NUTRITION AND CANCER, *37*(2), 161–168

Citrus Peel Use Is Associated With Reduced Risk of Squamous Cell Carcinoma of the Skin

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Abstract: *Limonene has demonstrated efficacy in preclinical models of breast and colon cancers. The principal sources of* d-*limonene are the oils of orange, grapefruit, and lemon. The present case-control study was designed to determine the usual citrus consumption patterns of an older Southwestern population and to then evaluate how this citrus consumption varied with history of squamous cell carcinoma (SCC) of the skin. In this Arizona population, 64.3% and 74.5% of the respondents reported weekly consumption of citrus fruits and citrus juices, respectively. Orange juice (78.5%), orange (74.3%), and grapefruit (65.3%) were the predominant varieties of citrus consumed. Peel consumption was not uncommon, with 34.7% of all subjects reporting citrus peel use. We found no association between the overall consumption of citrus fruits [odds ratio (OR) = 0.99, 95% confidence interval (CI) = 0.73–1.32] or citrus juices (OR = 0.97, 95% CI = 0.71–1.31) and skin SCC. However, the most striking feature was the protection purported by citrus peel consumption (OR = 0.66, 95% CI = 0.45–0.95). Moreover, there was a dose-response relationship between higher citrus peel in the diet and degree of risk lowering. This is the first study to explore the relationship between citrus peel consumption and human cancers. Our results show that peel consumption, the major source of dietary* d-*limonene, is not uncommon and may have a potential protective effect in relation to skin SCC. Further studies with large sample sizes are needed to more completely evaluate the interrelationships between peel intake, bioavailability of* d-*limonene, and other lifestyle factors.*

Introduction

Diet, including excess or scarcity of specific foods and nutrients, has been identified as a risk factor for various cancers. *d*-Limonene, a monocyclic monoterpene, is a natural nonnutritive component of a variety of foods and beverages and is found in many fruits, especially citrus fruit oils (1,2). Intake of *d*-limonene can vary considerably, however, depending on the types of food consumed. *d*-Limonene is probably present in high levels in the Mediterranean diet compared with US diets and may be an important component in the putative cancer-preventive effect of the Mediterranean diet. Experimental studies have demonstrated skin photoprotective activity (3) as well as inhibitory effects on skin carcinogenesis by *d*-limonene (4), suggesting the potential for a protective effect of citrus products and citrus peel consumption against human skin cancer. The association between citrus consumption and skin squamous cell carcinoma (SCC) in humans has not, however, been examined in epidemiological studies. Furthermore, no previous studies have explored the potential for differential effects between different citrus products and cancer risk.

The present case-control study was designed to determine the usual citrus consumption patterns of an older Southwestern population and to then evaluate how this citrus consumption varied with history of SCC of the skin. A detailed citrus questionnaire was developed to assess specific citrus food consumption, citrus use in food preparation, and patterns of citrus peel use.

Subjects and Methods

Overview

A population-based case-control study was conducted between 1993 and 1996 with cases of SCC of the skin randomly selected from persons identified through the Southeastern Arizona Skin Cancer Registry, the only population-based skin cancer registry in the United States that has been routinely collecting information on the incidence of nonmelanoma skin cancers since 1985 (5). Control 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 (UV) exposure information. A subset of subjects also completed 24-hour dietary recalls. This group of subjects was then asked to participate in the citrus consumption case-control study.

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Study Population

Cases were eligible if they were ≥30 years of age, had a histopathologically confirmed, nonmetastatic SCC of the skin diagnosed within four months before first contact, and had no prior history of a skin cancer. All cases were randomly selected from cases identified by the Southeastern Arizona Skin Cancer Registry and selected to reflect the age and gender distribution of SCC within the registry. On identification of a potential case from the registry, a letter was sent to the personal physician requesting permission to contact the patient regarding study participation. Once the physician granted permission, a letter describing the study was sent to the patient; a study interviewer then contacted the patient by phone to describe the study, determine eligibility, invite participation, and schedule the interview. Over 83% of identified cases from the registry were located and interviewed for eligibility and interest. Of these interviewed cases, 404 were eligible and participated in the baseline study.

Population-based controls were selected using random-digit dialing. Phone numbers were randomly generated from the cases' residential telephone numbers. These numbers were dialed until it was resolved as a nonworking number, nonresidential number, or residential telephone. Nonresidential or nonworking numbers were excluded and replaced. At least six attempts were made to interview a member of the selected residence before replacement. If no eligible persons were identified, a new telephone number was selected. Controls were frequency matched to the cases by age category (10-yr age groups) and gender. One control per household was invited to participate using modified Waksberg criteria (6). Control subjects were considered eligible if they had no prior history of skin cancer, lived within the Tucson region, and were within the age, gender, and ethnicity grouping.

In 1997, all persons who completed the initial SCC skin cancer study and the 24-hour dietary recalls were asked to participate in the evaluation of citrus consumption ($n = 566$). A total of 470 (242 cases and 228 controls) individuals completed the citrus questionnaire for a final response of 86.7% (24 subjects were deceased and 72 subjects could not be located or refused a second interview). This group is the sample for the present analyses.

Citrus Questionnaire

A citrus questionnaire was developed after a series of focus groups identified usual patterns of citrus consumption. The questionnaire sought detailed information on consumption of each type of citrus fruit and juice intake. In addition, the questionnaire asked how the juice was stored, i.e., types of containers, and whether citrus juices and citrus peel were added during food preparation and/or food serving.

This questionnaire was then evaluated for short-term (1 wk) and long-term (6 mo) reliability within a randomly selected sample of men and women ($n = 40$) who had participated in the original case-control study and who had not completed the 24-hour dietary recalls. The correlation coefficients between baseline and six-month interviews were as follows: $r = 0.91$ and 0.87 for frequency of orange juice and grapefruit juice consumption, respectively, $r = 0.96$ and 0.84 for frequency of orange and grapefruit consumption, respectively, and $r = 0.81$ for frequency of citrus peel consumption (7).

Other Variables

All participants completed a structured interview detailing personal, behavioral, and demographic characteristics. The interview instrument sought information on skin characteristics, sunburns and tanning history, use of suntan lotions and sunscreens during the last year, residential history, UV exposure during the past year, family history of skin cancer, past medical history, tobacco and alcohol use, physical characteristics, and demographic information. All subjects included in the citrus study also completed four 24-hour dietary recalls, which included four 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 (8).

Quality Control

A trained, experienced interviewer conducted all interviews. At the time of the citrus questionnaire, the interviewer was unaware of the case-control status of the subjects. She was given a computer-generated list that included only names and phone numbers of subjects. After each interview, questionnaires were reviewed for completeness and coded. Data entry was through screen-based entry programs that included range checks.

Data Analyses

Distribution of demographic characteristics and potential risk factors were compared between cases and controls by use of *t*-tests for continuous variables and χ^2 tests for categorical variables. Tests for trend were also calculated.

Crude odds ratios (ORs) and 95% confidence intervals were calculated as the effect measure to estimate relative risk, with the nonconsumers used as the reference category. Adjusted ORs were then calculated using multiple logistic regressions. We assessed the potential confounding or modifying effects of age, gender, education, energy intake (kcal/day), fat intake (mean percentage of kcal as fat), retinol and carotenoids (μg/day), vitamin C (mg/day), α-tocopherol (mg/day), foods rich in carotenoids (servings/wk), alcohol intake (mean alcohol intake/day), smoking history (never, former smoker, and current smoker), daily hours of sun exposure, history of actinic skin damage [self-reported diagnosed actinic keratosis (AK)], self-reported ability to tan after prolonged sun exposure (no suntan/possible freckling,

mildly tanned/possible freckling, moderately tanned, and deeply tanned), number of current freckles on the arms, and current use of sunscreen. The initial adjusted model included age, gender, and energy intake (kcal/day). Other multivariate models were evaluated to determine impact of other skin cancer risk factors as potential confounding factors. Inclusion of fat intake, antioxidant vitamin or carotenoid intake, alcohol intake, smoking status, education, and daily hours of sun exposure did not alter any of the results and were excluded from the final model. Age, gender, energy intake, inability to tan after prolonged sun exposure, history of diagnosed and treated AK, number of current freckles on the arms, and current use of sunscreen were included in the final multivariate model.

Citrus consumption was assessed by various methods. It was first assessed by asking participants to report frequency of consumption of citrus juices (orange, grapefruit, lemonade), citrus fruits (orange, grapefruit, lemon, others), citrus peel (never/rarely, sometimes, often, always), marmalade, and other citrus products. Participants were then asked to report any citrus use (lemon or lime) during cooking or addition during serving of foods or beverages. All statistical analyses were done by using STATA computer software (9).

Results

Population

A total of 274 men and 196 women who had participated in the Southeastern Arizona Skin Cancer Study completed the citrus consumption questionnaire. The mean time between the initial interview and the citrus questionnaire was two years, whereas the mean time between the citrus questionnaire and SCC diagnosis was two years nine months. The structure of the citrus questionnaire allowed us to identify patterns and dietary habits related to citrus consumption. Citrus fruit and juice consumption were compared with previously available data from the 24-hour dietary recalls (r = 0.31, $p < 0.001$). The sample for these analyses then included 242 cases and 228 controls.

Because this evaluation of citrus consumption and skin SCC was completed in a subset of the original participants, we compared subjects who participated in the citrus study with those who did not participate. We found no statistically significant difference between the two groups in relation to case-control status, gender, education, smoking, average hours in sun per day, and tanning ability. Although participants in the citrus study reported more AK history than subjects who did not participate, the difference was similar for cases and controls.

Table 1 shows the distribution of cases and controls according to gender, age, education, reported tanning ability, history of AK, daily hours of sun exposure, number of current freckles on the arms, and smoking status. Information from the 24-hour dietary recall data as energy, alcohol intake, percentage of calories from fat, and antioxidants is also included. The study population is composed of an older educated Southwestern US population with 68.8% of cases and 66.2% of controls reporting some college education. There was no difference between the cases and controls in the reported usual hours spent in the sun during the past year. Cases reported spending, on average, 1.38 hours in the sun during peak hours compared with 1.49 hours for controls. There was also no difference between cases and controls in the number of years they lived in Arizona. Only tanning ability, number of current freckles on the arms, use of sunscreen in the past year, and history of AK showed a significant difference between cases and controls ($p < 0.01$).

Pattern and Type of Citrus Consumption and SCC Risk

In this Arizona population, 64.3% and 74.5% reported weekly consumption of citrus fruits (orange, grapefruit, and/or other citrus fruits such as tangerine, kumquat, and pomelo) and citrus juices (orange, grapefruit, and/or lemonade), respectively. Orange juice (78.5%), orange (74.3%), and grapefruit (65.3%) were the predominant varieties of citrus consumed. Table 2 gives frequency of reported usual intake of specific citrus foods and lemon use among cases and controls. The most commonly used juice was orange juice, consumed at least daily by 36% of the cases and 29.4% of the controls.

Peel consumption was not uncommon, with 34.7% of all subjects reporting some citrus peel use. Orange peel was the predominant variety of citrus peel used. Overall, ~45.4% of women compared with 27% of men reported citrus peel use ($\chi^2 = 17.1$, $p = 0.000$), and 30.2% of the cases compared with 39.5% of the controls consumed citrus peel ($\chi^2 = 4.49$, p = 0.03). Only 7% of the cases compared with 11.8% of the controls reported relatively high levels of citrus peel intake.

Table 3 gives associations of intake of citrus foods, fruits, and vegetables with skin SCC risk. ORs are given, unadjusted and adjusted, for confounding variables. There were no statistically significant associations between overall consumption of any citrus fruits, citrus juices, total fruit and vegetable intake and SCC risk, nor were there suggestions of overall trends across levels of use (data not shown). No associations were found between antioxidant (vitamins and carotenoids) intake and skin SCC. Results were similar for each type of citrus fruit and citrus juice. However, although citrus peel was used by only one-third of the population, we found a significant protective effect of citrus peel consumption (adjusted OR = 0.66, 95% confidence interval = 0.45–0.95), as well as a significant protective trend (p = 0.03) with increased use.

Table 4 shows the characteristics of the participants who reported consuming citrus peel compared with those who did not. Citrus peel use was significantly more common among women (p = 0.000). Citrus peel consumption was not, however, associated with age, smoking history, average

Table 1. Selected Characteristics of Skin SCC Cases and Controls Participating in the SEAH Citrus Study[a]

	Cases, % (n = 242)	Controls, % (n = 228)	P Value
Gender			
Male	58.7	57.9	
Female	41.3	42.1	0.86
Age			
≤60 yr	19.8	24.6	
61–70 yr	40.5	37.2	
>70 yr	39.7	37.2	0.47
Smoking			
Never	36.0	36.4	
Former	52.4	48.6	
Current	11.6	15.0	0.51
AK history			
No	21.9	63.2	
Yes	78.1	36.8	0.000
Tanning ability[b]			
No	31.0	18.9	
Yes	69.0	81.1	0.002
Freckles on the arm			
≤25	50.8	65.4	
>25 to <50	38.9	29.8	
≥50	10.3	4.8	0.004
Use of sun screen			
No	13.6	29.4	
Yes	86.4	70.6	0.000
Sun exposure[c,d]	1.38 ± 0.08	1.49 ± 0.08	0.35
Energy intake			
≤1,498 kcal[f]	58.3	50.4	
>1,498 kcal	41.7	49.6	0.09
Fat intake			
<30% kcal[g]	47.5	49.6	
≥30% kcal	52.5	50.4	0.66
Alcohol intake[e]			
No	20.7	18.4	
Yes	79.4	81.6	0.54
Antioxidants[d]			
Retinol, μg/day	474.5 ± 23.2	449.4 ± 23.8	0.45
β-Carotene, μg/day	92.4 ± 7.1	87.6 ± 7.8	0.65
α-Carotene, μg/day	722.9 ± 52.3	777.9 ± 63.0	0.50
Lycopene, μg/day	5,882.9 ± 359.7	5,440.2 ± 419.4	0.42
α-Tocopherol, mg/day	7.1 ± 1.1	6.4 ± 0.5	0.57
Vitamin C, mg/day	110.7 ± 4.5	109.5 ± 4.0	0.84

a: Abbreviations are as follows: SEAH, Southeastern Arizona Health; AK, actinic keratosis; SCC, squamous cell carcinoma.
b: Ability to tan after prolonged sun exposure (no, no suntan/mild tan; yes, moderate or deep tan).
c: Average number of hours of sun exposure per day during peak sun in the last year.
d: Means ± SD.
e: Alcohol intake based on four 24-h dietary recalls.
f: Median of energy intake, among controls.
g: Cut point of 30% of kilocalories from fat based on Healthy 2000 Guidelines.

daily alcohol intake, or any of the known risk factors of skin SCC.

Citrus Peel Consumption, Risk Factors, and SCC Risk

The association between citrus peel use and skin SCC was further evaluated for potential effect modification by selected covariates (Table 5). Consumption of citrus peel was calculated on the basis of frequency of usual consumption (none, sometimes, and often) and serving size (<1 tablespoon, 1 tablespoon, and >1 tablespoon). The categories of citrus peel consumption were then identified as follows: sometimes consume with a usual serving size <1 tablespoon; II, sometimes consume with a serving size equal to 1 tablespoon or often consume but with a serving size <1 tablespoon; III, sometimes consume with a serving size >1 tablespoon or often with a serving size ≥1 tablespoon. Use of citrus peel appeared to be generally protective, with signifi-

Table 2. Distribution of Citrus Intake and Lemon Use in Cases and Controls

Frequency	Cases, % (n = 242)	Controls, % (n = 228)
Orange		
0	28.1	23.3
<1/wk	26.0	24.1
1–6/wk	37.2	45.6
≥7/wk	8.7	7.0
Grapefruit		
0	36.4	32.9
<1/wk	18.6	21.5
1–6/wk	38.4	37.7
≥7/wk	6.6	7.9
Orange juice		
0	21.9	21.0
<1/wk	16.9	18.0
1–6/wk	25.2	31.6
≥7/wk	36.0	29.4
Grapefruit juice		
0	69.0	67.5
<1/wk	14.9	15.4
1–6/wk	13.2	14.9
≥7/wk	2.9	2.2
Lemonade/limeade		
0	65.7	66.7
<1/wk	16.5	16.7
1–6/wk	16.1	14.9
≥7/wk	1.7	1.7
Citrus peel[a]		
0	69.8	60.5
Sometimes	24.4	33.3
Often	5.8	6.2
Always	0	0
Citrus peel categories[b]		
0	69.8	60.5
I	14.5	18.0
II	8.7	9.7
III	7.0	11.8
Add lemon to food[a]		
0	31.8	37.3
Sometimes	19.0	13.6
Often	29.3	32.0
Always	19.8	17.1
Add lemon/lime to tea[a]		
0	44.6	44.5
Sometimes	23.6	23.8
Often	19.4	15.4
Always	12.4	16.3

a: Sometimes, 25–50% of the time; often, 50–75% of the time; always, 75–100% of the time.

b: Based on frequency and serving size as follows: I, sometimes with <1 tablespoon; II, sometimes with 1 tablespoon or often with <1 tablespoon; III, sometimes with >1 tablespoon or often with ≥1 tablespoon.

cant inverse associations observed between citrus peel use and skin SCC for most of the strata considered.

Discussion

This Arizona population offers a unique opportunity to study potential associations between citrus consumption and risk of skin SCC. Arizona has one of the highest risks of skin SCC worldwide, and citrus is a commonly grown and consumed food product. In this older population, 64.3% and 74.5% reported weekly consumption of citrus fruits and citrus juices, respectively, during the past year. Our data showed that 32.8% of our study population consumed their daily citrus juices as orange juice, whereas only 2.6% of the subjects consumed it as grapefruit juice. Peel consumption was not uncommon, with 34.7% of all subjects reporting some citrus peel use.

Table 3. Estimated OR and 95% CI for the Association Between Consumption of Citrus Foods, Fruits, and Vegetables and Skin SCC

Food	OR (95% CI)[a]	OR (95% CI)[b]
Citrus juices	1.03 (0.78–1.34)	0.97 (0.71–1.31)
Orange juice	1.05 (0.89–1.23)	1.00 (0.83–1.20)
Grapefruit juice	0.98 (0.78–1.21)	0.94 (0.72–1.21)
Lemonade	1.03 (0.82–1.29)	1.08 (0.83–1.39)
Citrus fruits	0.99 (0.76–1.28)	0.99 (0.73–1.32)
Orange	0.89 (0.73–1.08)	0.89 (0.71–1.11)
Grapefruit	0.95 (0.78–1.14)	0.93 (0.75–1.15)
Marmalade	1.07 (0.94–1.22)	1.04 (0.90–1.21)
Citrus peel	0.81 (0.59–1.11)	0.66 (0.45–0.95)
Other citrus fruits[c]	1.00 (0.47–2.110)	1.17 (0.33–4.12)
Total fruits[d]	1.01 (0.98–1.04)	1.02 (0.98–1.05)
Mixed vegetables[d]	1.26 (0.92–1.71)	1.15 (0.83–1.61)

a: Crude odds ratios (OR) from univariate logistic regression analyses; CI, confidence interval.

b: Estimates from multiple logistic regression models including terms for age, gender, kilocalories, history of AK, number of current freckles on the arms, inability to tan after prolonged sun exposure, and use of sunscreen in the last year.

c: Other citrus fruits included tangerine, kumquat, and pomelo.

d: Consumption is based on number of servings per week.

Table 4. Characteristics of the Participants Who Reported Consuming Citrus Peel Compared to Those Who Did Not

	Peel Consumption		
Variable	Yes (n = 163)	No (n = 307)	P Value
Gender, %			
Male	45.4	65.7	0.000
Age, %			
≤60 yr	27.9	19.2	
61–70 yr	37.3	40.1	
>70 yr	34.8	40.71	0.11
Smoking, %			
Never	39.9	34.3	
Former	50.0	51.0	
Current	10.1	14.7	0.21
History of AK, %	62.6	55.5	0.15
No tanning ability, %	24.5	25.3	0.84
>75 current freckles on the arms	9.8	6.7	0.43
Energy intake, kcal/day	1,493.6	1,529.2	0.38
Average caloric intake from fat, %	29.7	30.2	0.52
Alcohol intake, g/day	7.1	6.7	0.69

Table 5. Relationship of Frequency and Amount of Citrus Peel Consumption to Skin SCC Risk for Selected Demographic and Behavioral Characteristics[a]

Variable	No Citrus Peel Consumption[b] (n = 307)	Citrus Peel Consumption (n = 163)	Consumption of Citrus Peel[c,d]			P Value (for trend)
			I (n = 76)	II (n = 43)	III (n = 44)	
Gender						
Females	1.0	0.46 (0.22–0.93)	0.33 (0.13–0.80)	0.83 (0.28–2.44)	0.745 (0.14–1.42)	0.16
Males	1.0	0.46 (0.24–0.84)	0.63 (0.26–1.46)	0.37 (0.12–1.13)	0.35 (0.12–0.92)	0.01
Age						
≤60 yr	1.0	0.34 (0.10–1.09)	0.09 (0.01–0.70)	1.34 (0.22–8.02)	0.30 (0.05–1.55)	0.23
61–70 yr	1.0	0.48 (0.22–0.99)	0.63 (0.25–1.59)	0.18 (0.04–0.74)	0.57 (0.16–1.94)	0.06
>70 yr	1.0	0.63 (0.30–1.30)	0.55 (0.21–1.42)	1.03 (0.34–3.08)	0.40 (0.10–1.56)	0.25
Energy[e]						
≤1,498 kcal	1.0	0.82 (0.43–1.56)	0.79 (0.33–1.87)	0.72 (0.25–2.00)	0.97 (0.35–2.61)	0.71
>1,498 kcal	1.0	0.27 (0.13–0.54)	0.29 (0.12–0.71)	0.51 (0.17–1.50)	0.08 (0.20–0.41)	0.00
Fat intake[f]						
<30% kcal	1.0	0.57 (0.29–1.10)	0.58 (0.25–1.34)	1.40 (0.46–4.22)	0.24 (0.08–0.72)	0.05
≥30% kcal	1.0	0.42 (0.21–0.83)	0.46 (0.18–1.17)	0.25 (0.09–0.72)	0.66 (0.21–1.99)	0.04
Smoking						
Never	1.0	0.33 (0.15–0.71)	0.36 (0.12–1.04)	0.30 (0.09–0.99)	0.32 (0.10–0.96)	0.01
Former	1.0	0.61 (0.32–1.13)	0.55 (0.24–1.20)	0.86 (0.30–2.39)	0.54 (0.18–1.58)	0.20
Current	1.0	0.21 (0.01–2.50)	0.36 (0.02–5.77)	0.09 (0.01–4.40)		0.18
Alcohol intake[g]						
No	1.0	0.49 (0.14–1.64)	0.59 (0.12–2.83)	0.23 (0.03–1.91)	0.62 (0.10–3.74)	0.31
Yes	1.0	0.45 (0.27–0.75)	0.43 (0.22–0.83)	0.63 (0.28–1.38)	0.35 (0.14–0.81)	0.01
AK						
No	1.0	0.77 (0.34–1.67)	0.52 (0.14–1.82)	2.13 (0.64–7.07)	0.48 (0.14–1.62)	0.52
Yes	1.0	0.37 (0.20–0.66)	0.43 (0.21–0.89)	0.33 (0.13–0.81)	0.29 (0.10–0.77)	0.00
Tanning ability[h]						
No	1.0	0.37 (0.12–1.07)	0.29 (0.07–1.13)	0.29 (0.05–1.50)	0.68 (0.11–3.97)	0.22
Yes	1.0	0.49 (0.29–0.82)	0.48 (0.24–0.93)	0.64 (0.28–1.44)	0.40 (0.17–0.92)	0.01
Freckles on the arm[i]						
≤25	1.0	0.42 (0.22–0.79)	0.40 (0.16–0.92)	0.65 (0.23–1.79)	0.32 (0.12–0.86)	0.01
>25 to ≤50	1.0	0.49 (0.22–1.17)	0.55 (0.18–1.61)	0.44 (0.10–1.85)	0.42 (0.09–1.81)	0.12
>50	1.0	0.68 (0.19–2.40)	0.62 (0.12–3.19)	0.54 (0.08–3.29)	1.02 (0.12–8.49)	0.90
Use of sunscreen						
No	1.0	0.29 (0.07–1.09)	0.20 (0.02–1.33)		1.03 (0.16–6.42)	0.29
Yes	1.0	0.51 (0.30–0.83)	0.53 (0.27–0.99)	0.72 (0.33–1.56)	0.32 (0.14–0.73)	0.01

a: Values are ORs, with 95% CIs in parentheses.
b: Reference group.
c: Consumption of citrus peel is based on frequency of consumption as follows: I, sometimes with <1 tablespoon; II, sometimes with 1 tablespoon or often with <1 tablespoon; III, sometimes with >1 tablespoon or often with ≥1 tablespoon.
d: Estimates from multiple logistic regression models including terms for age, gender, kilocalories, history of AK, number of current freckles on the arms, inability to tan after prolonged sun exposure, and use of sunscreen.
e: Median of energy intake among controls.
f: Cut point of 30% of kilocalories from fat based on Healthy 2000 Guidelines.
g: Alcohol intake based on four 24-h dietary recalls.
h: Ability to tan after prolonged sun exposure (no, no suntan/mild tanning; yes, moderate or deep tanning).
i: Number of current freckles on the arms.

In this population-based case-control study designed to investigate risk factors for skin SCC in Arizona, we found no evidence for a relationship between the overall consumption of any citrus fruit or juice and skin SCC. However, the most striking feature was the significant association between citrus peel use and skin SCC risk. Our data show that persons without skin cancer significantly consumed more citrus peel than did cases of skin SCC. This association was observed with multiple definitions of citrus peel consumption: frequency, serving size, and the product of frequency and serving size and with increasing consumption.

The finding of a protective effect of citrus peel in relation to skin SCC is consistent with other experimental studies. The chemopreventive efficacy of limonene during the initiation and promotion stages of carcinogenesis has been demonstrated in chemically induced rodent cancers (12–19). *d*-Limonene, which comprises >90% of citrus peel oil, has chemopreventive activity against rodent skin (4), mammary (12–14), liver (15), lung and forestomach (16,17), gastric (18), and colon (19) cancers. Several mechanisms of action may account for the antitumor activities of *d*-limonene. The blocking chemopreventive effects of limonene during the

initiation phase of carcinogenesis are likely due to the induction of phase II carcinogen-metabolizing enzymes, resulting in carcinogen detoxification (20). The postinitiation phase, tumor-suppressive chemopreventive activity of limonene may be due to inhibition of the posttranslational isoprenylation of cell growth-regulating proteins (21,22). Overall, dietary *d*-limonene has been shown to be effective in the chemoprevention and chemotherapy of cancer. As a result, its cancer chemotherapeutic activities are under evaluation in phase I human clinical trials (23,24). *d*-Limonene also possesses many characteristics of an ideal chemopreventive agent, namely, efficacious antitumor activity, dietary availability, oral bioavailability, low cost, and no toxicity.

The difference in finding an association with citrus peel consumption, but not with consumption of citrus fruit or juice, may be partially explained by the simple fact that citrus peel contains more *d*-limonene than other citrus products. Daily United States per capita consumption of *d*-limonene, as a result of its natural occurrence in food and its presence as a flavoring agent, is estimated to be 0.27 mg/kg body wt/day for a 60-kg individual (16.2 mg/day) (10). Citrus juice products are among the richest sources of *d*-limonene, with an intake approaching 1 mg/kg body wt/day for adults and 2 mg/kg body wt/day for young children (11). Citrus oil concentration may be the most important determinant of *d*-limonene concentration in a glass of citrus juice. It is possible that various citrus juice preparations vary in the amount of actual citrus oils and *d*-limonene in the product (7).

Our present work suggests that participants in this study with the highest citrus juice intake were consuming *d*-limonene at 20–40 mg/day. However, in human populations, the amount of *d*-limonene ingested is determined not only by the frequency and amount of citrus food intake but also by the amount of citrus oil (peel) consumed (7). In this study, individuals consuming peel were consuming *d*-limonene at 50–90 mg/day. Human exposure to *d*-limonene through the diet or environment is widespread. Food preparation may play an important role in determining the amount of limonene consumed, when orange zest or parts of the whole lemon fruit are often included during cooking to add a desirable sour taste to the food.

Some limitations and strengths of the study deserve consideration. In case-control studies, the possibilities for recall and interviewer bias are a major concern. Differential recall of diet and citrus consumption between cases and controls can lead to biased estimates of effect. Furthermore, because there was a lag between diagnosis of the skin cancer and interview (average of 4 mo), there is potential for cases to have altered their behavior and to then report their recently changed behaviors. Several steps were taken to reduce potential bias. Standard questionnaires were administered to all subjects by a trained interviewer who was not aware of the case-control status of the subjects at the time of the citrus questionnaire administration. There is some evidence, however, that skin cancer cases did recently alter their behavior for risk factors they thought were related to skin cancer. For instance, they reported recent (past-year) exposure to the sun similar to that of the controls and more current use of sunscreens. The interview did not record information on sun exposure experiences in the more distant past. However, given that skin SCC is related to other measures of high UV exposure (i.e., history of AK, history of sunburns), the lack of a finding for a differential sun exposure history for cases and controls argues for a change in behavior since the diagnosis of the skin cancer. Furthermore, there was no difference between the cases and controls in past use of sunscreens.

However, although it appears that the cases did modify some of their behaviors, there is no evidence that they recently altered their consumption of citrus products. Also, because there have been no prior studies of skin cancer occurrence and citrus consumption, it is unlikely that this population would have considered citrus consumption, or lack of consumption, to be related to their risk of skin cancer. The difference in risk patterns between overall consumption of citrus fruits or juices and specific consumption of citrus peel supports the lack of differential recall between cases and controls in their reporting of citrus consumption. Public perception of citrus consumption has been that there would be no difference in the potential effect of citrus on the basis of different products. Furthermore, there was a highly significant correlation between reported citrus consumption from the citrus questionnaire and citrus intake as estimated from the four 24-hour dietary recalls, which indicate the absence of significant changes in citrus consumption patterns among cases and controls. We believe that the most significant limitation of this study was that we did not collect a comprehensive and detailed history of different forms of citrus peel use and that the sample size was still limited.

This study controlled for important confounding factors, including history of physician-diagnosed AK, tanning ability, number of current freckles on the arms, and current use of sunscreen. Citrus peel use appeared to be generally protective. Significant inverse associations between citrus peel use and skin SCC were observed in most of the strata considered. In addition, control for covariates increased the magnitude of association of citrus peel use with skin SCC risk.

This is the first study to explore the relationship between citrus peel consumption, and hence dietary *d*-limonene, and human cancer of the skin. Although this study was only of moderate sample size, particularly when exploring relationships within subgroups of different citrus products' consumption patterns, we were able to observe a significant protective effect for citrus peel consumption but not for other citrus foods. Further studies of large sample sizes are needed to more completely evaluate the interrelationships between preparation techniques, forms of dietary citrus peel intake, and other lifestyle factors.

Acknowledgments and Notes

The authors thank Dr. David Alberts (Div. of Cancer Prevention and Control) for support, Steve Rodney for assistance with data management, and Mary Lurie for assistance with interviewing and data entry. This research was supported by National Cancer Institute Grant K07 CA-76008.

This publication was made possible by National Cancer Institute Grant P01 CA-27502. Its contents are solely the responsibility of the authors and do not necessarily represent the official views of the National Cancer Institute. Address correspondence to Iman Hakim, M.D., Ph.D., M.P.H., Arizona Cancer Center, College of Medicine, 1515 N. Campbell Ave., PO Box 245024, Tucson, AZ 85724. Phone: (520) 626-5355. FAX: (520) 626-5348. E-mail: ihakim@azcc.arizona.edu.

Submitted 3 February 2000, accepted in final form 8 May 2000.

References

1. US National Toxicology Program: *Toxicology and Carcinogenesis Studies of* d-*Limonene in F344/N Rats and B6c3F1 Mice*. Research Triangle Park, NC: National Toxicology Program, 1990. (NIH Publ 90-2802)
2. Kesterson, JW, Hendrickson, R, and Braddock, RJ: *Florida Citrus Oils*. Gainesville, FL: University of Florida, 1971, pp 3–174. (Tech Bull 749)
3. Saija, A, Tomaino, A, Trombetta, D, Giacchi, M, and Bonina, F: Influence of different penetration enhancers on *in vitro* skin permeation and *in vivo* photoprotective effect of flavonoids. *Int J Pharm* **175,** 85–94, 1998.
4. Van Duuren, BL, and Goldschmidt, BM: Cocarcinogenic and tumor-promoting agents in tobacco carcinogenesis. *JNCI* **56,** 1237–1242, 1976.
5. Harris, R, Griffith, K, and Rodney, S: *Report From the Southeastern Arizona Skin Cancer Registry*. Tucson, AZ: Arizona Cancer Center, 1999.
6. Waksberg, J: Sampling methods for random digital dialing. *J Am Stat Assoc* **56,** 33–43, 1978.
7. Hakim, IA, McClure, T, and Liebler, D: Assessing dietary *d*-limonene intake for epidemiological studies. *Abst 3rd Int Food Data Conf, July 1999, Rome, Italy*.
8. *Nutrition Data System*, version 2.9. Minneapolis, MN: Nutrition Coordinating Center, University of Minnesota, 1997.
9. STATA: *STATA Statistical Software, Intercooled Stata*, release 5.0. College Station, TX: STATA, 1997.
10. Lloyd, RJ: Instrumentation for automated thermal desorption-pyrolysis capillary gas chromatography. *J Chromatogr* **284,** 357–371, 1984.
11. Flavor and Extract Manufacturers' Association: *d-Limonene Monograph*. Washington, DC: Flavor and Extract Manufacturers' Association, 1991, pp 1–4.
12. Elson, CE, Maltzman, TH, Boston, JL, Tanner, MA, and Gould, MN: Anti-carcinogenic activity of *d*-limonene during the initiation and promotion/progression stages of DMBA-induced rat mammary carcinogenesis. *Carcinogenesis* **9,** 331–332, 1988.
13. Maltzman, TH, Hurt, LM, Elson, CE, Tanner, MA, and Gould, MN: The prevention of nitrosomethylurea-induced mammary tumors by *d*-limonene and orange oil. *Carcinogenesis* **10,** 781–783, 1989.
14. Elegbede, JA, Elson, CE, Tanner, MA, Qureshi, A, and Gould, MN: Inhibition of DMBA-induced mammary cancer by the monoterpene *d*-limonene. *Carcinogenesis* **5,** 661–664, 1984.
15. Giri, RK, Parija, T, and Das, BR: *d*-Limonene chemoprevention of hepatocarcinogenesis in AKR mice: inhibition of c-*jun* and c-*myc*. *Oncol Rep* **6,** 1123–1127, 1999.
16. Wattenberg, LW, and Coccia, JB: Inhibition of 4-(methylnitrosoamino)-1-(3-pyridyl)-1-butanone carcinogenesis in mice by *d*-limonene and citrus fruit oils. *Carcinogenesis* **12,** 115–117, 1991.
17. Wattenberg, LW, Sparnins, VL, and Barany, G: Inhibition of *N*-nitrosodiethylamine carcinogenesis in mice by naturally occurring organosulfur compounds and monoterpenes. *Cancer Res* **49,** 2689–2692, 1989.
18. Yano, H, Tatsuta, M, Iishi, H, Baba, M, Sakai, N, et al.: Attenuation by *d*-limonene of sodium chloride-enhanced gastric carcinogenesis induced by *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine in Wistar rats. *Int J Cancer* **82,** 665–668, 1999.
19. Kawamori, T, Tanaka, T, Hirose, M, and Mori, H: Inhibitory effects of *d*-limonene on the development of colonic aberrant crypt foci induced by azoxymethane in F-344 rats. *Carcinogenesis* **17,** 369–372, 1996.
20. Gelb, MH, Tamanoi, F, Yokoyama, K, Ghomashchi, F, Esson, K, et al.: The inhibition of protein prenyltransferases by oxygenated metabolites of limonene and perillyl alcohol. *Cancer Lett* **91,** 169–175, 1995.
21. Elegbede, JA, Maltzman, TH, Elson, CE, and Gould, MN: Effects of anticarcinogenic monoterpenes on phase II hepatic metabolizing enzymes. *Carcinogenesis* **14,** 1221–1223, 1993.
22. Hardcastle, IR, Rowlands, MG, Barber, AM, Grimshaw, RM, Mohan, MK, et al.: Inhibition of protein prenylation by metabolites of limonene. *Biochem Pharmacol* **57,** 801–809, 1999.
23. McNamee, D: Limonene trial in cancer (abstr). *Lancet* **342,** 801, 1993.
24. Vigushin, DM, Poon, GK, Boddy, A, English, J, Halbert, GW, et al.: Phase I and pharmacokinetic study of *d*-limonene in patients with advanced cancer. *Cancer Chemother Pharmacol* **42,** 111–117, 1998.