Original Research

Associations of Dietary Flavonoids with Risk of Type 2 Diabetes, and Markers of Insulin Resistance and Systemic Inflammation in Women: A Prospective Study and Cross-Sectional Analysis

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Objective: Flavonoids, as antioxidants, may prevent the progressive impairment of pancreatic β-cell function due to oxidative stress and may thus reduce the occurrence of type 2 diabetes. The aim of the present study was to examine the association of dietary flavonol and flavone intake with type 2 diabetes, and biomarkers of insulin resistance and systemic inflammation.

Methods: In 38,018 women aged ≥45 y and free of cardiovascular disease, cancer and diabetes with an average 8.8y of follow-up, we calculated relative risks (RRs) of incident type 2 diabetes (1,614 events) according to dietary intake of total or individual flavonols and flavones and flavonoid-rich foods. We also measured and examined plasma concentrations of insulin, HbA_{1C}, CRP, and IL-6 in relation to total flavonol and flavone intake among 344 nondiabetic women.

Results: During 332,905 person-years of follow-up, none of total flavonols and flavones, quercetin, kaempferol, myricetin, apigenin, and luteolin was significantly associated with risk of type 2 diabetes. Among flavonoid-rich foods, apple and tea consumption was associated with diabetes risk. Women consuming ≥1 apple/d showed a significant 28% reduced risk of type 2 diabetes compared with those who consumed no apples (the multivariate-adjusted RR = 0.72, 95% CI: 0.56, 0.92; $p = 0.006$ for trend). Tea consumption was also inversely associated with diabetes risk but with a borderline significant trend (≥4 cups/d vs. none: RR 0.73, 95% CI: 0.52–1.01; p for trend = 0.06). In 344 nondiabetic women, total intake of flavonols and flavones was not significantly related to plasma concentrations of fasting insulin, HbA_{1C}, CRP, or IL-6.

Conclusions: These results do not support the hypothesis that high intake of flavonols and flavones reduces the development of type 2 diabetes, although we cannot rule out a modest inverse association with intake of apples and tea.

INTRODUCTION

Experimental studies have suggested that oxidative stress due to increased production of reactive oxygen species and abnormal antioxidant status may facilitate the progressive impairment of β-cell function in the pathogenesis of type 2 diabetes [1–4]. Flavonoids are a group of naturally occurring polyphenolic compounds primarily from fruits and vegetables [5]. Several small dietary intervention trials have shown that consumption of flavonoid-rich foods such as tea and onions was associated with a significant increase in plasma levels of flavonoids in diabetic patients [6,7]. Flavonoids have high antioxidant activities as free radical scavengers and potent metal chelators [5,6,8]. Flavonoids may preserve β-cell function by reducing

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oxidative stress-induced tissue damage and therefore protect against the progression of insulin resistance to type 2 diabetes. However, the relation of dietary flavonoids to the development of type 2 diabetes is less well studied. A prospective study in Finland recently showed that the intakes of some specific types of flavonoids including quercetin and myricetin were inversely associated with risk of incident type 2 diabetes [9]. In addition, emerging evidence shows that oxidative stress may be involved in the pathogenesis of chronic inflammation underlying insulin resistance, diabetes and cardiovascular disease [10]. Yet few studies have specifically examined whether dietary intake of flavonoids is associated with biomarkers of insulin resistance and systemic inflammation, such as fasting insulin, CRP, and IL-6.

To investigate these hypotheses, we therefore prospectively examined the associations between dietary intake of total flavonols and flavones, specific types of flavonols and flavones, and major flavonoid-rich foods and the risk of incident type 2 diabetes in a large cohort of US middle-aged and older women in the Women's Health Study. We also conducted a cross-sectional study to examine the relation between flavonoids and biologic markers of insulin resistance and systemic inflammation in a sample of 344 apparently healthy women from this cohort.

MATERIAL AND METHODS

Study Population

The Women's Health Study (WHS) is a randomized, double-blind, placebo-controlled trial designed to evaluate the balance of benefits and risks of low-dose aspirin and vitamin E in the primary prevention of cardiovascular disease and cancer [11]. We randomized a total of 39,876 female health professionals aged ≥45 y, who were free of coronary heart disease, stroke, or cancer (except for non-melanoma skin cancer). Of them, 98% provided detailed information about their diet, completing a 131-item semiquantitative food frequency questionnaire in 1993 [11]. We excluded individuals with more than 70 items left blank in their questionnaire and with energy intake outside the range of 2,514 kJ/d (600 kcal/d) and 14,665 kJ (3,500 kcal/d), women with reported diabetes at baseline, and women with missing data for total flavonol and flavone intake, leaving 38,018 women for the analysis. The Institutional Review Board at Brigham and Women's Hospital approved all procedures, and written informed consent was obtained from all participants.

Dietary Assessment

We assessed dietary intake by using a 131-item semiquantitative food frequency questionnaire at baseline. For each food, a commonly used unit or portion size was specified, and each participant was asked how often she had consumed that amount, on average, during the previous year. Nine possible responses ranging from "never" to "≥6 times/d" were recorded. The food tables were originally generated in the Netherlands [12,13] and later supplemented with values for American foods [14]. Individual flavonols and flavones including quercetin, kaempferol, myricetin, apigenin, and luteolin were based on food tables maintained by the Department of Nutrition, Harvard School of Public Health, Boston. Total flavonoids represent the sum of the individual selected flavonols and flavones. Each nutrient was adjusted for total energy using the residual method [15]. In male health professionals, this food frequency questionnaire has demonstrated reasonably good validity as a measure of long-term average dietary intake of fruits and vegetables including flavonoid food sources. The correlations between the questionnaire and two 1-week diet records were 0.77 for tea, 0.70 for apples, and 0.46 for broccoli [16].

Ascertainment of Diabetes Cases

The status of type 2 diabetes was evaluated at baseline, and women with a history of diabetes were excluded. Thereafter, all of the participants were asked annually whether and when they had been diagnosed with diabetes since completing the previous questionnaire. Two complementary approaches have been used to confirm self-reported diagnoses in the WHS. First, we attempted to contact 473 women who provided bloods as part of a nested case-control study of diabetes to verify a diabetes diagnosis [17]. Using the American Diabetes Association (ADA) diagnostic criteria [18], the self-reported diagnosis of diabetes was confirmed in 406 (91%) of 446 women who responded via telephone interview. Second, a random sample of 147 women with self-reported diabetes was mailed a supplemental diabetes questionnaire, also using the ADA criteria to parallel the telephone interview. Among 136 respondents, 124 (91%) women were classified as having type 2 diabetes by the supplemental questionnaire. In addition, 113 of the 124 women gave permission to contact their primary care physician. Ninety-seven of the 113 physicians responded, of whom 90 provided adequate information to apply the ADA criteria. For these 90 women, 89 (99%) were confirmed to have type 2 diabetes on the basis of the combined information from the supplemental questionnaire and physician information. Thus, we believe that self-reported type 2 diabetes is valid in the WHS.

Blood Assays and Covariates

In our cross-sectional analysis, we included 349 apparently healthy women who had served as controls in a previous case-control study nested in the WHS [17]. After excluding 5 women with missing data on flavonoid intake, this sub-cohort comprised 344 healthy, nondiabetic, middle-aged and older women who remained diabetes-free during a period of 4 years subsequent to assessment of baseline clinical and biochemical parameters. Baseline fasting specimens from these 349 women

were assayed for insulin, CRP, IL-6, HbA_{1C} levels. Double antibody systems (Linco Research, St Louis, Mo), with less than 0.2% cross-reactivity between insulin and its precursors, were used to measure specific concentrations of plasma insulin. CRP, IL-6, and free plasma HbA_{1C} were measured as previously described [17]. All samples were handled identically and analyzed in random order to reduce systematic bias and interassay variation. Blinded quality control specimens were analyzed simultaneously with the study sample. Average intraassay coefficient of variation for insulin, CRP, and IL-6 were 14.7%, 12.0%, and 12.7%, respectively [19].

On the WHS baseline questionnaire, women also provided data on age (in y), weight and height (converted to body mass index, in kg/m^2), smoking status (categorized as never, former, or current), alcohol use (categorized as rarely or never, 1–3 drinks/mo, 1–6 drinks/wk, and ≥1 drink/d), frequency of vigorous exercise (categorized as rarely or never, <1 time/wk, 1–3 times/wk, and ≥4 times/wk), family history of diabetes (no, yes), history of hypertension (no, yes), and history of hypercholesterolemia (no, yes). In a similar population of the Nurses' Health Study, these self-reported covariates were found to be reasonable valid in a validation study of this cohort [20].

Data Analysis

We calculated the incidence rates of type 2 diabetes for each quintile of baseline flavonoid intake by dividing the number of incident cases by the person-years of follow-up from 1993 to 2003. We used Cox proportional hazards models to estimate the rate ratio (described as relative risk: RR) and 95% confidence interval (95% CI) of developing type 2 diabetes for each quintile of flavonoid intake, compared with the lowest quintile. The initial multivariate model was adjusted for age and total energy intake. Furthermore, we adjusted for BMI, smoking status, vigorous exercise, alcohol intake, history of hypertension, history of high cholesterol, and family history of diabetes. The final multivariate model added dietary factors (all in quintiles), including dietary fiber, total fat intakes, glycemic load, and total magnesium intake. Tests of linear trend across increasing quintiles of intake were conducted by assigning the medians of intakes in quintiles treated as a continuous variable. We used stratified analysis to assess the potential effect modifications by predefined covariates including BMI (< or ≥25 kg/m^2), smoking (never vs. ever smokers), exercise (<1 time/wk vs. ≥1 time/wk), and family history of diabetes (yes or no). Likelihood ratio tests were used to assess the significance of interaction terms. A similar analytic approach was used for analyses of total flavonols and flavones, and their subtypes, including quercetin, kaempferol, luteolin, myricetin, and apigenin. Analyses were also done for the intake of major food sources of flavonoids, *a priori* categorized for tea (none, <1 cup/d, 1–3 cups/d, and ≥4 cups/d), apples (none, ≤1 apple/wk, 2–6 apples/wk, and ≥1 apple/d), broccoli [none, ≤1 serving/wk, 2–4 servings/wk, and ≥5 servings/wk; 1 serving = ½ cup (113 g)], onion (none, ≤1 serving/wk, 2–4 servings/wk, and ≥5 servings/wk; 1 serving = 1 onion), and tofu [none, 1–3 servings/mo, and ≥1 serving/wk; 1 serving = 3–4 oz (85–113 g)]. In secondary analyses, we carried out all analyses excluding women with either a history of hypertension or a history of high cholesterol at baseline because these participants might have changed their dietary intake.

In our cross-sectional analysis in 344 apparently healthy nondiabetic women, we categorized total flavonoid intake into quintiles and then calculated the median plasma concentrations of these markers according to each quintile of intake. We then calculated geometric means of plasma concentrations of insulin, HbA_{1C}, CRP, and IL-6 by regressing the natural logarithm of plasma concentrations of markers on flavonoid intake and then taking an antilog of the resulting mean logarithmic value. Multiple linear regression models controlled for the same potential confounding factors included in the second multivariate Cox hazards model. Tests of linear trend across increasing quintiles of intake were conducted by assigning the medians of intakes in quintiles treated as a continuous variable. All statistical analyses were conducted using SAS (version 8.0; SAS Institute, Cary, NC).

RESULTS

There was an approximately 5-fold difference in total flavonol and flavone intake between the highest and lowest quintiles of the study population (median: 47.2 mg/d in the highest quintile vs. 8.85 mg/d in the lowest quintile). Of the selected flavonols and flavones, quercetin was the major contributor to total flavonoids (72%), followed by kaempferol (19.5%), myricetin (4.74%), apigenin (3.30%), and luteolin (0.43%).

Women with high intake of total flavonols and flavones were older, smoked and drank less, and exercised more than were women with low intake. High consumers of total flavonols and flavones were also more likely to have a history of high cholesterol than those with low intake of total flavonols and flavones (Table 1). Total flavonol and flavone intake was positively associated with intakes of dietary fiber and total magnesium and glycemic load, and inversely associated with total energy intake and fat intake.

During an average of 8.8 y of follow-up (332,905 person-years), we documented 1,614 cases of incident type 2 diabetes. Neither total flavonols and flavones nor types of flavonoids were significantly associated with the risk of developing type 2 diabetes (Table 2). The multivariate-adjusted relative risks (RR) comparing the highest quintile of intake with the lowest quintile were 0.89 (95% CI: 0.75, 1.04; p for trend = 0.52) for total flavonol and flavone, 0.93 (0.79, 1.09; p for trend = 0.83) for quercetin, 0.96 (0.82, 1.13; p for trend = 0.76) for kaempferol, 0.95 (0.81, 1.12; p for trend = 0.38) for myricetin, 0.98 (0.84, 1.15; p for trend = 0.90) for apigenin, and 1.00 (0.85, 1.17; p for trend = 0.96) for luteolin. After further

Table 1. Baseline Characteristics according to Quintiles of Total Flavonoid Intake among 38,018 Women in the Women's Health Study

Characteristic	Quintiles of total flavonoid intake					
	1	2	3	4	5	P^1
Median intake (mg/d)	8.85	13.9	19.1	27.1	47.2	
Age (y)[2]	53.2 ± 6.7	53.6 ± 6.9	54.1 ± 7.0	54.4 ± 7.2	54.1 ± 7.2	<0.0001
BMI (kg/m^2)[2]	26.2 ± 5.2	25.8 ± 4.8	25.8 ± 4.9	25.8 ± 4.9	25.8 ± 4.9	<0.0001
Smoking (%)						<0.0001
Never	46.9	50.5	51.7	53.1	53.1	
Past	35.0	36.6	36.9	36.4	34.5	
Current	18.2	12.9	11.4	10.6	12.4	
Exercise (%)						<0.0001
Rarely or never	46.4	36.5	35.0	34.7	37.2	
<1 time/wk	19.3	20.7	20.3	19.7	19.5	
1–3 times/wk	26.0	32.2	33.4	33.6	31.8	
≥4 times/wk	8.4	10.6	11.3	12.0	11.5	
Alcohol consumption (%)						<0.0001
Rarely or never	45.3	41.3	41.4	44.7	48.3	
1–3 drinks/mo	13.2	12.8	13.2	13.6	13.2	
1–6 drinks/wk	30.0	34.0	34.9	32.1	30.0	
≥1 drink/d	11.6	11.9	10.5	9.62	8.55	
Multivitamin use (%)	28.0	29.6	30.0	29.8	28.7	0.05
History of hypertension (%)	25.0	23.9	25.3	25.7	25.5	0.11
History of high cholesterol (%)	25.9	25.4	27.2	27.5	27.2	0.01
Family history of diabetes (%)	24.3	25.0	24.6	25.1	24.9	0.75
Total energy (kcal/d)[2]	1725 ± 539	1745 ± 518	1755 ± 534	1741 ± 550	1666 ± 522	<0.0001
Total fat (g/d/)[2,3]	62.0 ± 11.7	58.3 ± 10.9	56.4 ± 11.2	55.8 ± 11.5	55.5 ± 12.2	<0.0001
Fiber (g/d/)[2,3]	15.3 ± 4.48	18.0 ± 4.55	19.7 ± 5.48	20.5 ± 5.85	21.4 ± 6.90	<0.0001
Magnesium (mg/d)[2,3]	311 ± 69	332 ± 69	343 ± 73	347 ± 74	356 ± 77	<0.0001
Glycemic load[2,3]	162 ± 31	165 ± 28	169 ± 29	170 ± 30	170 ± 31	<0.0001

[1] ANOVA were used for continuous variables and chi-square tests were used for categorical variables.

[2] Data are means ± SD, unless otherwise indicated.

[3] All the means of nutrients are energy adjusted.

adjustment for dietary factors, the results were not materially altered. We found no apparent modification of the relation between flavonoids and type 2 diabetes by smoking status, BMI, exercise, and family history of diabetes (data not shown). To minimize the effects of dietary changes or residual confounding, we examined these associations by excluding women with a history of hypertension, or high cholesterol. None of the associations was statistically significant; the corresponding RRs across quintiles of total intake of flavonols and flavones were 1.00, 1.03 (0.77–1.38), 1.14 (0.85–1.51), 1.05 (0.79–1.41), and 1.04 (0.78–1.39) (p for trend = 0.93).

Among major flavonoid-rich foods, only apple consumption was significantly associated with a lower risk of type 2 diabetes. In multivariate analyses, the RRs of type 2 diabetes according to consumption categories (≤1 apple/wk, 2–6 apples/wk, and ≥1 apple/d) were 0.83, 0.72, and 0.72 (95% CI, 0.56, 0.92; p for trend = 0.006) as compared with women who reported no apple intake. The inverse association was attenuated but remained significant after additional adjustment for dietary factors including fiber, magnesium, glycemic load, and total fat intakes (Table 3); the multivariate-adjusted RRs across categories of apple intake (never, ≤1 apple/wk, 2–6 apple/wk, and ≥1 apple/d) were 1.00, 0.83, 0.73, and 0.72 (95% CI, 0.55, 0.94; p for trend = 0.02). This inverse association persisted even after further controlling for total intake of flavonoids or quercetin intake (data not shown). In addition, tea intake also had a similar pattern of an inverse association with type 2 diabetes in multivariate model, but was of borderline significance; the multivariate-adjusted RRs across categories of tea consumption (never, <1 cup/d, 1–3 cups/d, and ≥4 cups/d) were 1.00, 1.07, 1.04, and 0.73 (95% CI, 0.52, 1.01; p for trend = 0.06). If the highest category of tea consumption was further classified into two categories (4–5 cups/d and ≥6 cups/d), the inverse association still existed. Higher intake of tea was related to a further risk reduction; the multivariate-adjusted RRs were 0.77 (95% CI, 0.53, 1.11) for women who drank 4–5 cups/d and 0.63 (95% CI, 0.33, 1.22) for those who drank ≥6 cups/d compared to those who consumed no tea (p for trend = 0.05).

Among 344 nondiabetic women in our cross-sectional analysis, lifestyle and dietary characteristics were similar to the entire WHS cohort (data not shown). Neither crude plasma concentrations nor multivariate-adjusted mean plasma concentrations of fasting insulin, HbA_{1C}, CRP, or IL-6 were related to

Table 2. Relative Risks (RRs) and 95% CIs of Type 2 Diabetes according to Quintiles of Flavonoid Intakes among 38,018 Women in the Women's Health Study

Variable	Quintiles of intake					*p* for trend[1]
	1	2	3	4	5	
Total flavonoid intake						
Median intake (mg/d)	8.85	13.9	19.1	27.1	47.2	
No of cases	362	288	304	348	312	
Adjusted for age and energy	1.00	0.79 (0.67–0.92)	0.81 (0.69–0.95)	0.96 (0.82–1.11)	0.86 (0.74–1.00)	0.60
Multivariate RR[2]	1.00	0.87 (0.74–1.02)	0.88 (0.75–1.03)	1.02 (0.88–1.19)	0.89 (0.75–1.04)	0.52
Multivariate RR[3]	1.00	0.89 (0.76–1.05)	0.92 (0.78–1.08)	1.06 (0.90–1.25)	0.92 (0.78–1.09)	0.81
Quercetin intake						
Median intake (mg/d)	6.47	10.2	13.6	19.2	32.6	
No of cases	353	307	294	338	322	
Adjusted for age and energy	1.00	0.85 (0.73–0.99)	0.82 (0.70–0.96)	0.94 (0.81–1.10)	0.91 (0.78–1.06)	0.84
Multivariate RR[2]	1.00	0.90 (0.76–1.05)	0.91 (0.78–1.07)	1.00 (0.85–1.16)	0.93 (0.79–1.09)	0.83
Multivariate RR[3]	1.00	0.92 (0.79–1.09)	0.95 (0.80–1.12)	1.04 (0.88–1.22)	0.97 (0.82–1.15)	0.82
Kaempferol intake						
Median intake (mg/d)	0.85	1.79	3.29	5.67	13.0	
No of cases	318	304	340	333	319	
Adjusted for age and energy	1.00	0.92 (0.79–1.08)	1.02 (0.88–1.19)	1.03 (0.89–1.21)	1.00 (0.85–1.17)	0.68
Multivariate RR[2]	1.00	0.94 (0.80–1.11)	1.05 (0.89–1.23)	1.07 (0.91–1.25)	0.96 (0.82–1.13)	0.76
Multivariate RR[3]	1.00	0.96 (0.81–1.14)	1.08 (0.92–1.27)	1.11 (0.94–1.30)	0.99 (0.84–1.17)	0.93
Myricetin intake						
Median intake (mg/d)	0.15	0.47	0.77	1.33	2.82	
No of cases	340	310	345	322	297	
Adjusted for age and energy	1.00	0.88 (0.75–1.02)	1.01 (0.87–1.18)	0.94 (0.81–1.10)	0.89 (0.76–1.04)	0.25
Multivariate RR[2]	1.00	0.98 (0.83–1.15)	1.16 (0.99–1.35)	1.06 (0.90–1.24)	0.95 (0.81–1.12)	0.38
Multivariate RR[3]	1.00	1.00 (0.85–1.17)	1.19 (1.02–1.39)	1.10 (0.94–1.30)	0.98 (0.83–1.16)	0.60
Apigenin intake						
Median intake (mg/d)	0.13	0.29	0.43	0.66	1.35	
No of cases	370	335	304	297	308	
Adjusted for age and energy	1.00	0.94 (0.81–1.09)	0.85 (0.73–0.99)	0.83 (0.71–0.97)	0.84 (0.72–0.98)	0.04
Multivariate RR[2]	1.00	1.01 (0.87–1.18)	0.88 (0.75–1.04)	0.94 (0.80–1.10)	0.98 (0.84–1.15)	0.90
Multivariate RR[3]	1.00	1.02 (0.87–1.19)	0.90 (0.76–1.05)	0.96 (0.82–1.13)	1.01 (0.85–1.19)	0.83
Luteolin intake						
Median intake (mg/d)	0.01	0.04	0.05	0.08	0.20	
No of cases	399	314	332	264	305	
Adjusted for age and energy	1.00	0.86 (0.74–1.01)	0.84 (0.73–0.98)	0.84 (0.71–0.98)	0.81 (0.70–0.95)	0.04
Multivariate RR[2]	1.00	0.96 (0.82–1.12)	1.00 (0.86–1.17)	0.93 (0.79–1.10)	1.00 (0.85–1.17)	0.96
Multivariate RR[3]	1.00	0.97 (0.83–1.13)	1.02 (0.87–1.19)	0.96 (0.81–1.13)	1.04 (0.88–1.23)	0.60

[1] Test for trend based on ordinal variable containing median value for each quintile.

[2] Multivariate model: adjusted for age (continuous), BMI (continuous), total energy intake (continuous), smoking (current, past and never), exercise (rarely/never, <1 time/wk, 1–3 times/wk, and ≥4 times/wk), alcohol use (rarely/never, 1–3 drinks/mo, 1–6 drinks/wk, and >=1 drink/d), history of hypertension (yes/no), history of high cholesterol (yes/no), and family history of diabetes (yes/no).

[3] Further adjustment for dietary intakes of fiber intake (quintiles), glycemic load (quintiles), magnesium (quintiles), and total fat (quintiles).

total flavonol and flavone intake. The linear trends across quintiles of total flavonol and flavone intake were not statistically significant (Table 4).

DISCUSSION

In this large prospective study of US middle-aged and older women, neither the intake of total and individual flavonols and flavones nor most flavonoid-rich foods were associated with risk of type 2 diabetes. However, women who ate ≥1 apple/d or had ≥4 cups/d of tea had an approximately 30% lower risk of developing type 2 diabetes than those who consumed no apples or tea. In a separate cross-sectional study, we found no relation between total flavonol and flavone intake and markers of insulin resistance and systemic inflammation, such as fasting insulin, HbA_{1C}, CRP, and IL-6 concentrations, among 344 nondiabetic women.

Other prospective data on flavonoid and diabetes risk are confined to Knekt et al., who reported that both intakes of quercetin and myricetin were marginally associated with a decreased risk of type 2 diabetes in 10,054 Finnish men and women [9]. In contrast, our data based on a greater number of women and incident cases do not support an inverse association of either total or individual intakes of flavonols and flavones with the incidence of type 2 diabetes. Their finding that apple

Table 3. Relative Risks (RRs) and 95% CIs of Type 2 Diabetes according to Categories of Various Flavonoid-Rich Food Groups among 38,018 Women in the Women's Health Study

Variable	Category of intake				*p* for trend[1]
	1st (lowest)	2nd	3rd	4th (highest)	
Tea	None	<1 cup/d	1–3 cups/d	≥4 cups/d	
No of cases/Total	496/12,279	686/15,633	363/8,344	48/1,201	
Adjusted for age and energy	1.00	1.08 (0.96–1.21)	1.03 (0.90–1.19)	0.92 (0.68–1.26)	0.49
Multivariate Model[2]	1.00	1.07 (0.95–1.20)	1.04 (0.90–1.20)	0.73 (0.52–1.01)	0.06
Multivariate Model[3]	1.00	1.07 (0.95–1.21)	1.05 (0.91–1.21)	0.72 (0.52–1.01)	0.06
Broccoli	None	≤1 serving/wk	2–4 servings/wk	≥5 servings/wk	
No of cases/Total	111/2,163	963/22,839	446/10,841	87/1,974	
Adjusted for age and energy	1.00	0.79 (0.65–0.97)	0.73 (0.59–0.91)	0.71 (0.53–0.96)	0.04
Multivariate Model[2]	1.00	0.93 (0.76–1.14)	0.90 (0.72–1.12)	0.89 (0.66–1.21)	0.41
Multivariate Model[3]	1.00	0.95 (0.77–1.16)	0.94 (0.75–1.18)	0.95 (0.69–1.31)	0.84
Apples	None	≤1/wk	2–6/wk	≥1/d	
No of cases/Total	196/3,525	773/17,502	509/13,501	118/3,118	
Adjusted for age and energy	1.00	0.75 (0.64–0.88)	0.60 (0.51–0.72)	0.55 (0.44–0.70)	<0.0001
Multivariate Model[2]	1.00	0.83 (0.70–0.98)	0.72 (0.60–0.86)	0.72 (0.56–0.92)	0.006
Multivariate Model[3]	1.00	0.83 (0.70–0.98)	0.73 (0.60–0.88)	0.72 (0.55–0.94)	0.02
Onions	None	≤1 serving/wk	2–4 servings/wk	≥5 servings/wk	
No of cases/Total	625/16,130	698/15,791	167/3,700	107/1,997	
Adjusted for age and energy	1.00	1.10 (0.99–1.23)	1.06 (0.88–1.26)	1.21 (0.98–1.51)	0.19
Multivariate Model[2]	1.00	1.08 (0.97–1.21)	1.09 (0.91–1.30)	1.14 (0.92–1.43)	0.27
Multivariate Model[3]	1.00	1.09 (0.97–1.22)	1.10 (0.92–1.33)	1.18 (0.94–1.48)	0.18
Tofu	None	1–3 servings/mo	≥1 serving/wk		
No of cases/Total	1481/34,325	74/2,132	45/1,073		
Adjusted for age and energy	1.00	0.77 (0.61–0.98)	0.88 (0.64–1.21)		0.08
Multivariate Model[2]	1.00	0.92 (0.72–1.18)	1.02 (0.73–1.43)		0.82
Multivariate Model[3]	1.00	0.95 (0.74–1.22)	1.06 (0.75–1.49)		0.95

[1] Test for trend based on ordinal variable containing median value for each quintile.

[2] Multivariate model: adjusted for age (continuous), BMI (continuous), total energy intake (continuous), smoking (current, past and never), exercise (rarely/never, <1 time/wk, 1–3 times/wk, and ≥4 times/wk), alcohol use (rarely/never, 1–3 drinks/mo, 1–6 drinks/wk, and >=1 drink/d), history of hypertension (yes/no), history of high cholesterol (yes/no), and family history of diabetes (yes/no).

[3] Further adjustment for dietary intakes of fiber intake (quintiles), glycemic load (quintiles), magnesium (quintiles), and total fat (quintiles).

Table 4. Plasma Concentrations of Fasting Insulin, HbA_{1c}, CRP, and IL-6 according to Quintiles of Total Flavonoid Intake in 344 Nondiabetic Women from the Women's Health Study[1]

Biomarkers	Quintile of intake					*p* for trend[2]
	1 (n = 68)	2 (n = 69)	3 (n = 69)	4 (n = 69)	5 (n = 69)	
Total flavonoids, median intake (mg/d)	8.75	13.5	18.2	27.2	44.2	
Fasting Insulin, pmol/L						
Crude (median, interquartile range)	39.9 (28.2–57.5)	41.3 (29.9–64.9)	36.7 (28.1–63.1)	39.2 (29.8–53.1)	41.3 (27.4–58.8)	0.89
Multivariate-adjusted[3]	41.2 (36.5–46.5)	41.0 (36.4–46.1)	39.4 (34.9–44.3)	41.1 (36.3–46.5)	43.7 (38.6–49.4)	0.34
HbA_{1C}, %						
Crude (median, interquartile range)	5.50 (5.30–5.70)	5.60 (5.30–5.70)	5.50 (5.30–5.70)	5.50 (5.30–5.70)	5.50 (5.30–5.70)	0.96
Multivariate-adjusted[3]	5.53 (5.45–5.61)	5.54 (5.46–5.62)	5.55 (5.47–5.63)	5.53 (5.45–5.61)	5.53 (5.45–5.62)	0.98
CRP, mg/L						
Crude (median, interquartile range)	2.00 (0.70–6.05)	2.10 (0.80–5.70)	2.80 (1.10–7.50)	2.50 (0.90–4.50)	2.60 (1.20–5.10)	0.24
Multivariate-adjusted[3]	2.39 (1.81–3.15)	2.14 (1.64–2.81)	2.87 (2.19–3.77)	2.25 (1.70–2.98)	2.87 (2.17–3.80)	0.28
IL-6, pg/mL						
Crude (median, interquartile range)	1.45 (0.95–2.10)	1.29 (0.85–2.16)	1.55 (1.06–2.23)	1.23 (0.90–1.69)	1.29 (0.96–1.85)	0.46
Multivariate-adjusted[3]	1.56 (1.33–1.82)	1.31 (1.13–1.53)	1.50 (1.28–1.74)	1.39 (1.18–1.63)	1.53 (1.31–1.80)	0.37

[1] Data are expressed as geometric mean (95% CI). All covariate values are according to the quintiles of magnesium intake.

[2] Test for trend based on ordinal variable containing median value for each quintile.

[3] Multivariate model: adjusted for age (continuous), total energy intake (continuous), BMI (continuous), smoking (current, past and never), exercise (rarely/never, <1 time/wk, 1–3 times/wk, and ≥4 times/wk), alcohol use (rarely/never, 1–3 drinks/mo, 1–6 drinks/wk, and >=1 drink/d), history of hypertension (yes/no), history of high cholesterol (yes/no), and family history of diabetes (yes/no).

intake was inversely associated with the incidence of type 2 diabetes was intriguing, similar to our results. The relative risk of type 2 diabetes comparing the highest (>47 g/d) to the lowest quartile of apple intake (0 g/d) was 0.73 (95% CI: 0.57–0.92; p for trend = 0.003) [9]. It is possible that differences in dietary assessment and consumption pattern of flavonoid-rich foods in these two populations may account for the discrepant findings between our results and Knekt's.

They also attributed quercetin intake to the beneficial effect of apple intake since adjusting for quercetin intake but not other fruit and vegetables or vitamin C and β-carotene appreciably attenuated these inverse associations with apple intake [9]. In our study population, average quercetin intake was 72% of total flavonol and flavone, with the major sources being onions, tea, and apples. Nevertheless, further adjustment for quercetin intake did not change the inverse association between apple consumption and risk of type 2 diabetes. We speculate that apples are also important sources of other polyphenolic compounds such as catechins, other antioxidants including β-carotene and vitamin C, or some unidentified dietary factors and may thus provide protection against type 2 diabetes. Similarly, tea is a major source of flavonoids, including catechins and isoflavones, which are not included in our diet database. In addition, caffeine in tea may also have beneficial effects on diabetes risk [21]. Alternatively, higher apple or tea consumption may be a surrogate for unknown or unadjusted healthy lifestyle factors that could favorably affect the occurrence of type 2 diabetes.

Dietary antioxidants have attracted wide interest as potent agents that may protect β-cells from free radical-mediated damage, and thus slow progressive β-cell dysfunction. There is evidence to suggest that hyperinsulinemia and postprandial hyperglycemia can elicit oxidative stress by increasing reactive oxygen species (ROS) production and reducing intracellular antioxidant defense [22–24]. Further, both *in vitro* and *in vivo* studies observed that oxidative stress generation impaired pancreatic β-cell insulin secretion [2,25–27] and interfered with insulin signaling pathway, thereby accelerating the progression to overt type 2 diabetes from insulin resistance [3,24]. Excessive oxidative stress may also be actively involved in the genesis of systemic inflammation [4], which is a common manifestation for insulin resistance, diabetes and cardiovascular disease [28]. Flavonoids, which are capable of scavenging free radicals and chelating the deleterious oxidant-inducing metals such as iron and copper, have been associated with a reduced risk of cardiovascular disease and cancer [8,9,29–36]. Nevertheless, the relevance of dietary flavonoids to insulin resistance or diabetic risk is less well studied, and, likewise, results from epidemiologic studies for the roles of others potent antioxidants such as vitamin E, vitamin C, and β-carotene in relation to the incidence of type 2 diabetes have been inconsistent [37–41]. Of note, data from clinical trials that specifically focus on decreasing diabetes incidence are scarce. In a controlled trial, β-carotene supplementation did not appear to be effective in the primary prevention of type 2 diabetes in men [42].

Our study has several strengths. The prospective design and high follow-up rates in our study minimized the possibility of selection and recall bias. Our validation study showed a high accuracy of self-reported diabetes compared with medical records so that the misdiagnosis of type 2 diabetes is unlikely. Nevertheless, some limitations of the present study merit consideration. First, measurement error in assessing flavonoid intakes from food frequency questionnaire may bias our results towards the null, although the validity and reliability of FFQs have been evaluated in similar cohort of US female health professionals [43]. Therefore, our finding cannot exclude the possibility of a modest reduction of type 2 diabetes associated with high intakes of flavonoids. Second, a limited variation in the intake of individual flavonoids or flavonoids-rich food groups in our cohort may have limited the statistical power to detect any associations with type 2 diabetes. Third, because of a high degree of statistical collinearity, our ability to distinguish the effect of any single flavonoid or flavonoid-rich food was limited. Fourth, flavonoids consist of six major subgroups including flavonols, flavones, flavanones, isoflavones, anthocyanins, and catechins and have more than 4000 different compounds. Based on the food-composition database available on the subclasses of flavonols and flavones, we were unable to assess intakes of all constituents such as catechins and isoflavones and thus could not address the relationship between these specific compounds with potent antioxidant properties and diabetic risk. Fifth, our cross-sectional analysis was based on a small group of nondiabetic women who did not develop type 2 diabetes during the first 4 y of follow-up. It is likely that they might have lower risks for type 2 diabetes and less degree of underlying insulin resistance than those randomly chosen from the whole cohort. Our cross-sectional analysis among these nondiabetic women may have attenuated the true correlation between total flavonoid intake and fasting insulin, HbA_{1C}, CRP, and IL-6 concentrations. Finally, measurement errors in assessing these biomarkers are not likely associated with dietary intake but would bias the results toward the null.

CONCLUSION

Our findings from this large cohort of middle aged or elderly US women do not support the hypothesis that high intake of flavonols and flavones protects against the development of type 2 diabetes. Further research needs to determine what compounds are responsible for the potential beneficial effects of apple and/or tea consumption on diabetic risk. Also, future investigations are warranted to examine whether abundant flavonoid compounds other than flavonols and flavones offer potential protection against the development of type 2 diabetes or its complications.

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