Published in final edited form as:

J Periodontol. 2020 August ; 91(8): 1057–1066. doi:10.1002/JPER.19-0463.

DIETARY FLAVONOID INTAKE AND RISK OF PERIODONTITIS

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Abstract

BACKGROUND: The anti-inflammatory effect associated with flavonoids containing foods and beverages could potentially impact the risk of periodontal disease. We prospectively investigate the associations between habitual flavonoid intake and incidence of periodontitis.

METHODS: The study population was 34,940 men from the Health Professionals Follow-Up Study, who were healthy and free of periodontal disease at baseline (1986). Participants in the study provided medical and dental history through mailed questionnaires biennially, and provided

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Summary of finding: Despite multiple plausible mechanisms by which habitual flavonoid intake may reduce the risk of periodontitis, no association was detected in the study population over a 24-year follow-up.

CONFLICT OF INTEREST STATEMENT

The authors had no conflict of interest to disclose.

dietary data through semi-quantitative food frequency questionnaires every 4 years. We examined the associations between total flavonoids and 6 flavonoid subclasses (flavonoid polymers, anthocyanins, flavan-3-ols, flavanones, flavones and flavonols) and incidence of periodontitis using Cox proportional hazard models. We adjusted for age, smoking, body mass index, physical activity, alcohol consumption, Alternative Healthy Eating Index, and diabetes.

RESULTS: There was no association between total flavonoids and the risk of periodontitis. The hazard ratio comparing the highest quintile of total flavonoid to the lowest quintile was 0.97 (95% confidence interval: 0.87 -1.08, *p*-value for trend = 0.61). Similar comparisons for flavonoids subclasses also did not show significant associations.

CONCLUSIONS: No association was detected between habitual flavonoid intake and risk of periodontitis in the study population.

Keywords

periodontitis; epidemiology; inflammation; diet

INTRODUCTION

Periodontitis is an inflammatory disease that affects hard and soft tooth supporting tissue and could lead to tooth loss.[1] Periodontitis has been associated with a plethora of systemic conditions including, cardiovascular health, preterm low-weight birth, impaired glycemic control, and impaired cognitive function.[2, 3] It is estimated that 46% of the US population 30 y or older have some form of periodontal disease, with about 8.9% having severe periodontitis.[4]

One of the seminal historic nutritional discoveries was establishing the association between vitamin C deficiency and scurvy, which is characterized by impairment of periodontal tissue due to defective collagen synthesis.[5] Yet, the relationship between diet and periodontal disease is not well understood.[6] Better understanding of the relationship between diet and periodontal disease is vital to bolstering knowledge about periodontitis pathology. In addition, it should aid in identifying modifiable risk factors that could benefit both individual and community level prevention of periodontal disease.[6]

Flavonoids are naturally occurring chemical compounds found in a variety of plant food and beverages, such as fruits, vegetables, tea, coffee and red wine. Inverse associations between consumption of some fruits/vegetables, coffee and tea, and periodontal disease have been reported.[7-10] There is some evidence that flavonoid intake in humans is associated with weight maintenance,[11] reduced systemic inflammatory biomarkers,[12] and reduced risk of chronic diseases including type-2 diabetes and cardiovascular disease.[13-15] *In vitro* models showed that flavonoids are associated with inhibition of pro-inflammatory enzymes such as cyclooxygenase-2, lipoxygenase, and inducible nitric oxide synthase; reduction of tumor necrosis factor-α, interleukin (IL) 1β, IL-6, and IL8 expression; and release of anti-inflammatory IL-10.[16]

Flavonoid compounds have displayed a promising potential in the prevention and treatment of periodontal disease through anti-inflammatory and antibacterial activities, mainly in

animal/cellular models, or in short-term clinical trials (using flavonoid extracts).[17-22] The prospective relationship between flavonoid intake and incidence of periodontitis has not been evaluated in humans. We hypothesize that greater habitual intake of flavonoids is associated with a lower risk of periodontal disease. The aim of this study was to prospectively evaluate the relationship between flavonoid intake and incidence of periodontal disease in the Health Professionals Follow-up study (HPFS).

METHODS

Study population

The HPFS is an ongoing cohort consisting of 51,529 male health professionals (dentists, pharmacists, optometrists, osteopathic physicians, podiatrists, and veterinarians) who were 40-75-year-old at the study baseline (1986), and who answered the initial mailed questionnaire. The questionnaires collected information about medical and dental history, in addition to lifestyle behavior, and body measurements (e.g. height and weight). Data were updated biennially. Participants' dietary data were collected through validated semi-quantitative food frequency questionnaire (FFQs) every 4 years. Standard portion sizes were provided for each food, and participants were asked about the frequency of intake ranging from "almost never" to "≥ 6 times/day". The reproducibility and validity of the FFQ in measuring diet for this cohort has been reported previously, and they showed good correlation with diet records.[23, 24]

We excluded edentulous participants (n=485) and those who reported periodontitis at baseline (n=8,333). We also excluded participants with history of myocardial infarction (n=1,486), coronary artery surgery (n=671), diabetes (n=809), or cancer (n=1,317) at baseline, as these conditions may tend to modify dietary habits. Participants who only responded to the baseline questionnaire (n=3,309), those who had missing periodontal information (n=1,117), and those who were missing data on body mass index (BMI) (n=902), physical activity (n=128), or age (n=36) at baseline were also excluded. In addition, men who reported caloric intake that was outside the plausible range (800-4200 kcal/day), and those who left 70 or more out of 131 FFQ items blank were also excluded (n=1,033). FFQs in this cohort with less than 70 blank spaces, has been found valid if the missing items were considered zero, and there is was blank whole section.[23, 24] The analyses included 34,940 men at baseline, after all the exclusions. The study was approved by the institutional review boards of the Harvard T.H. School of Public and the Brigham and Women's Hospital, and completion of the questionnaires constituted informed consent.

Outcome Assessment

The outcome of the study was incidence of periodontal disease which was defined as answering "yes" to the question "Have you been professionally diagnosed with periodontal disease with bone loss?" in the biennial mailed questionnaires. Self-reported periodontal disease in this cohort was evaluated in a subsample against bitewing radiographs and the results showed good validity.[25, 26] The positive predictive value was 0.76, among dentists in the HPFS, and 0.83 among non-dentists, while the negative predictive value was 0.74 for dentists and was 0.69 for non-dentists.

Main Exposures Assessment

The main exposure of the study was dietary flavonoids estimated from the FFQs. The frequency of intake , including the portion size, of flavonoid containing food and beverages was multiplied by the flavonoid content, using the US Department of Agriculture (USDA) database.[27, 28] Six subclasses of flavonoids were calculated; flavanones (eriodictyol, hesperetin and naringenin), anthocyanins (cyanidin, delphinidin, malvidin, pelargonidin, petunidin and peonidin), flavan-3-ols (catechin, gallocatechin, epicatechin, epigallocatechin, epicatechin 3-gallate and epigallocatechin 3-gallate), flavonoid polymers (proanthocyanidins, theaflavins and thearubigins), flavonols (quercetin, kaempferol, myricetin and isorhamnetin), and flavones (luteolin, and apigenin), as well as the sum of all of the subclasses.[29] We did not investigate isoflavone in the current study due to the very low intake in this cohort. The cumulative average of each flavonoid subclass from baseline until the start of each 2-year follow-up interval was calculated, and then divided into quintiles. The same approach was used for total flavonoid intake.

Covariates Assessment

To control for confounding, the analysis was adjusted for significant risk factors of periodontitis i.e. age, smoking, BMI, physical activity, alcohol consumption, and diabetes [6, 30-34]. Incidence of diabetes during the follow-up was collected in the biennial questionnaire. Self-reported diabetes in this cohort has shown good validity.[35] BMI values (kg/m^2) were categorized as follows: underweight (<18.5), healthy weight (18.5–24.9), overweight (25–29.9), and obese (≥30) and we used the updated biennial measures, because updated recent BMI was significantly associated with risk of periodontal disease in this cohort.[34] Physical activity data were collected through the questionnaires. We used the Metabolic Equivalent of Task (MET) to calculate the total MET hours (MET-h) for each participant. Physical activity data was categorized into quintiles, and we used the updated measures for each follow up. Total alcohol intake was estimated from the FFQ, and we used the cumulative average of intake classified into: 0, 0.1–4.9, 5–14.9, 15–29.9 and ≥30 grams per day (g/day). The cumulative average caloric intake was also estimated from the FFQs and data was categorized into quintiles. We adjusted for smoking using the Comprehensive Smoking Index (CSI), an algorithm that combines smoking data from each questionnaire cycle, and takes into consideration: smoking duration in years, smoking intensity (i.e. number of cigarettes smoked per day), time since smoking cessation, and a specific biologic half-life of smoking effect on the disease, which is estimated to be 1.5 years for periodontitis.[36, 37] To adjust for other dietary factors, we used the Alternative Healthy Eating Index (AHEI) without fruits and vegetables components.[38]. Deaths in the HPFS were reported by next of kin, or coworkers, or were obtained from postal service authorities or from the National Death Index.

Statistical Analysis

Descriptive statistics at baseline by quintile of total flavonoid intake were calculated as means for continuous data and percentage for categorical data. We used Cox proportional hazard models with age in months as the underlying timescale to estimate the hazard ratios of periodontitis comparing each higher quintile of total or subclass flavonoid to the lowest.

Person-time was calculated from the return of the baseline questionnaire until incidence of periodontal disease, death, last available response, or end of follow-up (January 31st, 2010), whichever was first. Models were adjusted for smoking, BMI, physical activity, alcohol intake, diabetes, AHEI, and total caloric intake. To conduct the test for linear trend, we assigned the median value of each flavonoid's quintile and created continuous variables. For missing smoking data at baseline, we assigned a CSI value of 0. We handled missing exposure or covariates data during follow-up by carrying forward values from the last cycle. We did not adjust for multiple comparisons when investigating flavonoids subclasses.[39]

As flavonoid intake was associated with weight maintenance,[11] we evaluated whether BMI is an important mediator by comparing the models with and without including BMI as a covariate. We also stratified the models by updated BMI categories (18.5-24.9, 25-29.9, and ≥30). We excluded underweight BMI (<18.5) from the stratified analysis due to the small number in the cohort (contributed less than 0.5% of the total person-time). We also stratified the total flavonoid analysis by age (those ≥65 versus <65), physical activity (those above the median versus below), diabetes over the follow-up, smoking at baseline (current, former and never) and profession (dentist versus non-dentist). To test for the statistical significance of interaction by stratifying variables, we created indicator variables for being a dentist, updated binary physical activity level, updated binary age, and diabetes during follow up. We fitted adjusted models that included interaction terms between the continuous variables of total flavonoid intake (the same variables we used to test for linear trends) and the created indicator variables. For BMI, we created an interaction term between updated continuous BMI and the continuous variables of total flavonoid intake. For smoking, we created the interaction term using continuous CSI. We used a Wald test to calculate the *p*-value of interaction, a one degree of freedom test. We performed the analysis using SAS for UNIX statistical software.*

Results

Over the 24 years of follow up (747,517 person-years) 3,738 new cases of periodontal disease were reported. Table 1 shows the distribution of age-standardized baseline characteristics by quintiles of total flavonoid assessed from the baseline questionnaire. At baseline, participants with higher flavonoids intake were more physically active, had higher AHEI score and consumed more fruits and vegetables compared to participants with lower intake, whereas those in the lowest quintile were more likely to be current smokers.

Total flavonoid intake was not associated with the risk of periodontal disease. In the age adjusted and the fully adjusted models, none of the flavonoid subclasses other than flavonols, were associated with risk of periodontitis (table 2). Flavonols intake was associated with slightly higher risk of periodontal disease; multivariate hazard ratio in the fourth versus the lowest quintile was 1.16 (95% CI: 1.04-1.30), and the trend was significant in the age adjusted model only (table 2). For all flavonoids, adding BMI to the adjusted models did not change the results. There was no association between total flavonoid and periodontitis within any of the subgroups (table 3). Evidence of statistically significant

*SAS Institute, Inc., Cary, NC, USA, version 9.4.

interaction was detected for smoking, but the association was null in all the categories (table 3). In tables 2 and 3, we present the number of cases / the total person-time contributed by individuals within each quintile. Although the analysis was done using age in months as the underlying timescale, we presented person-years in the tables for easier interpretation of the time contributed by individuals from the return of the baseline questionnaire until the earliest of the following: incidence of periodontitis, death, last available response, or end of follow-up (January 31st, 2010).

We further conducted food based analyses for the main sources of total flavonoid, and main sources of flavonols. Tea is the main source of total flavonoid in this cohort. Other sources included orange juice, orange, apple, strawberry, blueberry, and red wine. The main sources of flavonols were: onion, apple, and tea. Data on onion intake was not available in the 1986 cycle. Starting in 1990, participants were asked if they consumed onion as a vegetable or as a garnish. Hence, we started the follow-up for onion analysis in 1990 (n = 20,897 and number of periodontitis cases = 2,009). Onion intake, especially as a garnish, was associated with a higher risk of periodontitis (table 4). Red wine intake was also associated with a slightly higher risk of periodontal disease, even though we controlled for total alcohol intake in the multivariate models. There was no association between any of the other foods and periodontitis. The slight association between flavonols and periodontitis was attenuated and lost significance after adding onion to the model, either as a garnish or as a vegetable. We also evaluated our results using an alternative method for control of smoking. i.e. never, former, current 1-14, current 15-24, and current 25+ cigarette/day. Missing data were handled using a missing indicator. The results were similar to our main analysis.

Discussion

In this prospective study of US health professional men, we did not find a significant association between total flavonoids intake and incidence of self-reported periodontal disease over the 24-y follow-up of the study. Except for flavonols, no association was detected between any of the flavonoids subclasses and incidence of periodontal disease. Intake of flavonols was associated with a slightly higher risk of periodontal disease. The main food sources of flavonols in this cohort were: onions, apples and tea. In the sub-analysis of flavonols sources, onion consumption was modestly associated with periodontitis, and the observed positive association with flovonols seemed attributable to onion consumption. However, this association between onion and periodontitis should be viewed with caution. Most of the reported onion consumption in our cohort was as a garnish. It is possible that this intake is correlated with other dietary/or non-dietary lifestyle factors, that we did not adjust for, and could confound the association. In addition, the association could be due to chance, because we did evaluate multiple flavonoid compounds and their dietary sources.

To our knowledge this is the first study that investigated the association between habitual intake of flavonoid and incidence of periodontal disease. The main strength of our study is the large cohort with available updated measures on exposure, outcome, and main confounding variables, and the long follow-up. The study participants were highly motivated health professionals which mitigates the risk of information bias. Our study is a secondary

analysis of an ongoing cohort. Edentulous and periodontitis patients at baseline were not at risk of developing the outcome and were not included. We did not include participants with major health issues at baseline, as they may have significantly modified their dietary and lifestyle habits, and the reported diet would not be representative of their long-term diet (and similarly for other lifestyle habits). We also did not include those with large amount of missing data as their data may not be valid. These exclusions may limit generalizability somewhat but is not expected to affect the validity of the results.

Our study has several limitations. Although we adjusted for multiple confounding factors, bias due to unmeasured and residual confounding is possible. In addition, the generalizability of the results to other populations, including females, is uncertain. However, the homogeneity of the study population does enhance the internal validity of the study, as confounding by education and socioeconomic factors was minimized. Another important limitation of the study is the self-report method for data collection, which due to misclassification, could have attenuated the results. [40] However, the validity of self-administered FFQs has been evaluated previously, and was proven as an effective method for measuring long-term diet intake.[23, 24] In addition, we used the cumulative averages of flavonoids intake, and adjusted the analysis for total caloric intake, which should reduce measurement error issues. The outcome in our analysis was also self-reported consisting of a response to the question “have you been professionally diagnosed with periodontal disease with bone loss?” in the biennial questionnaire. When validated within sub-samples of the HPFS, the positive predictive value for self-reported periodontal disease was 0.76, among dentists in the cohort, and 0.83 among non-dentists, while the negative predictive value was 0.74 for dentists and 0.69 for non-dentists.[25, 26] The systematic review by Blicher et al. found the phrasing of the question about periodontitis in the HPFS questionnaire performed well when compared to other questions in other studies.[41] Hence, self-reported periodontal disease can be considered a valid, well-defined end-point in this cohort. However, periodontitis diagnosis on a patient-level clinical setting varies in severity and extent, and is rarely described as a yes/no dichotomy. It is possible that in spite of some existing impact of flavonoids on periodontal tissue (such as differences in periodontal probing depth, clinical attachment loss, or periodontal bleeding tendency), we were unable to detect these using our measure of self-reported “periodontal disease with bone loss”. We did not have enough data about periodontal treatment in our cohort, which could be viewed as a limitation. However, we excluded participants with periodontitis at baseline, hence treatment prior to baseline is not pertinent. In our survival analysis, we considered a participant as a periodontitis case, if they reported incidence of periodontitis, and they did not contribute person-time after that. Hence, treatment of periodontitis during follow up would not affect our results. Residual confounding due to variations in routine scaling and root planing could be a limitation.

Flavonoids are naturally available in a variety of fruits, vegetables, coffee, tea and red wine. Several studies found a protective influence of these foods/beverages for periodontal disease. Schwartz et al. reported reduced progression of periodontal disease with higher intake of high-fiber fruits among men 65 and older.[9] They reported that per serving of high-fiber fruits, there was 14% risk reduction of alveolar bone loss (HR: 0.86, 95% CI = 0.78-0.95), and 5% risk reduction in periodontal probing depth (HR: 0.95, 95% CI =

0.91-0.99). Yoshihara et al. found that intake of vegetables was inversely associated with incidence of periodontal disease.[8] Kushiyama et al. reported a slight inverse association between green tea consumption and periodontal disease.[7] One cup per day was associated with a 0.02 mm reduction in mean probing depth, and 0.03 mm reduction in mean clinical attachment. Ng et al. reported that coffee consumption was slightly inversely associated with the number of teeth with periodontal bone loss over the follow-up.[10] All the above-mentioned studies however used individual diet items and clinical measures of periodontal disease that allowed detection of minor differences between groups. In a cross-sectional study among Japanese young females (18-22 y), Tanaka et al. found an inverse association between soy and isoflavone intake and self-reported periodontal disease.[42] We were unable to evaluate isoflavone in our study due to the extremely low-intake in our cohort that is far from the minimal dose required for biological effects.[43] In a cross-sectional study, Susin et al. defined periodontitis as having ≥ 30% of the teeth with periodontal attachment loss of ≥ 5 mm, and found an inverse association between moderate wine intake and periodontitis among Brazilian men but not women.[44] Red wine in our analysis had a positive association with periodontal disease, even when adjusting for total alcohol intake.

Conclusion

In conclusion, we observed no association between habitual flavonoid intake and the risk of periodontal disease in our study. In the food-based analysis, we detected an increased risk of periodontitis associated with onion and red wine intake. Future research could further investigate the relationship between onion and periodontal disease, as it is unlikely related to its flavonoid. In addition, it would be of value to evaluate the association between flavonoid intake and periodontal health using clinical measures, and in other populations.

ACKNOWLEDGMENTS

The authors would like to thank the participants and staff of the Health Professionals Follow-Up Study. The study was supported by research grant UM1 CA167552, Health Professionals Follow-Up Study infrastructure grant, from the National Institutes of Health (NIH).

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Table 1

Age-standardized baseline (1986) characteristics by quintile of total flavonoids intake in the baseline questionnaire.

	Quintile 1	Quintile 2	Quintile 3	Quintile 4	Quintile 5
***N* (%)**	6,892 (20%)	6,945 (20%)	7,007 (20%)	7,045 (20%)	7,051 (20%)
Median intake (mg/day)	88.2	153.6	223.6	327.1	593.8
Age (years)	51.5 ± 9.3	52.2 ± 9.4	52.5 ± 9.4	52.6 ± 9.5	52.7 ± 9.4
White	95%	96%	96%	95%	95%
BMI (kg/m^2)	25.7 ± 3.4	25.5 ± 3.4	25.4 ± 3.2	25.4 ± 3.3	25.4 ± 3.3
Smoking:					
Former	41%	40%	40%	39%	40%
Current	14%	9%	7%	6%	7%
Alcohol (gm/day)	11.2 ± 15.0	11.5 ± 15.3	11.3 ± 15.0	11.2 ± 15.2	10.5 ± 15.1
Total Activity (MET/Week)	17.4 ± 29.3	20.3 ± 26.0	22.6 ± 28.3	24.0 ± 33.1	24.1 ± 33.8
AHEI score	49.1 ± 11.1	51.0 ± 11.1	53.4 ± 11.1	54.1 ± 11.3	54.7 ± 11.7
AHEI score without fruits & vegetables.	37.5 ± 9.5	37.0 ± 9.3	37.4 ± 9.1	37.2 ± 8.8	37.1 ± 9.0
Intake (servings/day)					
Total fruits	0.7 ± 0.6	1.2 ± 0.7	1.7 ± 0.9	1.9 ± 1.2	2.3 ± 1.8
Total vegetables	2.2 ± 1.4	2.7 ± 1.3	3.1 ± 1.6	3.3 ± 1.8	3.6 ± 2.0
Dentist	55%	57%	58%	58%	57%
Number of teeth:					
25-32	87%	88%	89%	89%	89%
17-24	10%	9%	9%	9%	9%
11-16	2%	1%	1%	1%	1%
1-10	1%	1%	1%	1%	1%

Values are means ± SD or percentages and are standardized to the age distribution of the study population.

AHEI= Alternative healthy eating index.

Table 2.

Hazard ratios and 95% CIs relating quintiles of intake of total flavonoids and subclasses with incidence of periodontitis.

	Quintile 1	Quintile 2	Quintile 3	Quintile 4	Quintile 5	*P* for tend[†]
Total Flavonoids						
Cases/p-yrs[≠]	783/140,734	750/141,527	718/141,702	749/141,636	738/141,571	
Model 1	1.0 (ref)	0.96 (0.87-1.06)	0.93 (0.84-1.03)	0.98 (0.88-1.08)	0.94 (0.85-1.04)	0.37
Model 2	1.0	0.99 (0.90-1.10)	0.97 (0.87-1.08)	1.02 (0.91-1.13)	0.96 (0.87-1.07	0.60
Model 3	1.0	0.99 (0.90-1.10)	0.97 (0.87-1.08)	1.02 (0.91-1.13)	0.97 (0.87-1.08)	0.61
Polymers						
Cases/p-yrs[≠]	803/140,690	691/141,712	762/141,624	760/141,496	722/141,649	
Model 1	1.0 (ref)	0.88 (0.79-0.97)	0.99 (0.89-1.09)	0.98 (0.89-1.08)	0.91 (0.82-1.01)	0.30
Model 2	1.0	0.91 (0.82-1.01)	1.03 (0.93-1.14)	1.03 (0.92-1.14)	0.94 (0.85-1.05)	0.53
Model 3	1.0	0.91 (0.82-1.01)	1.03 (0.93-1.14)	1.03 (0.92-1.14)	0.94 (0.85-1.05)	0.53
Anthocyanins						
Cases/p-yrs[≠]	823/141,011	735/141,609	744/142,130	735/ 141,411	701/141,010	
Model 1	1.0 (ref)	0.92 (0.83-1.01)	0.95 (0.86-1.06)	0.95 (0.86-1.05)	0.97 (0.87-1.07)	1.00
Model 2	1.0	0.94 (0.85-1.04)	0.98 (0.88-1.09)	0.99 (0.89-1.10)	1.00 (0.89-1.11	0.61
Model 3	1.0	0.94 (0.85-1.04)	0.98 (0.88-1.09)	0.99 (0.89-1.10)	1.00 (0.89-1.12)	0.60
Flavan-3-ols						
Cases/p-yrs[≠]	801/140,804	716/141,376	714/141,774	798/141,720	709/141,497	
Model 1	1.0 (ref)	0.91 (0.83-1.01)	0.93 (0.84-1.03)	1.04 (0.94-1.14)	0.89 (0.81-0.99)	0.17
Model 2	1.0	0.93 (0.84-1.03)	0.93 (0.84-1.04)	1.04 (0.94-1.16)	0.90 (0.81-0.99	0.15
Model 3	1.0	0.93 (0.84-1.03)	0.94 (0.84-1.04)	1.05 (0.94-1.16)	0.90 (0.81-1.00)	0.15
Flavanones						
Cases/p-yrs[≠]	780/140,875	726/141,610	756/141,605	719/141,300	757/141,781	
Model 1	1.0 (ref)	0.93 (0.84-1.03)	0.95 (0.86-1.05)	0.87 (0.79-0.97)	0.92 (0.83-1.02)	0.07
Model 2	1.0	0.96 (0.87-1.06)	0.98 (0.89-1.09)	0.91 (0.82-1.01)	0.95 (0.86-1.06	0.27
Model 3	1.0	0.96 (0.86-1.06)	0.98 (0.89-1.09)	0.91 (0.82-1.01)	0.96 (0.86-1.06)	0.32
Flavones						
Cases/p-yrs[≠]	765/141,279	748/141,753	727/141,487	755/141,536	743/141,117	
Model 1	1.0 (ref)	0.97 (0.88-1.08)	0.93 (0.84-1.03)	0.97 (0.88-1.08)	0.99 (0.90-1.10)	1.00
Model 2	1.0	1.00 (0.90-1.10)	0.96 (0.86-1.06	1.00 (0.90-1.12)	1.02 (0.91-1.13	0.70
Model 3	1.0	1.00 (0.90-1.10)	0.96 (0.86-1.06)	1.01 (0.91-1.12)	1.02 (0.91-1.13)	0.66
Flavonols						
Cases/p-yrs[≠]	729/140,291	700/141,474	745/141,633	812/141,901	752/141,872	
Model 1	1.0 (ref)	0.97 (0.87-1.07)	1.03 (0.93-1.14)	1.15 (1.04-1.27)	1.06 (0.95-1.17)	<.05*
Model 2	1.0	0.98 (0.88-1.09)	1.05 (0.94-1.17)	1.16 (1.04-1.30)	1.07 (0.95-1.20)	0.08
Model 3	1.0	0.98 (0.88-1.09)	1.05 (0.94-1.17)	1.16 (1.04-1.30)	1.06 (0.95-1.19)	0.10

Model 1: age adjusted

Model 2: adjusted for age, energy intake (quintiles), smoking (CSI), physical activity (METs quintiles), alcohol (g/day: 0, 0.1–4.9, 5–14.9, 15–29, 30+), occupation (dentist vs non-dentist), race (White/Black/Asian/Other), incidence of diabetes during follow up, and AHEI w/o fruits and vegetables.

Model 3: model 2 and adjusted for BMI (<18.5, 18.5-24.9, 25–29.9, 30+).

* Statistically significant.

† *p*-value for trend: each quintile was assigned the median value and treated as a continuous variable.

‡ The total number of cases over the total person-years on each quintile.

Table 3.

Multivariate association between total flavonoid intake and periodontitis incidence within subgroups

Subgroup		Quintile 1	Quintile 2	Quintile 3	Quintile 4	Quintile 5	*P* for trend†	*P* for interaction‡
BMI								
18.5-29.9	Cases/p-yrs§	291/53,652	283/57,333	280/60,410	329/61,101	293/61,028		
	HR (95% CI)	**1.0 (ref)**	**0.96 (0.81-1.14)**	**0.92 (0.78-1.10)**	**1.08 (0.91-1.28)**	**0.93 (0.78-1.10)**	**0.62**	
25-29.9	Cases/p-yrs§	382/68,612	354/67,055	344/ 65,588	332/64,539	357/64,472		
	HR (95% CI)	**1.0**	**0.99 (0.85-1.15)**	**1.01 (0.86-1.17)**	**1.00 (0.86-1.17)**	**1.02 (0.88-1.19)**	**0.71**	
≥30	Cases/p-yrs§	107/17,981	110/16,578	91/15,088	86/15,573	89/15,595		
	HR (95% CI)	**1.0**	**1.11 (0.84-1.47)**	**1.02 (0.76-1.36)**	**0.91 (0.67-1.24)**	**0.94 (0.69-1.28)**	**0.38**	0.68
Age								
<65	Cases/p-yrs§	524/99,482	450/88,615	421/83939	401/82,655	404/83,841		
	HR (95% CI)	**1.0 (ref)**	**1.02 (0.90-1.16)**	**1.03 (0.90-1.17)**	**1.00 (0.87-1.15)**	**0.95 (0.82-1.09)**	**0.25**	
≥65	Cases/p-yrs§	259/41,252	300/52,929	297/57,757	347/58,976	335/57,745		0.13
	HR (95% CI)	**1.0**	**0.96 (0.81-1.14)**	**0.91 (0.76-1.08)**	**1.04 (0.87-1.23)**	**0.99 (0.83-1.17)**	**0.35**	
Smoking (baseline)								
Current	Cases/p-yrs§	187/17,531	105/10,386	83/8,348	81/7,394	83/8,374		
	HR (95% CI)	**1.0 (ref)**	**0.89 (0.70-1.15)**	**0.86 (0.66-1.13)**	**0.98 (0.74-1.29)**	**0.83 (0.63-1.10)**	**0.39**	
Former	Cases/p-yrs§	325/53,595	315/53,883	302/53,472	295/51,416	301/53,615		
	HR (95% CI)	**1.0**	**0.98 (0.83-1.15)**	**0.96 (0.82-1.13)**	**0.98 (0.83-1.15)**	**0.91 (0.77-1.08)**	**0.40**	
Never	Cases/p-yrs§	271/69,609	330/77,274	333/79,876	372/82,820	355/79,596		
	HR (95% CI)	**1.0**	**1.10 (0.93-1.30)**	**1.09 (0.92-1.29)**	**1.15 (0.98-1.36)**	**1.13 (0.96-1.35)**	**0.22**	<0.001*
Physical Activity								
<Median	Cases/p-yrs§	498/83,779	415/71,434	352/64,588	335/61,911	365/62,501		
	HR (95% CI)	**1.0 (ref)**	**1.01 (0.88-1.15)**	**0.95 (0.82-1.09)**	**0.94 (0.82-1.09)**	**0.97 (0.84-1.12)**	**0.83**	
≥Median	Cases/p-yrs§	285/56,954	335/70,110	366/77,108	413/79,720	374/79,085		
	HR (95% CI)	**1.0**	**0.96 (0.82-1.13)**	**0.99 (0.84-1.16)**	**1.09 (0.93-1.28)**	**0.95 (0.81-1.12)**	**0.88**	0.81
Diabetes								
No	Cases/p-yrs§	752/136,203	716/136,102	686/135,559	711/135,927	713/136,589		

Subgroup		Quintile 1	Quintile 2	Quintile 3	Quintile 4	Quintile 5	*P* for trend[†]	*P* for interaction[‡]
	HR (95% CI)	**1.0 (ref)**	**0.99 (0.89-1.10)**	**0.98 (0.88-1.09)**	**1.02 (0.91-1.13)**	**0.97 (0.87-1.09)**	**0.95**	
Yes	Cases/p-yrs[§]	31/4,531	34/5,441	32/6,137	37/5704	26/4,997		
	HR (95% CI)	**1.0**	**1.06 (0.63-1.79)**	**0.94 (0.55-1.60)**	**1.18 (0.69-2.03)**	**0.87 (0.49-1.55)**	**0.49**	0.50
Dentist								
No	Cases/p-yrs[§]	350/63,131	326/61,714	317/59,231	280/58,856	295/60,642		
	HR (95% CI)	**1.0 (ref)**	**0.96 (0.83-1.13)**	**1.00 (0.85-1.17)**	**0.92 (0.78-1.09)**	**0.86 (0.73-1.02)**	**0.06**	
Yes	Cases/p-yrs[§]	433/77,604	424/79,829	401/82,465	468/82,775	444/80,944		
	HR (95% CI)	**1.0**	**1.05(0.91-1.20)**	**0.99 (0.86-1.14)**	**1.15 (1.00-1.32)**	**1.08 (0.93-1.24)**	**0.22**	0.09

Models adjusted for age, energy intake (quintiles), smoking (CSI), physical activity (METs quintiles), alcohol (g/day: 0, 0.1–4.9, 5–14.9, 15–29, 30+), occupation (dentist vs non-dentist), race (White/Black/Asian/Other), BMI (<18.5, 18.5-24.9, 25–29.9, 30+), and for incidence of diabetes during follow up, except for the stratification variable.

[*] Statistically significant.

[†] *p*-value for trend: each quintile was assigned the median value and treated as a continuous variable.

[‡] *p*-value for the interaction term between an indicator variable for the stratifying term and the continuous variable for dietary pattern (using median value of each quintile).

[§] The total number of cases over the total person-years on each quintile.

Table 4.

Hazard ratios (95% CIs) relating quintiles of main sources of total flavonoid intake and incidence of periodontitis.

	< 1/mon	1-3/mon	1/week	2-4/week	≥5/ week	*P* Trend[†]
Tea (1 cup)						
Model 1	1.0 (ref)	1.09 (0.99-1.20)	0.98 (0.85-1.13)	1.04 (0.94-1.15)	0.97 (0.89-1.06)	0.15
Model 2	1.0	1.09 (0.99-1.20)	0.99 (0.86-1.14)	1.06 (0.96-1.17)	0.99 (0.90-1.07)	0.23
Model 3	1.0	1.09 (0.99-1.20)	0.99 (0.86-1.14)	1.05 (0.94-1.17)	0.96 (0.84-1.08)	0.16
Onion-Garnish (1 slice) [ǂ]						
Model 1	1.0 (ref)	1.16 (0.97-1.39)	1.25 (1.03-1.50)	1.33 (1.13-1.57)	1.46 (1.22-1.74)	<0.001 *
Model 2	1.0	1.14 (0.95-1.36)	1.19 (0.99-1.44)	1.25 (1.06-1.48)	1.37 (1.14-1.64)	<0.001 *
Model 3	1.0	1.14 (0.95-1.36)	1.19 (0.99-1.44)	1.25 (1.06-1.48)	1.38 (1.15-1.65)	<0.001 *
Onion-Vegetable (1 onion) [ǂ]						
Model 1	1.0 (ref)	1.10 (0.96-1.25)	1.21 (1.04-1.41)	1.18 (1.03-1.36)		0.03 *
Model 2	1.0	1.06 (0.92-1.21)	1.16 (0.99-1.35)	1.12 (0.97-1.29)		0.16
Model 3	1.0	1.06 (0.92-1.21)	1.16 (0.99-1.35)	1.13 (0.97-1.30)		0.14
Apple (1 apple)						
Model 1	1.0 (ref)	0.95 (0.82-1.11)	0.84 (0.72-0.98)	0.88 (0.77-1.02)	0.90 (0.78-1.04)	0.52
Model 2	1.0	0.98 (0.85-1.14)	0.90 (0.76-1.05)	0.95 (0.83-1.10)	0.99 (0.85-1.15)	0.50
Model 3	1.0	0.98 (0.85-1.14)	0.90 (0.77-1.05)	0.96 (0.83-1.11)	1.00 (0.85-1.17)	0.39
Orange-Juice (small glass)						
Model 1	1.0 (ref)	1.02 (0.90-1.16)	0.96 (0.83-1.11)	1.02 (0.91-1.15)	0.93 (0.83-1.04)	0.03 *
Model 2	1.0	1.01 (0.89-1.15)	0.96 (0.83-1.11)	1.03 (0.91-1.16)	0.94 (0.84-1.06)	0.09
Model 3	1.0	1.01 (0.89-1.15)	0.96 (0.83-1.11)	1.03 (0.91-1.16)	0.94 (0.84-1.06)	0.09
Orange-Fruit (1 orange)						
Model 1	1.0 (ref)	0.92 (0.82-1.02)	0.91 (0.81-1.03)	0.86 (0.77-0.96)	0.85 (0.75-0.96)	0.02 *
Model 2	1.0	0.94 (0.84-1.04)	0.95 (0.85-1.08)	0.90 (0.81-1.01)	0.89 (0.78-1.01)	0.12
Model 3	1.0	0.93 (0.84-1.04)	0.95 (0.84-1.08)	0.90 (0.81-1.01)	0.89 (0.78-1.02)	0.13
Red wine (4 oz. glass)						
Model 1	1.0 (ref)	1.12 (1.03-1.21)	1.06 (0.94-1.20)	1.17 (1.07-1.27)		<0.01 *
Model 2	1.0	1.06 (0.97-1.16)	1.06 (0.93-1.20)	1.18 (1.06-1.31)		<0.01 *
Model 3	1.0	1.06 (0.97-1.16)	1.06 (0.93-1.20)	1.18 (1.06-1.31)		<0.01 *
Strawberry (1/2 cup)						
Model 1	1.0 (ref)	0.93 (0.85-1.01)	0.93 (0.84-1.02)			0.13
Model 2	1.0	0.95 (0.87-1.03)	0.95 (0.87-1.05)			0.39
Model 3	1.0	0.95 (0.87-1.03)	0.96 (0.87-1.05)			0.41
Blueberry (1/2 cup)						
Model 1	1.0 (ref)	1.00 (0.93-1.08)	1.02 (0.91-1.13)			0.79
Model 2	1.0	1.02 (0.95-1.10)	1.05 (0.94-1.17)			0.35
Model 3	1.0	1.03 (0.95-1.11)	1.06 (0.94-1.18)			0.31

Model 1: age adjusted

Model 2: adjusted for age, energy intake (quintiles), smoking (CSI), physical activity (METs quintiles), alcohol (g/day: 0, 0.1–4.9, 5–14.9, 15–29, 30+), occupation (dentist vs non-dentist), race (White/Black/Asian/Other), , incidence of diabetes during follow up, AHEI w/o fruits and vegetables, and adjusted for BMI (<18.5, 18.5-24.9, 25–29.9, 30+).

Model 3: model 2 and adjusted for total flavonoid (quintiles).

[*] Statistically significant.

[†] *p*-value for trend: each quintile was assigned the median value and treated as a continuous variable.

[‡] Follow-up started in 1990.