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Citrus consumption and risk of basal cell carcinoma and squamous cell carcinoma of the skin

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Abstract

Animal experiments have demonstrated the photocarcinogenic properties of furocoumarins, a group of naturally occurring chemicals that are rich in citrus products. We conducted a prospective study for citrus consumption and risk of basal cell carcinoma (BCC) and squamous cell carcinoma (SCC) of the skin based on data from 41 530 men in the Health Professionals Follow-up Study (1986–2010) and 63 759 women in the Nurses' Health Study (1984–2010) who were free of cancers at baseline. Over 24–26 years of follow-up, we documented 20 840 incident BCCs and 3544 incident SCCs. Compared to those who consumed citrus products less than twice per week, the pooled multivariable-adjusted hazard ratios were 1.03 [95% confidence interval (95% CI): 0.99–1.08] for BCC and 1.14 (95% CI: 1.00–1.30) for SCC for those who consumed two to four times per week, 1.06 (95% CI: 1.01–1.11) for BCC and 1.15 (95% CI: 1.02–1.28) for SCC for five to six times per week, 1.11 (95% CI: 1.06–1.16) for BCC and 1.22 (95% CI: 1.08–1.37) for SCC for once to 1.4 times per day and 1.16 (95% CI: 1.09–1.23) for BCC and 1.21 (95% Cl: 1.06–1.38) for SCC for 1.5 times per day or more (P_{trend} = 0.001 for BCC and 0.04 for SCC). In contrast, consumption of non-citrus fruit and juice appeared to be inversely associated with risk of BCC and SCC. Our findings support positive associations between citrus consumption and risk of cutaneous BCC and SCC in two cohorts of men and women, and call for further investigations to better understand the potential photocarcinogenesis associated with dietary intakes.

Introduction

Basal cell carcinoma (BCC) and squamous cell carcinoma (SCC) of the skin are the most frequently diagnosed malignancies in the population (1,2). They are more common than all other cancers combined and can cause substantial morbidity and rising cost to the health-care system (3,4). Knowledge on the modifiable risk factors of these skin cancers is important for the prevention of cancer incidence. Solar ultraviolet (UV) radiation has been recognized as the major environmental factor for skin cancer. However, previous experimental studies suggest that certain photoactive agents, such as furocoumarins, may also increase the risk of skin cancer in the presence of UV radiation (5–11). Furocoumarins (furanocoumarins) are a group of naturally occurring chemicals that are rich in certain plants, including citrus products (12–15). Furocoumarins have high UV absorbance and mutagenic properties (16,17). Oral application of psoralens (a group of furocoumarin derivatives, e.g. 8-methoxypsoralen and 5-methoxypsoralen/bergapten) and UVA radiation (PUVA) has been used as an effective therapy for severe psoriasis and other cutaneous problems (18,19). Interestingly, epidemiologic studies have demonstrated an increased risk of BCC and SCC

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Abbreviations

BCC	basal cell carcinoma
CI	confidence interval
FFQ	food frequency questionnaire
HR	hazardous ratio
HPFS	Health Professionals Follow-up Study
NHS	Nurses' Health Study
SCC	squamous cell carcinoma
UV	ultraviolet

among patients receiving PUVA treatment (8–10). Commonly consumed citrus products, such as grapefruit and orange, may contain varying amounts of psoralens/furocoumarins (12,13). However, whether dietary consumption of furocoumarin-rich foods may increase the risk of skin cancer is unknown.

Our previous investigation based on data from two large ongoing cohort studies, the Health Professionals Follow-up Study (HPFS, 1986–2010) and the Nurses' Health Study (NHS, 1984–2010), identified an increased risk of cutaneous malignant melanoma associated with citrus consumption (20). In the present study, we further examined the association between citrus consumption and risk of two other major forms of skin cancer (i.e. BCC and SCC) in the HPFS and NHS.

Methods

Study population

The study population consisted of participants from two ongoing longitudinal cohort studies: HPFS and NHS. The HPFS consisted of 51 529 male health professionals who were aged 40–75 and completed their initial questionnaire in 1986. Information on medical history and lifestyle factors was collected biennially via mailed questionnaires in the two cohorts. The NHS was established in 1976 when 121 701 married, registered, female nurses who were aged 30–55 years and residing in the USA at the time of enrollment responded to an initial questionnaire regarding their medical history and lifestyle risk factors. The present study was approved by the Institutional Review Boards of Brigham and Women's Hospital and Harvard School of Public Health. We consider the participants' completion and return of the self-administered questionnaire as informed consent.

We followed participants for incident BCC and SCC starting from 1986 in the HPFS and 1976 in the NHS. At baseline, 49 617 HPFS men and 81 685 NHS women completed the dietary questionnaires. Participants who had a history of cancer at baseline were excluded. Owing to small number and low risk of skin cancer in non-white participants (1), the present study only included participants of Caucasian ancestries. After exclusions, 41 530 men and 63 759 women (total n = 105 289) remained in the present study.

Assessment of dietary consumption and other skin cancer risk factors

The present study used a food frequency questionnaire (FFQ) to collect dietary information every 4 years since 1986 in the HPFS and NHS, with an additional dietary questionnaire completed in 1984 in the NHS. Participants responded to the questions regarding how often on average (in nine categories ranging from never to six+ servings per day) during the previous year they had consumed grapefruit ('a half'), oranges ('one'), grapefruit juice and orange juice ('one small glass' of six fluid ounces) and other food items. Grapefruit and grapefruit juice were asked as a single item in the 2002 and 2006 FFQs. Overall citrus consumption was calculated as the sum of the reported frequencies of these individual citrus products. Dietary intake collected using the FFQ has been demonstrated to be a valid estimator of relative food intake when compared with multiple diet records (21,22). The correlation coefficients ranged from 0.75 to 0.84 for the correlations between intakes of individual citrus products assessed on the baseline FFQ and intakes assessed on two 1-week dietary records (21). Information on other dietary factors, including intakes of total energy, alcohol, coffee, other fruits and juices (13 items) and vegetables (26 items), was also collected by the FFQs.

In the biennial follow-up questionnaires, we inquired and updated information on body weight and height, physical activity, cigarette smoking and menopausal status and post-menopausal hormone use among women. Data on the following skin cancer risk related variables were also collected through the questionnaires (23,24): natural hair color at an early age; number of moles on arms; family history of melanoma in first-degree relatives; skin reaction to sun exposure for 2 h or more as a child/adolescent; number of lifetime blistering sunburns; average time spent in direct sunlight since high school; cumulative UV flux at residence since baseline and use of sunscreen.

Assessment of BCC and SCC cases

Biennial questionnaires mailed to all study participants included questions on diagnoses of BCC and SCC during the previous 2 years. We obtained permission from participants who reported new diagnoses of SCC to review their medical and pathological reports. Study physicians who were blinded of the exposure status reviewed the records to validate the diagnoses and retrieve information on tumor stage and location if available. SCCs were further classified into the following two subgroups according to tumor location: tumors occurred on the body sites with higher continuous sun exposure (head, neck and extremities), and tumors occurred on the body sites with lower continuous sun exposure (truncal sites including shoulder, back, hip, genitals, abdomen and chest). Although medical records were not obtained for self-reported BCC, previous validation studies have demonstrated high validity of self-reported BCC in the two cohorts, with 96% women and 84% men confirmed by pathological records (25,26). Over 2 million person-years of follow-up, we documented a total of 20 840 incident BCCs (9033 in men and 11 807 in women) and 3544 incident SCCs (1540 in men and 2004 in women). SCCs include 2329 invasive cases and 1215 *in situ* cases, among which 2758 occurred on the body sites with higher continuous sun exposure (head, neck and extremities) and 483 occurred on the body sites with lower continuous sun exposure (truncal sites).

Statistical analysis

To create the best estimates of long-term intake and to minimize within-person variation, each dietary intake was calculated as the average of all reported intakes up to that time prior to every 2-year follow-up interval. Because grapefruit and grapefruit juice were asked as a single item in the 2002 and 2006 FFQs, analyses for separate grapefruit and grapefruit juice used cumulative average intakes up to 1998 for the subsequent follow-up. We created a new intake variable for combined grapefruit and grapefruit juice for sensitivity analyses. Each participant contributed person-time from the return month of the baseline questionnaire to the date of the first report of any cancer, date of death, or the end of follow-up (1 January 2010 for men; 1 June 2010 for women), whichever came first. We used SAS software version 9.2 (SAS Institute, Cary, NC) for all statistical analyses. All statistical tests were two-tailed, and the significance level was set at $P < 0.05$.

Cox proportional hazards models were used to compute the hazard ratios (HR) with 95% confidence intervals (CI) of BCC and SCC associated with dietary intakes. Multivariable analyses were performed with adjustment for other skin cancer risk factors and potential lifestyle and dietary confounders. We used the most recent information for time-varying variables (e.g. body mass index) prior to each follow-up interval to take into account potential changes over the follow-up. Missing data during any follow-up period were coded as a missing indicator category for categorical variables (e.g. smoking status) and with carried-forward values for continuous variables (e.g. body mass index). Trend tests for a given citrus product were performed by assigning median values for citrus intake categories and treating the new variable as a continuous term in the models. The analyses were performed among men and women separately and then pooled using a random-effects model. We also performed analyses for SCC subtypes divided by tumor stage and site. The two highest consumption categories for each citrus variable were combined to maintain the statistical power in subtype analyses.

Several sensitivity analyses were performed to test the specificity and consistency of the reported associations. To examine whether the positive

association with risk of BCC and SCC was specific to citrus products, we computed the HRs of BCC and SCC in association with consumption of non-citrus fruits and juices and vegetables. To address the concern about potential reverse causality between dietary assessment and cancer diagnosis, we performed lag analyses by adding a 2-year interval between dietary intake and cohort follow-up (e.g. we used citrus consumption from the 1984 questionnaire for the follow-up period from 1986 to 1988 in the NHS).

Results

Table 1 shows the baseline characteristics of the study population. There was no appreciable difference in known skin cancer risk factors including phenotypic traits (e.g. red/blonde hair color and skin reaction to sun as a child/adolescent) and sun exposure related variables (i.e. number of lifetime blistering sunburns, average time spent in direct sunlight since high school and annual UV flux at residence) over the citrus intake categories, suggesting homogeneous characteristics of the study participants in terms of host risk profile and sun exposure. Consumption levels of citrus products remained relatively constant over the follow-up, and orange juice was the major contributor of overall citrus consumption (Supplementary Table 1, available at *Carcinogenesis* Online).

No significant heterogeneity was detected between sex-specific results for BCC and SCC (Supplementary Tables 2 and Table 3, available at *Carcinogenesis* Online, all *P* for heterogeneity > 0.05), and therefore the pooled risk estimates were presented as the main results. Overall citrus consumption was significantly associated with increased risk of BCC in the pooled analyses (Table 2). Among the individual citrus products, grapefruit and orange juice showed significant positive associations with risk of BCC. Neither grapefruit juice nor oranges was significantly associated with risk of BCC. Similarly, overall citrus consumption was also positively associated with risk of SCC (Table 3). Among the individual citrus products, grapefruit showed the most apparent association with risk of SCC, followed by orange juice, and neither grapefruit juice nor oranges was significantly associated with risk of SCC.

Associations between citrus consumption and risk of BCC and SCC remained essentially unchanged when we added a 2-year lag between dietary assessment and cohort follow-up (data not shown). Subtype analyses for SCC showed similar associations with citrus consumption for invasive and *in situ* cases (data not shown). Interestingly, the positive association of SCC with citrus consumption appeared to be more apparent for tumors occurred on the body sites with higher continuous sun exposure (head, neck and extremities) than for tumors occurred on the body sites with lower continuous sun exposure (truncal sites) (Table 4). There were significant trends towards higher risk for SCC on head, neck and extremities (all $P_{trend} < 0.05$) but not

Table 1. Baseline characteristics of study participants according to frequency of overall citrus consumption in the HPFS and NHS

	<2 per week	2–4 per week	5–6 per week	1–1.4 per day	≥1.5 per day
Men (1986)					
No. of participants	10 617	6236	6921	9496	8260
Age (year)[a]	52.0 (9.2)	52.2 (9.4)	53.3 (9.6)	54.3 (9.8)	54.7 (9.8)
Red/blonde hair (%)	13.3	14.2	13.0	13.8	12.6
Arm with moles (%)	31.4	32.2	32.7	31.9	32.0
Painful burn/blisters skin reaction as a child/adolescent (%)	23.7	23.7	23.9	24.0	22.7
Family history of melanoma (%)	4.2	3.6	3.9	4.3	3.7
No. of lifetime blistering sunburns	13.0 (12.0)	13.1 (12.1)	13.1 (12.1)	12.6 (12.0)	12.6 (12.2)
Average time spent in direct sunlight since high school (h/week)	9.2 (5.4)	9.1 (5.3)	9.2 (5.4)	9.1 (5.5)	9.0 (5.6)
Annual UV flux at residence ($\times 10^{-4}$ RB count)	132.0 (27.5)	130.1 (27.1)	129.1 (26.7)	127.4 (26.2)	126.3 (26.2)
Body mass index (kg/m^2)	25.1 (5.2)	25.1 (5.3)	25.1 (5.1)	24.9 (4.6)	24.8 (5.0)
Physical activity level (metabolic equivalents h/week)	17.1 (27.0)	19.7 (29.3)	21.1 (30.7)	22.0 (28.9)	25.8 (32.3)
Current smoker (%)	14.1	10.1	8.4	7.7	6.3
Alcohol intake (g/day)	12.6 (17.9)	11.8 (16.0)	11.8 (15.8)	11.8 (15.2)	10.6 (14.6)
Coffee (cup/day)	1.6 (1.8)	1.4 (1.6)	1.3 (1.5)	1.2 (1.5)	1.1 (1.4)
Women (1984)					
No. of participants	17 660	10 217	9771	15 269	10 842
Age (year)[a]	49.2 (7.1)	49.5 (7.2)	50.0 (7.2)	50.7 (7.2)	51.3 (7.0)
Red/blonde hair (%)	15.5	16.1	15.4	15.4	16.6
Arm with moles (%)	36.5	37.6	36.5	37.5	39.2
Painful burn/blisters skin reaction as a child/adolescent (%)	15.6	14.8	14.0	13.8	14.6
Family history of melanoma (%)	6.7	6.8	7.1	7.3	7.3
No. of lifetime blistering sunburns	8.6 (7.0)	8.8 (7.0)	8.8 (6.9)	8.7 (6.9)	8.6 (7.0)
Average time spent in direct sunlight since high school (h/week)	4.8 (2.7)	4.9 (2.7)	5.0 (2.7)	4.9 (2.7)	5.0 (2.7)
Annual UV flux at residence ($\times 10^{-4}$ Robertson-Berger count)	124.4 (25.9)	123.0 (25.1)	122.0 (24.3)	119.7 (23.0)	118.8 (22.1)
Body mass index (kg/m^2)	25.0 (4.9)	25.3 (4.8)	25.2 (4.7)	24.9 (4.7)	25.0 (4.8)
Physical activity level (metabolic-equivalents h/week)	12.1 (19.7)	13.1 (18.8)	14.1 (19.1)	14.2 (19.1)	17.3 (24.6)
Current smoker (%)	32.1	25.2	21.0	20.0	18.6
Menopausal status (%)	46.5	46.1	45.8	45.9	46.3
Postmenopausal hormone use (%)[b]	24.5	24.0	25.0	24.9	24.8
Alcohol intake (g/day)	7.2 (12.7)	6.7 (11.4)	6.9 (11.0)	7.3 (11.3)	7.1 (11.0)
Coffee (cup/day)	1.9 (1.9)	1.8 (1.8)	1.7 (1.7)	1.6 (1.6)	1.6 (1.7)

Values are means (SD) or percentages and have been standardized to the age distribution of the study population.

[a]Values are not age adjusted.

[b]Percentages among postmenopausal women.

Table 2. Pooled risk of basal cell carcinoma according to frequency of citrus consumption in the HPFS (1986–2010) and NHS (1984–2010)

	Serving category					*P* for trend
Overall citrus	<2 per week	2–4 per week	5–6 per week	1–1.4 per day	≥1.5 per day	
No. of person-years	400456	371958	465228	418628	319669	
No. of cases	3505	3665	5022	4787	3861	
Age-adjusted HR (95% CI)	1.00	1.07 (0.99–1.16)	1.11 (1.06–1.16)	1.17 (1.12–1.22)	1.20 (1.15–1.26)	<0.001
Multivariable-adjusted HR1 (95% CI)	1.00	1.03 (0.99–1.08)	1.06 (1.01–1.11)	1.11(1.06–1.16)	1.16 (1.09–1.23)	0.001
Grapefruit	Never	<1 per week	1 per week	2 per week	≥3 per week	
No. of person-years	509252	639008	349832	221228	256619	
No. of cases	4507	6460	3827	2795	3251	
Age-adjusted HR (95% CI)	1.00	1.08 (1.04–1.13)	1.15 (1.10–1.20)	1.21 (1.15–1.27)	1.19 (1.14–1.25)	<0.001
Multivariable-adjusted HR1 (95% CI)	1.00	1.03 (0.99–1.08)	1.09 (1.02–1.17)	1.13 (1.07–1.19)	1.13 (1.05–1.21)	<0.001
Multivariable-adjusted HR2 (95% CI)	1.00	1.03 (0.99–1.08)	1.09 (1.01–1.18)	1.13 (1.06–1.20)	1.14 (1.04–1.24)	0.002
Grapefruit juice	Never	<1 per week	1 per week	2 per week	≥3 per week	
No. of person-years	1092966	476005	165397	110093	131479	
No. of cases	11062	5256	1768	1278	1476	
Age-adjusted HR (95% CI)	1.00	1.05 (1.00–1.11)	1.03 (0.98–1.08)	1.07 (1.01–1.13)	1.08 (1.02–1.15)	0.01
Multivariable-adjusted HR1 (95% CI)	1.00	1.00 (0.96–1.04)	0.99 (0.94–1.04)	1.02 (0.96–1.08)	1.06 (1.00–1.12)	0.08
Multivariable-adjusted HR2 (95% CI)	1.00	0.98 (0.93–1.04)	0.96 (0.91–1.01)	0.98 (0.92–1.04)	1.02 (0.97–1.08)	0.80
Oranges	Never	<1 per week	1 per week	2 per week	≥3 per week	
No. of person-years	228853	518773	401333	338726	488254	
No. of cases	2213	5157	4075	3940	5455	
Age-adjusted HR (95% CI)	1.00	1.00 (0.95–1.06)	1.03 (0.97–1.08)	1.07 (0.93–1.25)	1.03 (0.94–1.12)	0.37
Multivariable-adjusted HR1 (95% CI)	1.00	0.96 (0.91–1.01)	0.95 (0.90–1.01)	0.97 (0.86–1.10)	0.94 (0.86–1.03)	0.59
Multivariable-adjusted HR2 (95% CI)	1.00	0.95 (0.90–1.00)	0.94 (0.89–1.00)	0.96 (0.84–1.10)	0.93 (0.84–1.04)	0.56
Orange juice	<1 per week	1–2 per week	3–4 per week	5–6 per week	≥1 per day	
No. of person-years	499507	451276	395566	310206	319385	
No. of cases	4723	4567	4223	3706	3621	
Age-adjusted HR (95% CI)	1.00	1.05 (1.01–1.09)	1.09 (1.04–1.13)	1.15 (1.10–1.20)	1.17 (1.12–1.22)	<0.001
Multivariable-adjusted HR1 (95% CI)	1.00	1.02 (0.97–1.06)	1.06 (1.01–1.10)	1.09 (1.04–1.14)	1.13 (1.05–1.21)	<0.001
Multivariable-adjusted HR2 (95% CI)	1.00	1.01 (0.97–1.05)	1.05 (1.01–1.10)	1.08 (1.03–1.14)	1.12 (1.05–1.20)	<0.001

Multivariable hazard ratios were adjusted for age, natural hair color (red, blonde, light brown, dark brown, black), number of arm moles (0, 1–2, 3–9, ≥10), sunburn susceptibility as a child/adolescent (none/some redness, burn, painful burn/blisters), family history of melanoma (yes, no), number of lifetime blistering sunburns (0, 1–4, 5–9, ≥10), cumulative UV flux since baseline (quintiles), average time spent in direct sunlight since high school (<2, 2–5, 6–10, ≥11 h/week), sunscreen use (yes, no), body mass index (<25.0, 25.0–29.9, 30.0–34.9, ≥35.0 kg/m^2), physical activity (quintiles), smoking status (never, past, current with 1–14, 15–24, or ≥25 cigarettes/day), alcohol intake (0, 0.1–4.9, 5.0–9.9, 10.0–19.9, ≥20.0 g/day), coffee intake (0, <1, 1, 2, ≥3 cup/day), and consumption of total other fruit and juice (except citrus products, <0.75, 0.75–1.2, 1.3–1.9, 2.0–2.9, ≥3.0 per day) and total vegetable (<2.0, 2.0–2.9, 3.0–3.9, 4.0–4.9, ≥5.0 per day). Analyses for women were also adjusted for menopausal status and postmenopausal hormone use (premenopausal, postmenopausal never, past or current use). The second multivariable hazard ratios were additionally adjusted for consumption of the other individual citrus products listed in the tables. Results in the HPFS and NHS were pooled using the random-effects model.

for SCC on truncal sites (all $P_{trend} > 0.50$) in association with consumption of overall citrus, grapefruit and orange juice.

We did not find any significant positive association between consumption of other non-citrus fruits, juices and vegetables and risk of BCC or SCC. Instead, we found primarily inverse associations between these food items and disease risk. For example, the fully adjusted HRs comparing the extreme consumption categories of total non-citrus fruit and juice (13 items, ≥3.0 per day versus <0.75 per day) were 0.90 (95% CI: 0.84–0.96, $P_{trend} = 0.049$) for BCC and 0.87 (95% CI: 0.74–1.03, $P_{trend} = 0.06$) for SCC (Supplementary Table 4, available at *Carcinogenesis* Online).

We conducted interaction tests to evaluate whether the association between citrus consumption and risk of SCC and BCC varied by other potential confounders, and found that there was no significant interaction between citrus consumption and other variables adjusted in the analysis (all $P_{interaction} > 0.10$, data not shown). We further examined the risk of other major non-skin cancers (e.g. breast cancer, prostate cancer, lung cancer and colorectal cancer) in association with citrus consumption, and did not find any similar positive associations (data not shown).

Discussion

In the present study, we performed a detailed analysis for the association between citrus consumption and risk of cutaneous BCC and SCC based on data from two large cohorts of men and women. After adjusting for other known skin cancer risk factors and potential confounders, citrus consumption was positively associated with increased risk of BCC and SCC. Among the individual citrus products, grapefruit and orange juice showed consistent positive associations with risk of BCC and SCC. These findings are generally consistent with our previous investigation for citrus consumption and risk of cutaneous malignant melanoma (20), both of which support a potentially increased risk of skin cancer associated with consumption of furocoumarin-containing foods.

The potential photocarcinogenesis of furocoumarins has well-documented biological plausibility. Furocoumarins/psoralens have been identified as a group of carcinogens for decades (5,7). Previous animal experiments have well demonstrated that furocoumarins are able to induce skin tumors in the presence of UV radiation (5–7,11). Mechanistic investigations have revealed a linear relation between epidermal and serum concentrations of psoralens after oral administration, and the appearance of phototoxicity is associated with the serum concentrations of psoralens (27). Furocoumarins plus UV radiation could induce skin erythema, edema, delayed pigmentation and increased activity of epidermal ornithine decarboxylase, which may serve as a biomarker for cutaneous tumor promotion (28,29). Furocoumarins could also induce lethal,

Table 3. Pooled risk of squamous cell carcinoma according to frequency of citrus consumption in the HPFS (1986–2010) and NHS (1984–2010)

	Serving category					*P* for trend
Overall citrus	<2 per week	2–4 per week	5–6 per week	1–1.4 per day	≥1.5 per day	
No. of person-years	400456	371958	465228	418628	319669	
No. of cases	530	672	913	825	604	
Age-adjusted HR (95% CI)	1.00	1.19 (1.02–1.39)	1.20 (1.07–1.33)	1.26 (1.13–1.41)	1.20 (1.07–1.35)	0.001
Multivariable-adjusted HR1 (95% CI)	1.00	1.14 (1.00–1.30)	1.15 (1.02–1.28)	1.22 (1.08–1.37)	1.21 (1.06–1.38)	0.04
Grapefruit	Never	<1 per week	1 per week	2 per week	≥3 per week	
No. of person-years	509252	639008	349832	221228	256619	
No. of cases	635	1182	641	530	556	
Age-adjusted HR (95% CI)	1.00	1.26 (1.14–1.39)	1.24 (1.09–1.42)	1.34 (1.19–1.51)	1.33 (1.18–1.49)	<0.001
Multivariable-adjusted HR1 (95% CI)	1.00	1.16 (1.05–1.28)	1.15 (0.93–1.41)	1.23 (1.05–1.45)	1.30 (1.14–1.48)	<0.001
Multivariable-adjusted HR2 (95% CI)	1.00	1.16 (1.04–1.28)	1.15 (0.94–1.41)	1.25 (1.07–1.47)	1.31 (1.15–1.49)	<0.001
Grapefruit juice	Never	<1 per week	1 per week	2 per week	≥3 per week	
No. of person-years	1092966	476005	165397	110093	131479	
No. of cases	1776	988	301	212	267	
Age-adjusted HR (95% CI)	1.00	1.10 (1.02–1.19)	1.00 (0.87–1.14)	0.95 (0.82–1.09)	1.17 (0.97–1.40)	0.37
Multivariable-adjusted HR1 (95% CI)	1.00	1.03 (0.95–1.11)	0.96 (0.84–1.11)	0.90 (0.78–1.04)	1.17 (1.02–1.36)	0.42
Multivariable-adjusted HR2 (95% CI)	1.00	0.99 (0.91–1.07)	0.91 (0.80–1.04)	0.84 (0.73–0.98)	1.11 (0.97–1.27)	0.85
Oranges	Never	<1 per week	1 per week	2 per week	≥3 per week	
No. of person-years	228853	518773	401333	338726	488254	
No. of cases	295	892	747	752	858	
Age-adjusted HR (95% CI)	1.00	1.11 (0.97–1.27)	1.20 (1.05–1.38)	1.16 (1.01–1.33)	1.02 (0.89–1.17)	0.45
Multivariable-adjusted HR1 (95% CI)	1.00	1.01 (0.88–1.16)	1.06 (0.92–1.22)	1.01 (0.87–1.17)	0.93 (0.80–1.08)	0.12
Multivariable-adjusted HR2 (95% CI)	1.00	0.99 (0.86–1.13)	1.04 (0.90–1.20)	0.99 (0.85–1.15)	0.91 (0.78–1.06)	0.12
Orange juice	<1 per week	1–2 per week	3–4 per week	5–6 per week	≥1 per day	
No. of person-years	499507	451276	395566	310206	319385	
No. of cases	723	816	768	662	575	
Age-adjusted HR (95% CI)	1.00	1.10 (0.95–1.26)	1.17 (1.00–1.36)	1.15 (1.03–1.28)	1.22 (1.10–1.37)	<0.001
Multivariable-adjusted HR1 (95% CI)	1.00	1.04 (0.94–1.15)	1.12 (1.01–1.24)	1.06 (0.95–1.18)	1.18 (1.06–1.32)	0.009
Multivariable-adjusted HR2 (95% CI)	1.00	1.03 (0.93–1.14)	1.11 (0.99–1.23)	1.05 (0.94–1.17)	1.17 (1.05–1.31)	0.01

Multivariable hazard ratios were further adjusted for the covariates listed in the Table 2. Results in the HPFS and NHS were pooled using the random-effects model.

mutagenic and clastogenic effects in mammalian cells and other organisms (16,17). Photoexcited furocoumarins can react with biomolecules, especially with pyrimidine bases in DNA, and form mono- and di-adducts (30,31), and photocycloaddition reactions initiated by furocoumarins play an important role in the formation of DNA adducts (18). Although DNA is generally assumed as the primary site of action for furocoumarins (31,32), they may also bind to other specific and high-affinity sites in mammalian cells which may in turn mediate the furocoumarin-induced phototoxicity in part (33).

Citrus products are known to contain furocoumarins (12–15), and orange and grapefruit are the two mostly consumed citrus fruits in the population over the past several decades, accounting for over 90% of citrus market shares (34). Although grapefruit and orange juice were significantly and positively associated with risk of BCC and SCC, grapefruit juice and oranges did not showed appreciable positive associations with these outcome diseases. Several reasons may help explain the different associations. Grapefruit generally contains higher levels of furocoumarins than oranges (12,13). The null association of grapefruit juice with skin cancer risk may be explained by its much lower consumption levels (Supplementary Table 1, available at *Carcinogenesis* Online) and a much larger number of non-consumers (Table 2) when compared with the other individual citrus products. In contrast, orange juice contributed to more than 50% of the overall citrus consumption, and the significant association of orange juice with skin cancer risk may be explained by its much higher consumption levels when compared with the other individual citrus products (Supplementary Table 1, available at *Carcinogenesis* Online).

In addition, we found that the positive association between citrus consumption and SCC risk appeared to be more apparent for tumors occurred on the body sites with higher continuous sun exposure (head, neck and extremities) than for tumors occurred on the body sites with lower continuous sun exposure (truncal sites). Interestingly, animal experiments have demonstrated that exposure to furocoumarins or UVA alone is not tumorigenic in mice whereas exposure to furocoumarins plus UVA substantially increases the number of mice with skin tumors (6,11). Therefore, our findings may suggest a potential synergistic effect between citrus consumption and UV radiation.

Our study has several strengths, including the prospective design, the large sample size and large number of skin cancer cases, the long-term follow-up over 24–26 years, the repeated assessment of dietary and lifestyle factors, and the ability to include a number of potential confounders. Nevertheless, our study also has several limitations. Our study populations consisted of well-educated Caucasian health professionals and may not be representative of the general population. Nevertheless, restricting the sample to health professionals also reduces potential residual confounding from socioeconomic status. Our results need to be replicated in future studies with sufficient power to detect similar associations among other populations. In addition, the diagnosis of BCC was assessed based on self-reports without pathological validation. However, the health care background of our study participants suggest that their reports were likely to be highly accurate, as proven in previous validation studies (25,26). The positive association between citrus consumption and BCC risk as reported herein is also consistent between the two study cohorts. These data suggest that the bias due to self-reported BCC

Table 4. Pooled risk of site-specific squamous cell carcinoma according to frequency of citrus consumption in the HPFS (1986–2010) and NHS (1984–2010)

	Serving category				*P* for trend
Overall citrus	<2 per week	2–4 per week	5–6 per week	≥1 per day	
No. of person-years	400 456	371 958	465 228	738 297	
Risk of SCC on the body sites with higher continuous sun exposure (head, neck and extremities)					
No. of cases	406	526	722	1104	
Multivariable-adjusted HR (95% CI)	1.00	1.15 (1.01–1.32)	1.17 (1.02–1.33)	1.23 (1.08–1.39)	0.004
Risk of SCC on the body sites with lower continuous sun exposure (truncal sites)					
No. of cases	78	91	126	188	
Multivariable-adjusted HR (95% CI)	1.00	1.01 (0.74–1.38)	1.07 (0.63–1.81)	1.07 (0.76–1.50)	0.70
Grapefruit	Never	<1 per week	1 per week	≥2 per week	
No. of person-years	509 252	639 008	349 832	477 847	
Risk of overall SCC on the body sites with higher continuous sun exposure (head, neck and extremities)					
No. of cases	471	935	496	856	
Multivariable-adjusted HR (95% CI)	1.00	1.21 (1.08–1.36)	1.18 (0.94–1.47)	1.33 (1.15–1.53)	0.01
Risk of overall SCC on the body sites with lower continuous sun exposure (truncal sites)					
No. of cases	93	167	84	139	
Multivariable-adjusted HR (95% CI)	1.00	1.09 (0.83–1.43)	1.03 (0.75–1.43)	1.15 (0.84–1.57)	0.58
Grapefruit juice	Never	<1 per week	1 per week	≥2 per week	
No. of person-years	1 092 966	476 005	165 397	241 572	
Risk of overall SCC on the body sites with higher continuous sun exposure (head, neck and extremities)					
No. of cases	1364	765	236	393	
Multivariable-adjusted HR (95% CI)	1.00	0.97 (0.88–1.06)	0.91 (0.79–1.05)	1.02 (0.91–1.15)	0.89
Risk of overall SCC on the body sites with lower continuous sun exposure (truncal sites)					
No. of cases	247	140	45	51	
Multivariable-adjusted HR (95% CI)	1.00	0.99 (0.80–1.24)	0.98 (0.65–1.47)	0.74 (0.54–1.02)	0.18
Oranges	Never	<1 per week	1 per week	≥2 per week	
No. of person-years	228 853	518 773	401 333	826 980	
Risk of overall SCC on the body sites with higher continuous sun exposure (head, neck and extremities)					
No. of cases	210	699	603	1246	
Multivariable-adjusted HR (95% CI)	1.00	1.03 (0.88–1.21)	1.10 (0.93–1.30)	0.96 (0.81–1.13)	0.41
Risk of overall SCC on the body sites with lower continuous sun exposure (truncal sites)					
No. of cases	43	117	92	231	
Multivariable-adjusted HR (95% CI)	1.00	0.82 (0.57–1.18)	0.80 (0.54–1.18)	0.88 (0.60–1.29)	0.97
Orange juice	<1 per week	1–2 per week	3–4 per week	≥5 per week	
No. of person-years	499 507	451 276	395 566	629 591	
Risk of overall SCC on the body sites with higher continuous sun exposure (head, neck and extremities)					
No. of cases	542	656	587	973	
Multivariable-adjusted HR (95% CI)	1.00	1.06 (0.93–1.21)	1.10 (0.96–1.25)	1.13 (1.01–1.26)	0.03
Risk of overall SCC on the body sites with lower continuous sun exposure (truncal sites)					
No. of cases	102	107	111	163	
Multivariable-adjusted HR (95% CI)	1.00	0.92 (0.70–1.21)	1.13 (0.75–1.72)	1.02 (0.64–1.64)	0.73

Multivariable hazard ratios were adjusted for the covariates listed in the Table 2. Results in the HPFS and NHS were pooled using the random-effects model.

is likely to be minimal and unlikely to affect the study results materially. Furthermore, we do not have data on exposure to arsenic, which has been linked to the risk of skin cancer (35). However, our study participants have better health awareness than the general population and are therefore less likely to be exposed to arsenic, either occupationally or non-occupationally.

In conclusion, our study based on two large cohorts of men and women demonstrated that citrus consumption was associated with an increased risk of cutaneous BCC and SCC, the most commonly diagnosed malignancies in the population. These findings together with our previous investigation on citrus consumption and risk of cutaneous malignant melanoma (20) provide evidence for the potential photocarcinogenic effect of commonly consumed foods. However, our study findings need to be confirmed in future studies before a clear causal inference could be obtained. Nevertheless, given the high prevalence of citrus consumption as well as skin cancers in the population, our findings hold general public health significance and may serve as the first effort to initiate future research in this area.

Supplementary material

Supplementary Tables 1-4. can be found at http://carcin.oxfordjournals.org/

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References

1. American Cancer Society. (2014) Cancer Facts & Figures 2014. American Cancer Society, Atlanta, GA.
2. Wehner, M.R. et al. (2012) Indoor tanning and non-melanoma skin cancer: systematic review and meta-analysis. BMJ, 345, e5909.
3. Rogers, H.W. et al. (2010) Incidence estimate of nonmelanoma skin cancer in the United States, 2006. Arch. Dermatol., 146, 283–287.
4. Guy, G.P. et al. (2011) Years of potential life lost and indirect costs of melanoma and non-melanoma skin cancer: a systematic review of the literature. Pharmacoeconomics, 29, 863–874.
5. GRIFFIN, A.C. et al. (1958) The wave length effect upon erythemal and carcinogenic response in psoralen treated mice. J. Invest. Dermatol., 31, 289–295.
6. Cartwright, L.E. et al. (1983) Psoralen-containing sunscreen is tumorigenic in hairless mice. J. Am. Acad. Dermatol., 8, 830–836.
7. Mullen, M.P. et al. (1984) Carcinogenic effects of monofunctional and bifunctional furocoumarins. Natl. Cancer Inst. Monogr., 66, 205–210.
8. Stern, R.S. et al. (1984) Cutaneous squamous-cell carcinoma in patients treated with PUVA. N. Engl. J. Med., 310, 1156–1161.
9. Stern, R.S. et al. (1998) Oral psoralen and ultraviolet-A light (PUVA) treatment of psoriasis and persistent risk of nonmelanoma skin cancer. PUVA Follow-up Study. J. Natl. Cancer Inst., 90, 1278–1284.
10. Stern, R.S. (2012) The risk of squamous cell and basal cell cancer associated with psoralen and ultraviolet A therapy: a 30-year prospective study. J. Am. Acad. Dermatol., 66, 553–62.
11. Zajdela, F. et al. (1981) 5-Methoxypsoralen, the melanogenic additive in sun-tan preparations, is tumorigenic in mice exposed to 365 nm u.v. radiation. Carcinogenesis, 2, 121–127.
12. Dugo, P. et al. (2000) LC-MS for the identification of oxygen heterocyclic compounds in citrus essential oils. J. Pharm. Biomed. Anal., 24, 147–154.
13. Dugo, P. et al. (2009) Determination of oxygen heterocyclic components in citrus products by HPLC with UV detection. J. Agric. Food Chem., 57, 6543–6551.
14. Frérot, E. et al. (2004) Quantification of total furocoumarins in citrus oils by HPLC coupled with UV, fluorescence, and mass detection. J. Agric. Food Chem., 52, 6879–6886.
15. Lin, Y.K. et al. (2009) Development of a reversed-phase high-performance liquid chromatographic method for analyzing furanocoumarin components in citrus fruit juices and Chinese herbal medicines. J. Chromatogr. Sci., 47, 211–215.
16. Ashwood-Smith, M.J. et al. (1980) 5-Methoxypsoralen, an ingredient in several suntan preparations, has lethal, mutagenic and clastogenic properties. Nature, 285, 407–409.
17. Scott, B.R. et al. (1976) Molecular and genetic basis of furocoumarin reactions. Mutat. Res., 39, 29–74.
18. Kitamura, N. et al. (2005) Molecular aspects of furocoumarin reactions: Photophysics, photochemistry, photobiology, and structural analysis. J. Photochem. Photobiol. C Photochem. Rev., 6, 168–185.
19. Parrish, J.A. et al. (1974) Photochemotherapy of psoriasis with oral methoxsalen and longwave ultraviolet light. N. Engl. J. Med., 291, 1207–1211.
20. Wu, S., et al. (2015) Citrus consumption and risk of cutaneous malignant melanoma. J. Clin. Oncol., pii: JCO.2014.57.4111, epub ahead of print.
21. Feskanich, D. et al. (1993) Reproducibility and validity of food intake measurements from a semiquantitative food frequency questionnaire. J. Am. Diet. Assoc., 93, 790–796.
22. Salvini, S. et al. (1989) Food-based validation of a dietary questionnaire: the effects of week-to-week variation in food consumption. Int. J. Epidemiol., 18, 858–867.
23. Wu, S. et al. (2013) Basal-cell carcinoma incidence and associated risk factors in U.S. women and men. Am. J. Epidemiol., 178, 890–897.
24. Wu, S. et al. (2014) Cumulative ultraviolet radiation flux in adulthood and risk of incident skin cancers in women. *Brit. J. Cancer*, 110, 1855–1861.
25. Colditz, G.A. et al. (1986) Validation of questionnaire information on risk factors and disease outcomes in a prospective cohort study of women. Am. J. Epidemiol., 123, 894–900.
26. van Dam, R.M. et al. (2000) Diet and basal cell carcinoma of the skin in a prospective cohort of men. Am. J. Clin. Nutr., 71, 135–141.
27. Kornhauser, A. et al. (1982) Psoralen phototoxicity: correlation with serum and epidermal 8-methoxypsoralen and 5-methoxypsoralen in the guinea pig. Science, 217, 733–735.
28. Lowe, N.J. (1980) Epidermal ornithine decarboxylase, polyamines, cell proliferation, and tumor promotion. Arch. Dermatol., 116, 822–825.
29. Walter, J.F. et al. (1982) Psoralen-containing sunscreen induces phototoxicity and epidermal ornithine decarboxylase activity. J. Am. Acad. Dermatol., 6, 1022–1027.
30. Tessman, J.W. et al. (1985) Photochemistry of the furan-side 8-methoxypsoralen-thymidine monoadduct inside the DNA helix. Conversion to diadduct and to pyrone-side monoadduct. Biochemistry, 24, 1669–1676.
31. Vos, J.M. et al. (1987) Processing of psoralen adducts in an active human gene: repair and replication of DNA containing monoadducts and interstrand cross-links. Cell, 50, 789–799.
32. Dall'Acqua, F. et al. (1971) Formation of inter-strand cross-linkings in the photoreactions between furocoumarins and DNA. Z. Naturforsch. B., 26, 561–569.
33. Laskin, J.D. et al. (1985) A possible mechanism of psoralen phototoxicity not involving direct interaction with DNA. Proc. Natl. Acad. Sci. USA, 82, 6158–6162.
34. US Department of Agriculture Economic Research Service (2014) Fruit and tree nut data. http://www.ers.usda.gov/data-products/fruit-and-tree-nut-data/yearbook-tables.aspx. (31 January 2015, date last accessed).
35. Surdu, S. (2014) Non-melanoma skin cancer: occupational risk from UV light and arsenic exposure. Rev. Environ. Health, 29, 255–264.