mg/day) was 0.0–2.0.

‖Sum of the five flavonoids above. The interquartile range for total flavonoid intake was 23.5–68.9 mg/day.

CIs for lung cancer by increasing intake of the main food sources of flavonoids, after adjustment for smoking and β-carotene and saturated fat intakes. Statistically significant inverse associations were found for onions, apples, and white grapefruit, with a 40%–50% decreased risk in the highest compared with the lowest category of intake. No clear association was observed for pink grapefruit, other citrus fruits, broccoli, celery, soy products, red wine, white wine, black tea, and green tea. Similarly, beer, hard liquor, and ethanol intakes were not associated with lung cancer risk in our data. Onions, apples, black tea, green tea, and red wine are important dietary sources of quercetin, whereas white grapefruit is a rich source of naringin. Because onions are also rich in potentially protective diallyl sulfides, we also estimated the lung cancer risk associated with intake of garlic, another allium vegetable. The OR by increasing quartile of garlic intake was 1.0 (referent), 0.9 (95% CI = 0.6–1.4), 0.8 (95% CI = 0.5–1.2), and 0.7 (95% CI = 0.4–1.1), and the *P* value for the trend test was .12. Given this weak association with garlic, we interpreted the effect for onions as being related more to the intake of quercetin than to the intake of diallyl sulfides.

The ORs for lung cancer by increasing intake of specific flavonols and flavanones are shown in Table 2, B. Consistent with the associations in Table 2, A, we found a suggestion of an association with quercetin (*P* for trend = .07). Although not statistically significant (*P* for trend = .17), the risk pattern for naringin was also consistent with the inverse association observed with its main food source. There was no association with intake of the other flavonoids (kaempferol, myricetin, and hesperidin) and with the sum of the five flavonoids.

To examine the homogeneity of the results, we stratified our analysis of foods by ethnicity. With the single exception of apples in Hawaiians, the inverse associations with onions, apples, and white grapefruit were observed in all ethnic groups (data not shown). An analysis stratified on pack-years (greater than or, at most, the median) showed that these inverse associations were suggested at both low and high levels of smoking but were somewhat stronger for those who smoked heavily (data not shown).

Table 3 presents risk estimates for the main cell types of lung cancer by intake levels of onions, apples, white grapefruit, quercetin, and naringin. The association with onions was clearly weaker for adenocarcinoma than for squamous cell carcinoma or the other cell types combined. The OR for adenocarcinoma for the highest compared with the lowest quartile of onion intake was 0.6 (95% CI = 0.3–1.2) (*P* for trend = .24). The corresponding OR for squamous cell carcinoma was 0.1 (95% CI = 0.0–0.6) (*P* for trend = .003). For the other foods and both flavonoids, the reduction in risk in the highest quantile was also greater for squamous cell carcinoma than for adenocarcinoma, although none of the ORs or trends was statistically significant (Table 3).

We tested the modifying effect of the CYP1A1 *Msp*I polymorphism on the association between onions and lung cancer (Table 4). When we cross-classified the subset of subjects who donated a blood sample by their CYP1A1 genotype and onion intake, we found that the protective effect of onions against squamous cell carcinoma was about twofold greater in individuals with the homozygous wild-type genotype (CYP1A1*1/*1) than in those carrying the CYP1A1*2 variant allele, which suggests that the effect of onions was stronger for the low enzymatic activity (wild-type) genotype. Despite our limited sample size in this subgroup (72 case patients and 453 control subjects), the *P* for interaction was of borderline significance (*P* = .07). No interaction was found between onions and CYP1A1*2 for all cell types combined or adenocarcinoma or between this food and other polymorphisms (GSTM1 and CYP2E1) found to be associated with lung cancer in this study *(36)*. Our sample size and the lower consumption of apples and white grapefruit in our population did not allow for similar interaction analyses.

DISCUSSION

The flavonol quercetin is quantitatively one of the most important flavo-

Table 3. Odds ratios* (95% confidence intervals) for lung cancer cell types by quantile of intake of onions, apples, white grapefruit, quercetin, and naringin

Cell type†	Odds ratio (95% confidence interval) by				Two-sided *P* for trend‡
	Q_1 (low)	Q_2	Q_3	Q_4 (high)	
Onions					
SCC	1.0 (referent)	1.6 (0.4–5.6)	0.7 (0.2–2.8)	0.1 (0.0–0.6)	.003
ADC	1.0 (referent)	1.0 (0.5–2.0)	0.7 (0.4–1.5)	0.6 (0.3–1.2)	.24
Other	1.0 (referent)	1.0 (0.4–3.0)	0.8 (0.3–2.2)	0.3 (0.1–0.7)	.005
Apples					
SCC	1.0 (referent)	0.6 (0.2–1.8)	0.6 (0.2–1.9)	0.5 (0.2–1.8)	.42
ADC	1.0 (referent)	0.8 (0.4–1.5)	1.2 (0.6–2.4)	0.7 (0.3–1.3)	.23
Other	1.0 (referent)	1.0 (0.5–2.1)	0.5 (0.2–1.1)	0.6 (0.3–1.4)	.26
White grapefruit					
SCC	1.0 (referent)	0.2 (0.0–2.0)	0.3 (0.1–1.6)		.16
ADC	1.0 (referent)	1.4 (0.6–3.5)	0.6 (0.3–1.5)		.39
Other	1.0 (referent)	0.2 (0.0–1.2)	0.3 (0.1–1.5)		.09
Quercetin					
SCC	1.0 (referent)	0.9 (0.3–3.1)	1.6 (0.4–5.4)	0.5 (0.2–1.9)	.44
ADC	1.0 (referent)	1.2 (0.6–2.2)	0.7 (0.4–1.4)	0.9 (0.4–2.0)	.65
Other	1.0 (referent)	0.8 (0.4–1.8)	0.8 (0.4–1.7)	0.5 (0.2–1.2)	.13
Naringin					
SCC	1.0 (referent)	0.2 (0.1–1.1)	0.2 (0.1–1.0)		.06
ADC	1.0 (referent)	0.9 (0.5–1.7)	1.4 (0.7–2.5)		.32
Other	1.0 (referent)	0.7 (0.2–1.8)	0.3 (0.1–0.9)		.03

*Adjusted by matching for age, sex, and ethnicity and for the following covariates: smoking status, duration, (duration)^2, number of cigarettes smoked per day, and β-carotene and saturated fat intakes.

†SCC = squamous cell carcinoma, 136 case–control pairs; ADC = adenocarcinoma, 253 pairs; other = 193 pairs.

‡*See* "Statistical Methods" section for further details.

Table 4. Odds ratios* for lung squamous cell carcinoma by CYP1A1 genotype and onion intake

Onion intake	CYP1A1	n†	Odds ratio (95% confidence interval)
≤median	*1/*1	22/124	1.0 (referent)
>median	*1/*1	6/126	0.2 (0.1–0.5)
≤median	*1/*2 or *2/*2	24/86	1.7 (0.7–4.0)
>median	*1/*2 or *2/*2	20/117	1.0 (0.4–2.4)
Two-sided *P* for interaction‡ = .07			

*Adjusted for age, sex, ethnicity, smoking status, duration, (duration)^2, number of cigarettes smoked per day, and β-carotene and saturated fat intakes.

†No. of case patients/No. of control subjects.

‡*See* "Statistical Methods" section for further details.

noids in the diet *(20)*. Previous attempts to estimate average daily intake of the flavonoids quercetin, kaempferol, myricetin, apigenin, and luteolin yielded total intakes varying between 2.6 mg in Finland and 68.2 mg in Japan *(40)*. Daily quercetin intake varied from 2.6 mg in Finland to 38.2 mg in Croatia *(40)*. A study in the United States reported an average intake of 20.1 mg/day for the same five flavonoids and 15.4 mg/day for quercetin *(41)*. Although two of the five flavonoids that we assessed were different, the mean intake estimates for flavonoids (59 mg/day) and quercetin (15.3 mg/day) for our population were comparable to those for other populations and exceeded those of most other antioxidants, such as β-carotene and vitamin E *(25)*. We are not aware of published estimates of population intake for naringin.

The flavonol content of vegetables is known to vary by variety and growing conditions, by part of the food (except for onion), and by cooking methods *(42)*. Furthermore, the bioavailability of certain flavonoids is known to vary, depending on the food source. For example, the absorption of quercetin from onions is fourfold greater than that from apples or tea *(43,44)*. The food-composition data for flavonoids do not currently take these factors into account and, consequently, are likely to introduce additional error into the quantification of flavonoid intakes. Therefore, until these data are improved, it appears preferable to give greater emphasis to food sources of flavonoids than to specific flavonoids when investigating their associations with disease risk. Moreover, the inaccuracy in the food-composition values may explain why the results in this study were stronger for foods than for flavonoids.

In the present population-based, case–control study of lung cancer, inverse associations were found with intakes of onions, apples, and white grapefruit. Because onions and apples are excellent sources of quercetin and white grapefruit is an excellent source of naringin, the results suggest that quercetin and naringin may be protective against lung cancer. The lack of a significant association with red wine (another rich source of quercetin) may reflect the low wine consumption of our population. The stronger finding for white grapefruit compared with pink grapefruit may be because naringin is present in pink grapefruit in lesser amounts than in white grapefruit *(31)*. No associations were found with citrus fruits other than grapefruit (sources of hesperidin and tangeretin), black and green teas (which contain flavanols), celery (a good source of luteolin and apigenin), and soy products (an excellent source of isoflavonoids), suggesting that these other flavonoids may not confer the same protection against lung cancer. The findings for foods were supported by the finding for flavonoid intakes: An inverse association with lung cancer that approached statistical significance was observed for quercetin. Although not statistically significant, the risk estimates for naringin were also consistent with the reduction in risk seen for white grapefruit. The inverse associations with onions, apples, and white grapefruit were present in both sexes and in almost all ethnic groups and were stronger for squamous cell carcinoma. The association between onions and squamous cell carcinoma was modified by the CYP1A1 genotype.

Few past epidemiologic studies have examined the potential protective effect of flavonoids against lung or other cancers. A Dutch cohort study on elderly men *(45)* found an inverse association between flavonoids and cancers of the respiratory and alimentary tracts, which was limited to fruit and vegetable sources. A larger cohort study of 10 000 Finnish men and women followed for more than 20 years *(46)* also found a significant inverse association of flavonoid intake

(of which 95% was quercetin) with lung cancer. The main source of flavonoids (apples) in this Finnish population was also significantly inversely associated with risk *(46)*. Early results from another large cohort study in The Netherlands *(47)* were also suggestive of an inverse association with onion intake. However, the strength of this association was reduced after adjustment for smoking. Thus, overall, our results are consistent with the literature in suggesting that flavonoids from onions and apples may be inversely associated with lung cancer. We are not aware of previous reports on lung cancer and grapefruit.

Quercetin has been shown to inhibit carcinogen-induced tumors in rats and mice *(18,19)*. Experimental studies *(20)* have also shown that quercetin inhibits cytochrome P450 enzymes of the CYP1A family. We reasoned that, since CYP1A1 bioactivates polycyclic aromatic hydrocarbons (PAHs) and since a polymorphism in the CYP1A1 gene was associated with squamous cell carcinoma in our data *(36)*, an interaction between onions and this polymorphism would imply that this mechanism could be important in explaining the association with onions. Our finding of such an interaction (albeit of borderline statistical significance) suggests that inhibition of CYP1A1 by quercetin and onions and their effects on PAH activation should be investigated further in humans as a possible protective mechanism against squamous cell carcinoma of the lung. Similarly, naringenin has been shown in laboratory studies to inhibit CYP3A4, an enzyme involved in the metabolism of many xenobiotics, including PAHs *(36)*. In humans, grapefruit juice is well-known to increase the bioavailability of various drugs (e.g., nifedipine and cyclosporine) metabolized by CYP3A4 *(48)*. Thus, CYP3A4 inhibition may be a mechanism by which naringenin and grapefruit could protect against lung cancer.

Among the other flavonoids, flavanols, such as catechins from tea, have been extensively studied in animals. Green tea and black tea have been shown to inhibit lung tumors induced with 4-(methylnitrosoamino)-1-(3-pyridyl)-1-butanone (NNK) in mice *(49,50)*. However, in agreement with our data, past studies reporting on black tea *(45,51,52)* have found no association with lung cancer. We are not aware of any past studies on green tea and lung cancer. However, Shim et al. *(53)* reported that the frequency of sister chromatid exchange in mitogen-stimulated peripheral blood lymphocytes from smokers consuming green tea (two to three cups per day) was comparable to that of nonsmokers and significantly lower than that of smokers who did not drink green tea. In the present study, only ethnic Japanese consumed green tea in substantial amounts (average: 124 g/day compared with 12 g/day for Caucasians). Nevertheless, no association was found with lung cancer in this ethnic group (ORs for increasing quartiles: 1.0, 1.0, 0.9, 0.9; P for trend = .76) or in the total sample (Table 2, A).

Experimental studies have suggested that citrus fruit flavonoids may also be protective against lung cancer. Orange and lemon oils included in the diet of mice resulted in a reduction of more than 80% in the yield of NNK-induced lung tumors *(54)*. In contrast, neither citrus fruits (other than grapefruit) nor hesperidin was associated with risk in our data. Koo *(55)* found an inverse association between soy-product consumption (the main source of isoflavonoids in the diet) and risk of lung cancer among Chinese women who never smoked (OR = 0.3; 95% CI = 0.1–1.1). In this study, we found no association between soy consumption and lung cancer risk, despite soy-consumption levels that are relatively high in ethnic Japanese and Hawaiians in Hawaii (average, 43 g/day and 33 g/day, respectively, in this study).

Much of the interest in the potential chemopreventive effect of flavonoids comes from their antioxidant activity. Flavonoids, like other phenolic antioxidants, eliminate free radicals by donating one electron while remaining chemically unreactive. Since an inverse association was found only for a subset of the flavonoids considered in this study, our data do not suggest a major role for the overall antioxidant effect of flavonoids in lung cancer.

Limitations of this study that need to be considered include possible residual confounding by smoking. We collected detailed information on smoking and gave great care to the way in which we adjusted our analysis for this variable so that residual confounding should have been minimized. To investigate a possible bias from our use of surrogate interviews, we repeated the analyses excluding surrogate respondents. We found very similar results in this subset analysis. In particular, the ORs for increasing quartiles or tertiles of intake were 1.0 (referent), 1.4 (95% CI = 0.8–2.5), 0.9 (95% CI = 0.5–1.6), and 0.4 (95% CI = 0.2–0.8) for onions (P for trend <.001); 1.0 (referent), 0.8 (95% CI = 0.5–1.6), 1.0 (95% CI = 0.5–1.7), and 0.6 (95% CI = 0.3–1.1) for apples (P for trend = .12); and 1.0 (referent), 0.9 (95% CI = 0.4–2.1), and 0.4 (95% CI = 0.2–0.8) for white grapefruit (P for trend = .009). Finally, although interviewed and noninterviewed subjects were similar in their demographics, case patients who were interviewed tended to have a less advanced disease despite our efforts to keep the time short between diagnosis and interview. Thus, the relevance of our findings to particularly fast-growing tumors is unclear. In addition, differences in recall may have occurred between case patients and control subjects. However, it appears unlikely that such a bias would selectively apply to onions, apples, and grapefruit and not to other sources of flavonoids. Nevertheless, replication of our findings in other settings is desirable, particularly in prospective studies.

In summary, in this case–control study in Hawaii, we found inverse associations between lung cancer and main food sources of quercetin (onions and apples) and naringin (white grapefruit). Decreased bioactivation of PAHs and other carcinogens by inhibition of CYP1A1 (by quercetin) and CYP3A4 (by naringenin) could be important mechanisms by which these foods may protect against lung cancer.

REFERENCES

(1) World Cancer Research Fund/American Institute for Cancer Research. Food, nutrition and the prevention of cancer: a global perspective. Washington (DC): American Institute for Cancer Research; 1997.

(2) Peto R, Doll R, Buckley JD, Sporn MB. Can dietary beta-carotene materially reduce human cancer rates? Nature 1981;290:201–8.

(3) Hennekens CH, Burling JE, Manson JE, Stampfer M, Rosner B, Cook NR, et al. Lack of effect of long-term supplementation with beta-carotene on the incidence of malignant neoplasms and cardiovascular disease. N Engl J Med 1996;334:1145–9.

(4) The Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study Group. The effect of vitamin E and beta-carotene on the incidence of lung cancer and other cancers in male smokers. N Engl J Med 1994;330:1029–35.

(5) Omenn GS, Goodman GE, Thornquist MD, Balmes J, Cullen MR, Glass A, et al. Effects of a combination of beta-carotene and vitamin A

on lung cancer and cardiovascular disease. N Engl J Med 1996;334:1150–5.
(6) Le Marchand L, Yoshizawa CN, Kolonel LN, Hankin JH, Goodman MT. Vegetable consumption and lung cancer risk: a population-based case–control study in Hawaii. J Natl Cancer Inst 1989;81:1158–64.
(7) Le Marchand L, Hankin JH, Kolonel LN, Beecher GR, Zhao LP. Intake of specific carotenoids and lung cancer risk. Cancer Epidemiol Biomarkers Prev 1993;2:183–7; 1994; 3:523.
(8) Steinmetz KA, Potter JD. Vegetables, fruit, and cancer. I. Epidemiology. Cancer Causes Control 1991;2:325–57.
(9) Steinmetz KA, Potter JD. Vegetables, fruit, and cancer. II. Mechanisms. Cancer Causes Control 1991;2:427–42.
(10) Block G, Patterson B, Subar A. Fruit, vegetables and cancer prevention: a review of epidemiological evidence. Nutr Cancer 1992; 18:1–29.
(11) Fontham ET. Diet and lung cancer. Cancer Causes Control 1997;8:819–20.
(12) Ziegler RG, Colavito EA, Hartge P, McAdams MJ, Schoenberg JB, Mason TJ, et al. Importance of β-carotene and other phytochemicals in the etiology of lung cancer. J Natl Cancer Inst 1996;88:612–5.
(13) Harbone JB, editor. The flavonoids. Advance in research since 1986. London (U.K.): Chapman & Hall; 1993.
(14) Kandaswami C, Middleton E Jr. Free radical scavenging and antioxidant activity of plant flavonoids. Adv Exp Med Biol 1994;366: 351–76.
(15) Robak J, Gryglewski RJ. Flavonoids are scavengers of superoxide anion. Biochem Pharmacol 1988;37:83–8.
(16) Husain SR, Cillard P. Hydroxy radical scavenging activity of flavonoids. Phytochemistry 1987;26:2489–92.
(17) Cerutti PA. Prooxidant states and tumor promotion. Science 1985;227:375–81.
(18) Deschner EE, Ruperto J, Wong G, Newmark HL. Quercetin and rutin as inhibitors of azoxymethanol-induced colonic neoplasia. Carcinogenesis 1991;7:1193–6.
(19) Verma AK, Johnson JA, Gould MN, Tanner MA. Inhibition of 7,12-dimethylbenz[*a*]anthracene and *N*-nitrosomethylurea induced rat mammary cancer by dietary flavonol quercetin. Cancer Res 1988;48:5754–8.
(20) Obermeier MT, White RE, Yang CS. Effects of bioflavonoids on hepatic P450 activities. Xenobiotica 1995;25:575–84.
(21) Hankin JH. A diet history method for research, clinical and community use. 23rd Lenna Frances Cooper Memorial Lecture. J Am Diet Assoc 1986;86:868–72.
(22) Hankin JH, Wilkens LR, Kolonel LN, Yoshizawa CN. Validation of a quantitative diet history method in Hawaii. Am J Epidemiol 1991;133:616–28.
(23) U.S. Department of Agriculture (USDA). Nutrient database for standard reference, release 10. Bethesda (MD): Human Nutrition Information Service, USDA; 1992.
(24) Hertog MG, Hollman PC, Katan MB. Content of potentially anticarcinogenic flavonoids of 28 vegetables and 9 fruits commonly consumed in The Netherlands. J Agric Food Chem 1992;40: 2379–83.
(25) Hertog MG, Hollman PC, Katan MB, Kromhout D. Intake of potentially anticarcinogenic flavonoids and their determinants in adults in The Netherlands. Nutr Cancer 1993; 20:21–9.
(26) Hertog MG, Hollman PC, van de Putte B. Content of potentially anticarcinogenic flavonoids of tea infusions, wines, and fruit juices. J Agric Food Chem 1993;41:1242–6.
(27) Hertog MG, Hollman PC, Venema DP. Optimization of a quantitative HPLC determination of potentially anticarcinogenic flavonoids in vegetables and fruits. J Agric Food Chem 1992;40:1591–8.
(28) Crozier Alan, Lean ME, McDonald MS, Black C. Quantitative analysis of the flavonoid content of commercial tomatoes, onions, lettuce, and celery. J Agric Food Chem 1997;45: 590–5.
(29) Bilyk A, Sapers GM. Distribution of quercetin and kaempferol in lettuce, kale, chive, garlic chive, leek, horseradish, red radish, and red cabbage tissues. J Agric Food Chem 1985;33: 226–8.
(30) Vlahov G. Flavonoids in three olive (*Olea europaea*) fruit varieties during maturation. J Sci Food Agric 1992;58:157–9.
(31) Mouly PP, Arzouyan CR, Gaydou EM, Estienne JM. Differentiation of citrus juices by factorial discriminant analysis using liquid chromatography of flavanone glycosides. J Agric Food Chem 1994;42:70–9.
(32) Ooghe WC, Ooghe SJ, Detavernier CM, Huyghebaert A. Characterization of orange juice (*Citrus sinensis*) by flavanone glycosides. J Agric Food Chem 1994;42:2183–90.
(33) Rouseff RL, Martin SF, Youtsey CO. Quantitative survey of narirutin, naringin, hesperidin, and neohesperidin in citrus. J Agric Food Chem 1987;35:1027–30.
(34) Bronner WE, Beecher GR. Extraction and measurement of prominent flavonoids in orange and grapefruit juice concentrates. J Chromatogr 1995;705:247–56.
(35) Kiyohara C, Hirohata T, Inutsuka S. The relationship between aryl hydrocarbon hydroxylase and polymorphisms of the CYP1A1 gene. Jpn J Cancer Res 1996;87:18–24.
(36) Le Marchand L, Sivaraman L, Pierce L, Seifried A, Lum A, Wilkens LR, et al. Associations of CYP1A1, GSTM1, and CYP2E1 polymorphisms with lung cancer suggest cell type specificities to tobacco carcinogens. Cancer Res 1998;58:4858–63.
(37) Breslow NE, Day NE. Statistical methods for cancer epidemiology. The analysis of case–control studies. Vol. I. Lyon (France): IARC Sci Publ No. 32. IARC; 1980.
(38) Willett W, Stampfer MJ. Total energy intake: implications for epidemiologic analyses. Am J Epidemiol 1986;124:17–27.
(39) Goodman MT, Hankin JH, Wilkens LR, Kolonel LN. High-fat foods and the risk of lung cancer. Epidemiology 1992;3:288–99.
(40) Hertog MG, Kromhout D, Aravanis C, Blackburn H, Buzina R, Fidanza F, et al. Flavonoid intake and long-term risk of coronary heart disease and cancer in the Seven Countries Study. Arch Intern Med 1995;155:381–6.
(41) Rimm EB, Katan MB, Ascherio A, Stampfer MJ, Willett WC. Relation between intake of flavonoids and risk for coronary heart disease in male health professionals. Ann Intern Med 1996;125:384–9.
(42) K. Hermann, editor. Flavonols and flavones in food plants: a review. J Food Technol 1976; 11:433–42.
(43) Hollman PC, van Trijp JM, Buysman MN, van der Gaag MS, Mengelers MJ, de Vries JH, et al. Relative bioavailability of the antioxidant flavonoid quercetin from various foods in man. FEBS Lett 1997;418;152–6.
(44) De Vries JH, Hollman PC, Meyboom S, Buysman MN, Zock PL, van Staveren WA, et al. Plasma concentrations and urinary excretion of the antioxidant flavonols quercetin and kaempferol as biomarkers for dietary intake. Am J Clin Nutr 1998;68:60–5.
(45) Hertog MG, Feskens EJ, Hollman PC, Katan MB, Kromhout D. Dietary flavonoids and cancer risk in the Zutphen elderly study. Nutr Cancer 1994;22, 175–84.
(46) Knekt P, Jarvinen R, Seppanen R, Heliovaara M, Teppo L, Pukkala E, et al. Dietary flavonoids and the risk of lung cancer and other malignant neoplasms. Am J Epidemiol 1997; 146:223–30.
(47) Dorant E, van den Brandt PA, Goldbohm RA. A prospective cohort study on *Allium* vegetable consumption, garlic supplement use, and the risk of lung carcinoma in The Netherlands. Cancer Res 1994;54:6148–53.
(48) Ha HR, Chen J, Leuenberger PM, Freiburghaus AU, Follath F. *In vitro* inhibition of midazolam and quinidine metabolism by flavonoids. Eur J Clin Pharmacol 1995;48:367–71.
(49) Xu Y, Ho CT, Amin SG, Han C, Chung FL. Inhibition of tobacco-specific nitrosamine-induced lung tumorigenesis in A/J mice by green tea and its major polyphenols as antioxidants. Cancer Res 1992;52:3875–9.
(50) Shi ST, Wang ZY, Smith TJ, Hong JY, Chen WF, Ho CT, et al. Effect of green tea and black tea on 4-(methylnitrosoamino)-1-(3-pyridil)-1-butanone bioactivation, DNA methylation, and lung tumorigenesis in A/J mice. Cancer Res 1994;54:4641–7.
(51) Zheng W, Doyle TJ, Kushi LH, Sellers TA, Hong CP, Folsom AR. Tea consumption and cancer incidence in a prospective study of postmenopausal women. Am J Epidemiol 1996; 144:175–82.
(52) Goldbohm RA, Hertog MG, Brants HA, van Poppel G, van den Brandt PA. Consumption of black tea and cancer risk: a prospective cohort study. J Natl Cancer Inst 1996;88:93–100.
(53) Shim JS, Kang MH, Roh JK, Roberts C, Lee IP. Chemopreventive effect of green tea (*Camellia sinensis*) among cigarette smokers. Cancer Epidemiol Biomarkers Prev 1995;4: 387–91.
(54) Wattenberg LW, Coccia JB. Inhibition of 4-(methylnitrosoamino)-1-(3-pyridyl)-1-butanone carcinogenesis in mice by D-limonene and citrus fruit oils. Carcinogenesis 1991;12:115–7.

(55) Koo LC. Dietary habits and lung cancer risk among Chinese women in Hong Kong who never smoked. Nutr Cancer 1988;11:155–72.

NOTES

[1]*Editor's note:* SEER is a set of geographically defined, population-based, central cancer registries in the United States, operated by local nonprofit organizations under contract to the National Cancer Institute (NCI). Registry data are submitted electronically without personal identifiers to the NCI on a biannual basis, and the NCI makes the data available to the public for scientific research.

Supported in part by Public Health Service grant R01CA55874 and contract N01CP67001 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services; and by grant EDT-78 from the American Cancer Society.

We thank the Hawaii Tumor Registry, Castle Medical Center, Kaiser-Permanente Medical Center, Kuakini Medical Center, Queen's Medical Center, Straub Clinic and Hospital, St. Francis Medical Center, Tripler Medical Center, and Wahiawa General Hospital for their collaboration. We also thank Ronette Hunt, Barbara Burden, Geraldine Kaneshiro, Carol Lau, and Yun Oh Jung for their technical assistance.

Manuscript received August 20, 1999; revised October 28, 1999; accepted November 15, 1999.