Flavonoid intake and risk of pancreatic cancer in the National Institutes of Health-AARP Diet and Health Study Cohort

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Background: Limited epidemiological studies show inverse associations between dietary flavonoid intake and pancreatic cancer risk, but results are inconsistent and are based on few cases. We examined the association between intake of flavonoids and pancreatic cancer risk in the large, prospective National Institutes of Health-AARP Diet and Health Study Cohort.

Methods: During follow-up through 2006 (median follow-up 10.6 years), 2379 pancreatic cancer cases were identified. We used Cox proportional hazards modelling to estimate hazard ratios (HRs) and 95% confidence intervals (CIs).

Results: We found no association between total flavonoid intake (Q5 *vs* Q1 HR = 1.09, 95% CI: 0.96–1.24) or any flavonoid subtypes and pancreatic cancer risk. Significant interactions were not observed by age, sex, smoking status, BMI or diabetes.

Conclusion: Our results do not support the hypothesis that flavonoids have a protective role in pancreatic cancer carcinogenesis.

Flavonoids are the most common of the plant polyphenolics (Chun *et al*, 2007) and are thought to have chemoprotective properties (Ramos, 2008). Flavones, a flavonoid subtype, have shown inhibition of pancreatic cancer cell growth *in vitro* (Ujiki *et al*, 2006), and quercetin (a flavonol) has demonstrated inhibition of pancreatic cancer growth and prevention of metastasis in mice and *in vitro* (Mouria *et al*, 2002).

Some epidemiological studies on flavonoids and pancreatic cancer suggest an inverse association with intake of specific flavonoids, but results are inconsistent and based on few cases (range *n* = 130–529) (Arts *et al*, 2002; Nöthlings *et al*, 2007; Bobe *et al*, 2008). We aimed to investigate the association between total flavonoid intake and six subtypes (flavan-3-ols, flavanones, flavonols, anthocyanidins, flavones, and isoflavones) and pancreatic cancer risk in the prospective National Institutes of Health (NIH)-AARP Diet and Health cohort.

MATERIALS AND METHODS

Study population. The NIH-AARP Diet and Health Study has been described previously (Schatzkin *et al*, 2001). In short, 566 399 individuals aged 50–71 years completed mailed questionnaires in 1995–1996. After exclusions (proxy respondents, *n* = 15 760; prevalent registry-confirmed cancers (except non-melanoma skin cancer), *n* = 8585; death/moved before baseline questionnaire, *n* = 24; > 2 interquartile ranges above the 75th or below the 25th percentile on a logarithmic scale for calories, *n* = 4810; and total flavonoid intake, *n* = 116), the analytic cohort consisted of 537 104 persons.

Dietary assessment. Each participant completed a 124-item food frequency questionnaire (FFQ) developed at the US National Cancer Institute. The FFQ queried on usual frequency and portion size of foods and beverages consumed over the past year (10

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categories of intake). Flavonoid intakes associated with each FFQ line item were calculated by multiplying amounts reported in the FFQ with food values from the database associated with 24-h dietary recalls collected in the 1994–1996 United States Department of Agriculture (USDA) Continuing Survey of Food Intakes by Individuals (CSFII) (Subar *et al*, 2000). Because the CSFII nutrient database did not originally include flavonoids, the 2007 USDA flavonoid database (385 foods) and the 2002 USDA isoflavonoid database were linked to the CSFII survey to provide nutrient values. Flavonoid values for processed foods were estimated assuming standard recipes. For processed foods that had only flavonoid or isoflavonoid values for raw foods, a 50% loss of flavonoid content during processing and preparation was assumed based on comparison of foods in the flavonoid databases that had both raw and processed values. Responses to the FFQ were validated against two non-consecutive, telephone administered, 24-h dietary recalls obtained within a year of the baseline questionnaire among a stratified random sample of participants (*n* = 2053) (Thompson *et al*, 2008); deattenuated flavonoid correlation coefficients were 0.57 for males and 0.65 for females.

Case ascertainment. Incident cancers were identified by linkage to 11 state registries. Pancreatic cancer deaths ascertained using the US National Death Index were also counted as cases. Cancer registry case ascertainment is estimated to be about 90% complete (Michaud *et al*, 2005). Vital status was determined annually by linkage to the Social Security Administration Death Master File and cancer registry. We included exocrine pancreatic cancer cases diagnosed through 2006 (codes C250-C259 in the *International Classification of Diseases for Oncology*, third edition).

Statistical analysis. Average daily flavonoid intakes were nutrient density adjusted (Willett *et al*, 1997) and divided into sex-specific quintiles. Hazard ratios (HRs) and two-sided 95% confidence

intervals (CIs) were estimated using Cox proportional hazards models with age as the underlying time metric. Person-years of follow-up were calculated from baseline questionnaire to cancer diagnosis, death, emigration from study area or end of follow-up. We examined characteristics in Table 1 as potential confounders. We adjusted for putative risk factors and those that changed log parameter estimates by > 10%. Final models were adjusted for sex, smoking, diabetes, BMI, and intakes of alcohol, calories, saturated fat and red meat.

We evaluated the proportional hazards assumption using interaction terms of the continuous exposure with follow-up time. To calculate *P*-trend, we created ordinal variables using quintile median intake levels and assessed the variable as linear. Potential effect modification was explored using stratified analyses and multiplicative interaction testing. In sensitivity analyses, we excluded subjects with < 1 and < 4 follow-up years to test the influence of subclinical disease on observed associations. Statistical analyses were performed using SAS version 9.2 (SAS Institute, Inc, Cary, NC, USA).

RESULTS

Over a median follow-up of 10.6 years, 2379 pancreatic cancer cases were identified (1541 men and 838 women). Participants with higher flavonoid intake were more educated, less likely to smoke, and consumed less red meat, fat, and alcohol (Table 1). Primary flavonoid dietary sources are presented in Supplementary Table 1.

Total flavonoid intake was not associated with risk of pancreatic cancer (Q5 *vs* Q1 HR = 1.09, 95% CI: 0.96–1.24) (Table 2). Specific flavonoid subtypes showed no significant associations with pancreatic cancer risk comparing extreme quintiles, although anthocyanidin intake showed a slight positive trend ($P_{trend} = 0.02$).

Table 1. Baseline characteristics of National Institutes of Health-AARP Diet and Health Study participants (*n* = 537 104) by sex-specific quintile of energy-adjusted total flavonoid intake

	Men (*n* = 316 556)			Women (*n* = 220 548)		
Characteristics	**Quintile 1**	**Quintile 3**	**Quintile 5**	**Quintile 1**	**Quintile 3**	**Quintile 5**
Median daily flavonoid intake (mg per 1000 kcal)	31.6	90.8	326.9	39.6	120.5	481.6
Median age at baseline (years)	62.7	63.2	63.1	62.1	62.5	62.7
Non-Hispanic White (%)	92.7	92.9	92.0	90.0	88.7	91.0
College or greater education (%)	38.4	47.9	44.9	25.9	32.0	30.1
Mean body mass index (kg m^{-2})	27.3	27.2	27.3	27.0	26.6	26.7
Smoking history (%)[a]						
Never smoker	23.6	30.4	31.3	37.8	45.3	45.7
Former	53.9	56.4	54.1	36.1	38.0	35.0
Current smoker	18.7	9.5	10.5	22.5	13.3	15.5
Self-reported diabetes (%)	11.1	9.8	10.5	8.1	7.3	7.4
Mean daily nutrient intake						
Calories (kcal per day)	2158	1994	1830	1633	1573	1447
Saturated fat (g per 1000 kcal)	11.9	10.0	10.4	11.5	9.8	10.2
Red meat (g per 1000 kcal)	43.8	35.4	36.4	33.9	27.7	28.6
Folate (mg per 1000 kcal)	118.4	154.9	166.1	133.2	175.3	188.5
Alcohol (g per day)	19.3	19.2	10.5	7.0	6.0	4.4
Multivitamin (% reporting any use)	48.8	53.9	51.8	56.9	62.3	60.1
Physical activity ⩾ 20 min, at least 5 times per week (%)	18.9	22.5	20.6	14.1	17.5	15.5

[a]Column percentages may not add up to 100% due to rounding or missing data.

Table 2. Hazard ratios and 95% confidence intervals for pancreatic cancer by quintiles of energy-adjusted intakes (mg per 1000 kcal) of total flavonoids and subtypes among 537 104 participants in the National Institutes of Health-AARP Diet and Health Study

	Q1	Q2	Q3	Q4	Q5	P-trend
Total flavonoids (mg per day)[a]	34.3	65.2	101.7	171.4	388.0	
Cases (n)	483	450	475	476	495	
Model 1[b]	1.00	0.90 (0.79–1.02)	0.94 (0.83–1.07)	0.95 (0.84–1.08)	0.99 (0.87–1.12)	0.477
Model 2[c]	1.00	0.98 (0.86–1.11)	1.06 (0.93–1.20)	1.07 (0.94–1.22)	1.09 (0.96–1.24)	0.113
Flavan-3-ols (mg per day)[a]	8.6	22.9	51.1	118.7	331.2	
Cases (n)	496	455	456	484	488	
Model 1	1.00	0.92 (0.81–1.04)	0.93 (0.82–1.05)	0.99 (0.87–1.12)	0.99 (0.87–1.12)	0.477
Model 2	1.00	0.97 (0.85–1.10)	0.98 (0.86–1.11)	1.04 (0.92–1.18)	1.03 (0.91–1.17)	0.335
Flavanones (mg per day)[a]	2.6	9.0	18.8	30.6	55.4	
Cases (n)	484	486	473	467	469	
Model 1	1.00	0.99 (0.87–1.12)	0.91 (0.80–1.03)	0.87 (0.77–0.99)	0.87 (0.77–0.99)	0.017
Model 2	1.00	1.07 (0.94–1.21)	1.02 (0.90–1.17)	1.01 (0.89–1.16)	1.06 (0.92–1.21)	0.663
Flavonols (mg per day)[a]	4.5	7.2	9.6	12.9	21.2	
Cases (n)	483	469	482	466	479	
Model 1	1.00	0.96 (0.84–1.09)	0.99 (0.87–1.13)	0.96 (0.85–1.09)	0.99 (0.87–1.13)	0.868
Model 2	1.00	1.02 (0.89–1.16)	1.08 (0.93–1.21)	1.06 (0.93–1.21)	1.09 (0.95–1.24)	0.264
Anthocyanidins (mg per day)[a]	2.1	4.3	6.4	9.2	15.2	
Cases (n)	498	476	459	458	488	
Model 1	1.00	0.90 (0.79–1.02)	0.85 (0.75–0.96)	0.83 (0.73–0.95)	0.88 (0.78– 1.00)	0.661
Model 2	1.00	0.99 (0.88–1.14)	0.98 (0.86–1.12)	1.01 (0.88–1.15)	1.10 (0.96–1.27)	0.022
Flavones (mg per day)[a]	0.2	0.4	0.6	0.9	1.6	
Cases (n)	473	445	517	483	461	
Model 1	1.00	0.91 (0.80–1.03)	1.03 (0.91–1.17)	0.95 (0.84–1.08)	0.91 (0.80–1.03)	0.224
Model 2	1.00	1.02 (0.86–1.12)	1.17 (1.03–1.34)	1.11 (0.97–1.27)	1.09 (0.95–1.25)	0.172
Isoflavones (mg per day)[a]	0.1	0.1	0.2	0.3	0.5	
Cases (n)	497	478	483	470	451	
Model 1	1.00	0.94 (0.83–1.07)	0.95 (0.83–1.07)	0.91 (0.80–1.03)	0.88 (0.77–1.00)	0.038
Model 2	1.00	0.97 (0.85–1.10)	0.99 (0.87–1.12)	0.96 (0.85–1.10)	0.96 (0.84–1.09)	0.435

[a]Reported intakes (mg per day) are flavonoid subtype-specific median values.
[b]Model 1 is adjusted for energy and sex, with age as the underlying time metric.
[c]Model 2 is adjusted for sex, smoking (never, quit >10 years before, quit 5–9 years before, quit 1–4 years before, quit <1 year prior or current smoker ≤20 cigarettes per day, quit <1 year prior, or current smoker >20 cigarettes per day), diabetes (yes/no), body mass index (18.5–<25, 25–<30, 30–≤50 kg m^{-2}, and missing), alcohol, calories, saturated fat and red meat intake (continuous variables), with age as the underlying time metric.

In additional analyses, sex, age, smoking (Supplementary Table 2), BMI, and diabetes did not appear to modify the association between flavonoids and pancreatic cancer risk (data not shown). Excluding subjects with <1 or <4 years of follow-up did not change the association.

DISCUSSION

In this large, prospective study, we observed no association between flavonoid intake and pancreatic cancer. In contrast, lower pancreatic cancer risk was observed with higher flavonol intake (Q5 vs Q1 HR = 0.77, 95% CI: 0.58–1.03) in the Multiethnic Cohort (MEC) (Nöthlings et al, 2007). The MEC participants might have unique tea-consumption patterns, given the large Japanese population, which was the largest ethnic group represented in the highest flavonol quintile. When MEC was stratified by ethnicity, no association was observed among Caucasians. Mean flavonol intake was estimated at 14.8 mg per day in MEC, compared with 20.4 mg per day in NIH-AARP, although it is difficult to compare values from different FFQs, as they are only semi-quantitative. In the Alpha Tocopherol Beta Carotene (ATBC) Study of male Finnish smokers, no overall association between flavonoid intake and pancreatic cancer risk was observed, but in stratified analysis, higher total flavonoid intake was associated with decreased pancreatic cancer risk in trial participants randomised to placebo (Q4 vs Q1 HR = 0.36, 95% CI: 0.17–0.78) but not to supplemental α-tocopherol and/or β-carotene ($P_{interaction}$ = 0.002) (Bobe et al, 2008). The ATBC study included fewer cases, and flavonoid intake was lower than in our population (median energy-adjusted daily total flavonoid intake 121.5 mg in ATBC highest quintile compared with 388.0 mg for NIH-AARP), perhaps because of differences in dietary patterns in Finland, dietary questionnaires, or the number and type of foods included in reference databases. The Iowa Women's Health Study showed no association (Q4 vs Q1 HR = 0.74, 95% CI: 0.46–1.20) between dietary catechins and pancreatic cancer (Arts et al, 2002). We captured catechins under the flavan-3-ol subtype, rather than isolating the specific flavonoid.

In vitro, polyphenols have demonstrated antioxidant properties (Halliwell, 2008). Antioxidants may protect against cancer risk by stimulating apoptosis and inhibiting reactive oxygen species (ROS) from inducing oxidative carcinogenic damage in DNA (Salganik, 2001). However, polyphenols have also shown pro-oxidant properties (Ahmad et al, 1992). Laboratory studies have reported that the role of ROS in pancreatic cancer cells is complicated, as some enzymes mediating ROS production may protect pancreatic cancer cells from apoptosis (Vaquero et al, 2004). The relationship

between antioxidant intake from flavonoids and pancreatic cancer may also differ *in vivo* from *in vitro*. One cross-over feeding study showed little variation in human F2-isoprostane plasma levels (biomarkers of oxidative damage) between flavonoid-rich and -poor diets (Halliwell, 2008). Generally, human plasma concentrations of flavonoids are low, and flavonoid metabolites show less antioxidant activity than parent compounds (Halliwell *et al*, 2005).

Strengths of our study include the largest sample of incident pancreatic cancer cases in a flavonoid analysis to date, allowing for exploration of effect modification by other risk factors. Our flavonoid intake data were linked to updated USDA food databases, including a greater scope of foods than in previous studies. Limitations of our study include measurement errors associated with FFQs and potential errors in the nutrient database used to calculate flavonoid values. The updated food databases in our study provided more foods with calculated flavonoid values, but make comparison of calculated flavonoid levels between studies difficult. Although our reported flavonoid values are higher than reported levels in other studies, our FFQ did not include specific questions on flavonoid-rich foods, such as specific types of tea, berries (other than strawberries), and tofu. Still, usual intake of these foods is low in the United States (US Population, 2001–04), and thus unlikely to greatly affect intake estimates. We could not account for absorption levels of various flavonoids because the understanding of flavonoid absorption and metabolism is still limited and differs by individual flavonoid (Scalbert *et al*, 2002). Finally, our participants were largely non-Hispanic white, and thus our results may not generalise to other populations.

In conclusion, we observed no association between flavonoid intake and pancreatic cancer risk. Future studies may want to evaluate flavonoid intake among non-white populations or populations with broader exposure ranges. However, our results suggest evaluating other modifiable risk factors for pancreatic cancer.

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