Dietary flavonoid intakes and risk of type 2 diabetes in US men and women[1–5]

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ABSTRACT

Background: Data from mechanistic studies support a beneficial effect of specific flavonoids on insulin sensitivity. However, few studies have evaluated the relation between intakes of different flavonoid subclasses and type 2 diabetes.

Objective: The objective was to evaluate whether dietary intakes of major flavonoid subclasses (ie, flavonols, flavones, flavanones, flavan-3-ols, and anthocyanins) are associated with the risk of type 2 diabetes in US adults.

Design: We followed up a total of 70,359 women in the Nurses' Health Study (NHS; 1984–2008), 89,201 women in the NHS II (1991–2007), and 41,334 men in the Health Professionals Follow-Up Study (1986–2006) who were free of diabetes, cardiovascular disease, and cancer at baseline.

Results: During 3,645,585 person-years of follow-up, we documented 12,611 incident cases of type 2 diabetes. Higher intakes of anthocyanins were significantly associated with a lower risk of type 2 diabetes (pooled HR for the 3 cohorts from a comparison of extreme quintiles: 0.85; 95% CI: 0.80, 0.91; *P*-trend < 0.001) after multivariate adjustment for age, BMI, and lifestyle and dietary factors. Consumption of anthocyanin-rich foods, particularly blueberries (pooled HR: 0.77 from a comparison of $\geq$2 servings/wk with $<$1 serving/mo; 95% CI: 0.68, 0.87; *P*-trend < 0.001) and apples/pears (pooled HR: 0.77 from a comparison of $\geq$5 servings/wk with $<$1 serving/mo; 95% CI: 0.65, 0.83; *P*-trend < 0.001), was also associated with a lower risk of type 2 diabetes. No significant associations were found for total flavonoid intake or other flavonoid subclasses.

Conclusion: A higher consumption of anthocyanins and anthocyanin-rich fruit was associated with a lower risk of type 2 diabetes. *Am J Clin Nutr* 2012;95:925–33.

INTRODUCTION

Flavonoids are polyphenolic compounds present in a wide variety of plants. Major dietary flavonoid subclasses are flavonols, flavones, flavanones, anthocyanins, flavan-3-ols, isoflavones, and their oligomeric and polymeric forms. Although early research focused on the capacity of flavonoids to scavenge free radicals and protect against lipid peroxidation, more recent attention has focused on the ability of specific flavonoids to modulate endothelial nitric oxide metabolism and NADPH oxidase activity (1–4). Results from mechanistic studies suggest that flavonoids may also decrease glycemia and improve insulin secretion and sensitivity with particular interest in the flavonol, flavan-3-ol, and anthocyanin subclasses (5). Studies in animal models have specifically shown that the anthocyanin subclass improved glucose metabolism, insulin resistance, and β cell dysfunction through GLUT4[6] regulation (6–9).

Few studies have evaluated dietary intake in the range of major flavonoid subclasses commonly consumed in the US diet in relation to risk of T2DM. To date, 3 prospective cohort studies (10–12) have been conducted, which found weak or null associations for T2DM; however, 2 of these studies (11, 12) relied on early versions of the USDA databases, which were less accurate and evaluated only a limited number of subclasses.

Given the heterogeneity in structural characteristics, bioavailability, absorption, and metabolism of the different flavonoid subclasses, it is essential to investigate each subclass individually. Recent developments in food composition data for flavonoids have enabled a more comprehensive analysis of the relative importance of the different subclasses. In the current study, we prospectively evaluated each of the major flavonoid subclasses and the association with T2DM in the 3 large cohorts: the NHS, NHS II, and HPFS.

SUBJECTS AND METHODS

Study population

We used data from 3 prospective cohort studies: NHS (started in 1976; n = 121,700; age range at baseline: 30–55 y), NHS II (es-

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[6] Abbreviations used: FFQ, food-frequency questionnaire; GLUT4, glucose transporter 4; HPFS, Health Professionals Follow-Up Study; NHS, Nurses' Health Study; T2DM, type 2 diabetes mellitus.

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tablished in 1989; n = 116,671; age range at baseline: 25–42 y), and HPFS (initiated in 1986; n = 51,529; age range at baseline: 40–75 y). Details of the 3 cohorts were previously described (13–15). In all 3 cohorts, questionnaires were administered at baseline and biennially thereafter to collect and update information on lifestyle practices and occurrence of chronic diseases. The follow-up rates of the participants in these cohorts all exceeded 90%.

In the current analysis, we used 1984 for NHS, 1991 for NHS II, and 1986 for HPFS as baseline, when a comprehensive FFQ with 118–131 food items was first distributed in these cohorts. We excluded men and women who reported a diagnosis of diabetes (including type 1 diabetes, T2DM, and gestational diabetes for women), cardiovascular disease, or cancer at baseline (n = 8453 for NHS, 5888 for NHS II, and 6834 for HPFS). We also excluded participants with missing information for dietary data or unusual total energy intakes (ie, daily energy intake $<$500 or $>$3500 kcal/d; n = 2945 for NHS, 363 for NHS II, and 4275 for HPFS). In addition, we excluded participants without follow-up information on diabetes diagnosis date. After exclusions, data from 70,359 NHS participants, 89,201 NHS II participants, and 40,420 HPFS participants were available for the analysis. The study protocol was approved by the institutional review boards of Brigham and Women's Hospital and Harvard School of Public Health. The completion of the self-administered questionnaire was considered to imply informed consent.

Assessment of flavonoid intakes

In 1984, a 118-item FFQ was administered among the NHS participants to collect information on their usual intake of foods and beverages in the previous year. In 1984, 1986, 1990, 1994, 1998, and 2002, similar but expanded FFQs with 131–166 items were sent to these participants to update their diet. The expanded FFQ used in the NHS was used to collect dietary data in 1986, 1990, 1994, 1998, and 2002 among the HPFS participants and in 1991, 1995, 1999, and 2003 among the NHS II participants. In all FFQs, we asked the participants how often, on average, they consumed each food of a standard portion size. There were 9 possible responses, ranging from "never or less than once per month" to "6 or more times per day." The reproducibility and validity of these FFQs were shown in detail elsewhere (16, 17). Validation studies were conducted among 173 NHS participants in 1980 and 127 HPFS participants in 1986. In both validation studies, the correlation coefficients between the FFQ and multiple 1-wk dietary records suggested reasonable validity for flavonoid-rich foods [eg, the correlation coefficients corrected for within-person variation were 0.80 for apple, r = 0.90 for wine, and r = 0.93 for tea in women (17), and 0.70, 0.83, and 0.77, respectively, in men (16)].

Quantification of the flavonoid content in various food sources is described in detail elsewhere (18). Briefly, a comprehensive database of levels of individual flavonoids in foods was established predominantly on the basis of the USDA flavonoid content of the foods database (19). Intake of each subclass of flavonoids was calculated by multiplying the frequency of consumption for a particular portion size by the flavonoid content in that particular food item and then summing the product across all food items. Total flavonoid intake was derived by summing up intakes of all subclasses of flavonoids. The 5 major flavonoid subclasses evaluated in the current analysis were flavonols, flavones, flavanones, flavan-3-ols, and anthocyanins. For anthocyanins, we additionally evaluated the 6 main constituents: cyanidin, delphinidin, malvidin, pelargonidin, peonidin, and petunidin (8).

Assessment of diabetes

In all 3 cohorts, a supplementary questionnaire regarding symptoms, diagnostic tests, and hypoglycemic therapy was mailed to participants who reported having received a diagnosis of diabetes. A case of T2DM was considered confirmed if at least one of the following was reported on the supplementary questionnaire according to the National Diabetes Data Group criteria (20): *1*) one or more classic symptoms (excessive thirst, polyuria, weight loss, and hunger) plus elevated glucose concentrations [fasting concentrations of $\geq$140 mg/dL (7.8 mmol/L), random plasma glucose concentrations of $\geq$200 mg/dL (11.1 mmol/L), and/or concentrations of $\geq$200 mg/dL after $\geq$2 h shown during oral-glucose-tolerance testing], *2*) elevated plasma glucose concentrations on $\geq$2 different occasions in the absence of symptoms, or *3*) treatment with hypoglycemic medication (insulin or oral hypoglycemic agent). The diagnostic criteria changed in June 1998, and a fasting plasma glucose concentration of 126 mg/dL (7.0 mmol/L) was considered the threshold for the diagnosis of diabetes instead of 140 mg/dL (21).

The validity of the supplementary questionnaire for the diagnosis of diabetes was documented previously (22). Of a random sample of 62 NHS participants who reported T2DM, which was confirmed by the supplementary questionnaire, 61 (98%) of them were reconfirmed after their medical records were reviewed by an endocrinologist blinded to the supplementary questionnaire. We conducted a similar validation study in the HPFS: of 59 T2DM cases who were confirmed by the supplementary questionnaire, 57 (97%) were reconfirmed by medical records (23).

Assessment of covariates

In the biennial follow-up questionnaires, we inquired and updated information on risk factors for chronic diseases, such as body weight, cigarette smoking, physical activity, multivitamin use, and a family history of diabetes. Among NHS and NHS II participants, we ascertained menopausal status, postmenopausal hormone use, and oral contraceptive use (NHS II only).

Statistical analysis

We calculated each individual's person-years from the date of return of the baseline questionnaire to the date of diagnosis of T2DM, death, or the end of the follow-up (30 June 2008 for NHS, 30 June 2007 for NHS II, or 31 January 2006 for HPFS), whichever came first. We used time-dependent Cox proportional hazards regression (24) to estimate the HR for flavonoid intake in relation to risks of T2DM by using the lowest quintile as the referent group. We used quintiles of intake to avoid assumptions about linearity and to also reduce the effect of potential outliers. The median intake value was assigned to each quintile category. A test for linear trend using the Wald test was performed by modeling the median values as a continuous variable. Analyses were first performed separately for the 3 cohorts and then the parameter estimates were pooled by using a random-effects model meta-analysis approach given the heterogeneities in age and sex among the cohorts.

To represent long-term diet and reduce within-person variation, we used the cumulative average of dietary intake from all FFQs

available before the beginning of each 2-y follow-up (25). We stopped cumulative updating once a participant reported a diagnosis of hypertension, hypercholesterolemia, gestational diabetes in women, cardiovascular disease, or cancer to reduce the potential for bias, given that the occurrence of these diseases may alter food choices and/or recall (26). For missing dietary intake values, values from baseline or the most recent available FFQ were carried forward. In a sensitivity analysis, we evaluated the association between baseline flavonoid intakes and risk of T2DM.

The analysis was stratified jointly by age and questionnaire year and controlled for various potential confounding factors, including BMI (in kg/m^2; <23, 23.0–24.9, 25.0–26.9, 27.0–28.9, 29.0–30.9, 31.0–32.9, 33.0–34.9, 35.0–36.9, 37.0–38.9, 39.0–40.9, 41.0–42.9, 43.0–44.9, or ≥45.0), ethnicity (white, African American, Hispanic, or Asian), physical activity (quintiles of MET-hours/wk), cigarette smoking [never, past, or current (1–14, 15–24, or ≥25 cigarettes/d)], alcohol intake (0, 0.1–4.9, 5.0–9.9, 10.0–14.9, or ≥15 g/d in women; 0, 0.1–4.9, 5.0–29.9, or ≥30 g/d in men), multivitamin use (yes or no), a family history of diabetes (yes or no), quintiles of total energy intake, polyunsaturated-to-saturated fat ratio, and intakes of *trans* fat, red meat, fish, whole grains, high-calorie sodas (including punch), and coffee. Among nurses, we adjusted for postmenopausal status and menopausal hormone use (NHS and NHS II) and for oral contraceptive use (NHS II only).

The primary exposures for this analysis were total flavonoids and the 5 major subclasses (ie, flavonols, flavones, flavanones, flavan-3-ols, and anthocyanins). Other analyses were secondary to provide more insight on the primary results. For example, we explored flavonoid-rich foods (eg, blueberries, strawberries, apples, or pears) for the flavonoid subclasses, for which significant results were found in the primary analysis. Statistical analyses were performed by using the Statistical Analysis System 9.2 (SAS Institute Inc). All *P* values were 2-sided. Statistical significance was defined at an α level of 0.05.

RESULTS

Baseline characteristics for participants in the NHS (1984), NHS II (1991), and HPFS (1986) are presented by quintiles of total flavonoid intake in **Table 1**. The mean (range) age of the participants was 50 (37–65) y in the NHS, 36 (26–45) y in the NHS II, and 53 (40–75) y in the HPFS. Among the 3 cohorts, with increasing consumption of flavonoids, participants tended to have a more health-conscious lifestyle pattern with more physical activity, a higher consumption of whole grains, less cigarette smoking, and a lower consumption of red meat, *trans* fat, and high-calorie soft drinks. During 3,645,585 person-years of observation, we documented a total of 12,611 incident cases of T2DM (n = 6878 in NHS, 3084 in NHS II, and 2649 in HPFS). The HRs for T2DM according to quintiles of flavonoid intakes in the 3 cohorts are shown in **Table 2** by cohort, followed by the pooled results. Significant inverse associations were observed for anthocyanins in the NHS II, flavonols in the HPFS, and flavonols, flavan-3-ols, anthocyanins, and total flavonoids in the NHS. After estimates from 3 cohorts were pooled,

TABLE 1
Baseline characteristics of participants in the 3 cohorts according to quintiles of total dietary flavonoid intake[1]

	NHS I (1984)			NHS II (1991)			HPFS (1986)		
	Q1	Q3	Q5	Q1	Q3	Q5	Q1	Q3	Q5
No. of subjects	14,170	14,068	14,025	17,811	17,814	17,865	8081	8085	8084
Age (y)	49.3 ± 7.1[2]	50.4 ± 7.1	50.1 ± 7.2	36.0 ± 4.7	36.0 ± 4.7	36.4 ± 4.6	52.0 ± 9.5	52.9 ± 9.6	53.4 ± 9.4
BMI (kg/m^2)	24.9 ± 4.8	24.8 ± 4.4	24.8 ± 4.3	24.9 ± 5.7	24.3 ± 5.0	24.6 ± 5.2	25.7 ± 3.4	25.4 ± 3.2	25.4 ± 3.2
Physical activity (MET-h/wk)	11.3 ± 16.8	15.6 ± 22.6	14.4 ± 20.7	17.0 ± 24.4	22.8 ± 28.4	21.6 ± 28.3	17.0 ± 27.9	23.4 ± 29.9	22.4 ± 33.2
Current smoker [*n* (%)]	5209 (36.8)	2771 (19.7)	2879 (20.5)	3235 (18.2)	1760 (9.9)	2011 (11.3)	1304 (16.1)	584 (7.2)	624 (7.7)
Race, white [*n* (%)]	13,836 (97.6)	13,738 (97.7)	13,794 (98.4)	16,380 (92.0)	16,492 (92.6)	16,784 (94.0)	7704 (95.3)	7716 (95.4)	7621 (94.3)
Family history of diabetes [*n* (%)]	3567 (25.2)	3502 (24.9)	3525 (25.1)	2919 (16.4)	2720 (15.3)	3054 (17.1)	1456 (18.0)	1486 (18.4)	1579 (19.5)
Hypertension [*n* (%)]	2713 (19.2)	2745 (19.5)	2792 (19.9)	1084 (6.1)	1004 (5.6)	1222 (6.8)	1553 (19.2)	1526 (18.9)	1613 (20.0)
Hypercholesterolemia [*n* (%)]	1038 (7.3)	1000 (7.1)	1008 (7.2)	2726 (15.3)	2418 (13.6)	2688 (15.0)	740 (9.2)	885 (11.0)	899 (11.1)
Multivitamin use [*n* (%)]	4559 (32.2)	5646 (40.1)	5182 (37.0)	6849 (38.5)	8390 (47.1)	7601 (42.6)	2995 (37.1)	3458 (42.8)	3446 (42.6)
Postmenopausal [*n* (%)]	7694 (54.3)	8274 (58.8)	8146 (58.1)	629 (3.5)	590 (3.3)	593 (3.3)	NA	NA	NA
Ever menopausal hormone use [*n* (%)]	2801 (19.8)	3182 (22.6)	3006 (21.4)	479 (2.7)	466 (2.6)	654 (3.7)	NA	NA	NA
Current oral conceptive use [*n* (%)]	NA	NA	NA	1900 (10.7)	1934 (10.9)	1837 (10.3)	NA	NA	NA
Total energy intake (kcal/d)	1694 ± 535	1784 ± 536	1705 ± 541	1700 ± 540	1848 ± 547	1730 ± 564	1912 ± 576	1994 ± 567	1901 ± 551
Alcohol intake (g/d)	8.0 ± 13.2	7.0 ± 10.6	5.8 ± 10.1	3.1 ± 6.4	3.3 ± 6.0	2.7 ± 5.7	12.5 ± 16.8	11.2 ± 14.7	9.6 ± 13.7
Red meat intake (servings/d)	1.4 ± 0.7	1.2 ± 0.6	1.2 ± 0.6	0.9 ± 0.6	0.7 ± 0.5	0.8 ± 0.5	1.4 ± 0.9	1.1 ± 0.7	1.0 ± 0.7
Fish intake (servings/d)	0.2 ± 0.1	0.2 ± 0.2	0.2 ± 0.2	0.2 ± 0.2	0.3 ± 0.2	0.3 ± 0.2	0.3 ± 0.3	0.3 ± 0.3	0.4 ± 0.3
Whole grain intake (g/d)	11.7 ± 12.8	15.0 ± 13.0	14.4 ± 13.2	18.0 ± 15.7	22.0 ± 15.9	20.1 ± 15.5	18.5 ± 19.0	23.1 ± 19.6	22.3 ± 19.2
Coffee intake (cups/d)[3]	2.8 ± 2.0	2.5 ± 1.8	1.8 ± 1.7	1.8 ± 1.8	1.6 ± 1.6	1.2 ± 1.5	2.3 ± 2.0	1.9 ± 1.8	1.6 ± 1.6
High-calorie soft drink intake (servings/d)	0.4 ± 0.8	0.3 ± 0.5	0.2 ± 0.5	0.5 ± 1.0	0.5 ± 0.8	0.4 ± 0.7	0.4 ± 0.7	0.2 ± 0.4	0.2 ± 0.4
Polyunsaturated:saturated fat	0.5 ± 0.2	0.6 ± 0.2	0.6 ± 0.2	0.5 ± 0.1	0.5 ± 0.2	0.5 ± 0.2	0.5 ± 0.2	0.6 ± 0.2	0.6 ± 0.2
trans Fat intake (% of energy)	2.0 ± 0.6	1.9 ± 0.6	1.9 ± 0.6	1.8 ± 0.7	1.5 ± 0.6	1.6 ± 0.6	1.4 ± 0.5	1.2 ± 0.5	1.2 ± 0.5

[1] HPFS, Health Professionals Follow-Up Study; MET, metabolic equivalent tasks; NA, not available; NHS, Nurses' Health Study; Q, quintile.
[2] Mean ± SD for continuous data (all such values).
[3] 1 cup = 8 oz = 237 mL.

TABLE 2
HRs (95% CIs) of type 2 diabetes risk according to flavonoid intake in the NHS (1984–2008), NHS II (1991–2007), and HPFS (1986–2006)[1]

	Frequency of consumption					
	Q1	Q2	Q3	Q4	Q5	*P*-trend
Flavonols						
NHS						
Median value (mg/d)	6.1	9.2	12.3	16.4	27.0	
Cases/person-years	1676/289,182	1389/305,484	1351/308,834	1242/313,506	1220/310,591	
Model 1[2]	1.00	0.85 (0.79, 0.91)	0.85 (0.79, 0.91)	0.78 (0.73, 0.84)	0.77 (0.71, 0.83)	<0.001
Model 2[3]	1.00	0.93 (0.87, 1.00)	0.96 (0.89, 1.03)	0.91 (0.84, 0.98)	0.84 (0.78, 0.91)	<0.001
NHS II						
Median value (mg/d)	7.5	11.4	15.1	20.6	33.6	
Cases/person-years	785/276,085	510/279,559	548/281,107	552/280,187	689/275,874	
Model 1[2]	1.00	0.75 (0.67, 0.84)	0.82 (0.74, 0.92)	0.83 (0.74, 0.92)	0.93 (0.84, 1.03)	0.74
Model 2[3]	1.00	0.85 (0.76, 0.95)	0.95 (0.85, 1.07)	0.94 (0.84, 1.05)	0.99 (0.89, 1.10)	0.46
HPFS						
Median value (mg/d)	6.6	10.0	13.1	17.2	26.8	
Cases/person-years	650/144,126	565/144,837	501/145,141	437/145,473	496/145,598	
Model 1[2]	1.00	0.90 (0.80, 1.01)	0.82 (0.73, 0.92)	0.73 (0.64, 0.82)	0.79 (0.71, 0.89)	<0.001
Model 2[3]	1.00	0.98 (0.87, 1.10)	0.92 (0.82, 1.04)	0.83 (0.73, 0.94)	0.88 (0.78, 1.00)	0.02
Pooled results[4]						
Random-effects model	1.00	0.92 (0.86, 0.99)	0.95 (0.90, 1.00)	0.90 (0.85, 0.95)	0.90 (0.81, 0.99)	0.18
P-heterogeneity	—	0.20	0.85	0.35	0.06	0.001
Flavones						
NHS						
Median value (mg/d)	0.7	1.3	1.8	2.4	3.4	
Cases/person-years	1503/298,859	1384/310,739	1413/309,181	1343/308,847	1235/299,971	
Model 1[2]	1.00	0.89 (0.83, 0.96)	0.92 (0.85, 0.99)	0.92 (0.85, 0.99)	0.90 (0.84, 0.97)	0.05
Model 2[3]	1.00	0.96 (0.89, 1.04)	1.03 (0.95, 1.11)	1.05 (0.97, 1.13)	1.07 (0.99, 1.16)	0.02
NHS II						
Median value (mg/d)	0.6	1.0	1.4	1.9	2.9	
Cases/person-years	806/273,758	650/278,077	607/278,825	541/281,259	480/280,892	
Model 1[2]	1.00	0.91 (0.82, 1.01)	0.92 (0.83, 1.02)	0.85 (0.76, 0.95)	0.86 (0.77, 0.97)	0.006
Model 2[3]	1.00	1.00 (0.90, 1.11)	1.07 (0.95, 1.19)	1.00 (0.89, 1.12)	1.02 (0.91, 1.16)	0.75
HPFS						
Median value (mg/d)	0.8	1.6	2.2	3.0	4.3	
Cases/person-years	553/144,874	582/145,336	478/144,999	533/145,027	503/144,941	
Model 1[2]	1.00	1.05 (0.94, 1.18)	0.88 (0.78, 0.99)	1.02 (0.91, 1.15)	0.94 (0.83, 1.06)	0.28
Model 2[3]	1.00	1.12 (1.00, 1.26)	0.96 (0.84, 1.08)	1.14 (1.01, 1.29)	1.07 (0.94, 1.22)	0.37
Pooled results[4]						
Random-effects model	1.00	1.02 (0.93, 1.11)	1.02 (0.97, 1.08)	1.05 (0.99, 1.12)	1.06 (1.00, 1.12)	0.02
P-heterogeneity	—	0.10	0.44	0.32	0.83	0.61
Flavanones						
NHS						
Median value (mg/d)	7.9	21.7	37.1	54.0	82.4	
Cases/person-years	1486/299,878	1404/312,037	1382/311,909	1357/306,655	1249/297,119	
Model 1[2]	1.00	0.89 (0.82, 0.95)	0.89 (0.83, 0.96)	0.93 (0.87, 1.00)	0.91 (0.84, 0.98)	0.13
Model 2[3]	1.00	0.96 (0.89, 1.03)	0.98 (0.91, 1.06)	1.05 (0.97, 1.13)	1.05 (0.97, 1.13)	0.05
NHS II						
Median value (mg/d)	6.2	14.7	25.0	40.0	70.6	
Cases/person-years	769/273,887	619/278,715	592/280,250	543/280,636	561/279,323	
Model 1[2]	1.00	0.88 (0.80, 0.98)	0.89 (0.80, 0.99)	0.84 (0.75, 0.94)	0.96 (0.86, 1.07)	0.63
Model 2[3]	1.00	0.95 (0.85, 1.06)	0.98 (0.88, 1.09)	0.94 (0.84, 1.05)	1.08 (0.97, 1.22)	0.13
HPFS						
Median value (mg/d)	8.7	25.6	44.6	64.6	100.1	
Cases/person-years	559/144,599	527/145,249	535/145,112	514/145,067	514/145,150	
Model 1[2]	1.00	0.97 (0.86, 1.10)	1.00 (0.89, 1.13)	0.99 (0.88, 1.12)	0.99 (0.88, 1.12)	0.96
Model 2[3]	1.00	1.03 (0.92, 1.17)	1.06 (0.94, 1.20)	1.08 (0.95, 1.22)	1.09 (0.96, 1.24)	0.14
Pooled results[4]						
Random-effects model	1.00	0.97 (0.92, 1.02)	1.00 (0.94, 1.06)	1.02 (0.95, 1.10)	1.06 (1.00, 1.13)	0.004
P-heterogeneity	—	0.52	0.52	0.19	0.79	0.94
Flavan-3-ols						
NHS						
Median value (mg/d)	8.4	15.6	27.0	54.6	135.1	
Cases/person-years	1614/295,137	1331/306,390	1261/312,298	1363/306,983	1309/306,789	
Model 1[2]	1.00	0.85 (0.79, 0.92)	0.82 (0.76, 0.88)	0.89 (0.83, 0.96)	0.85 (0.79, 0.91)	0.03
Model 2[3]	1.00	0.94 (0.87, 1.01)	0.91 (0.85, 0.98)	0.95 (0.88, 1.02)	0.87 (0.81, 0.94)	0.002

(Continued)

TABLE 2 (*Continued*)

	Frequency of consumption					
	Q1	Q2	Q3	Q4	Q5	*P*-trend
NHS II						
Median value (mg/d)	9.0	16.5	27.7	56.2	148.4	
Cases/person-years	784/274,916	510/279,651	510/281,582	575/279,240	705/277,424	
Model 1[2]	1.00	0.78 (0.70, 0.87)	0.84 (0.75, 0.94)	0.86 (0.78, 0.96)	0.98 (0.88, 1.08)	0.06
Model 2[3]	1.00	0.91 (0.81, 1.02)	0.98 (0.87, 1.10)	0.96 (0.86, 1.07)	1.01 (0.91, 1.12)	0.40
HPFS						
Median value (mg/d)	9.0	16.7	25.4	43.9	103.9	
Cases/person-years	653/144,321	527/145,054	457/145,424	487/145,311	525/145,066	
Model 1[2]	1.00	0.84 (0.75, 0.94)	0.76 (0.68, 0.86)	0.82 (0.73, 0.93)	0.85 (0.76, 0.96)	0.25
Model 2[3]	1.00	0.90 (0.80, 1.02)	0.85 (0.75, 0.96)	0.91 (0.80, 1.02)	0.88 (0.78, 0.99)	0.22
Pooled results[4]						
Random-effects model	1.00	0.92 (0.87, 0.98)	0.91 (0.85, 0.98)	0.94 (0.89, 0.99)	0.91 (0.84, 1.00)	0.32
P-heterogeneity	—	0.80	0.27	0.78	0.07	0.03
Anthocyanins						
NHS						
Median value (mg/d)	2.2	4.7	8.1	13.1	22.3	
Cases/person-years	1688/286,253	1513/303,189	1293/314,489	1251/314,333	1133/309,332	
Model 1[2]	1.00	0.87 (0.81, 0.93)	0.75 (0.70, 0.81)	0.75 (0.70, 0.80)	0.69 (0.64, 0.74)	<0.001
Model 2[3]	1.00	0.93 (0.86, 0.99)	0.84 (0.78, 0.91)	0.85 (0.79, 0.92)	0.83 (0.77, 0.90)	<0.001
NHS II						
Median value (mg/d)	2.0	4.5	8.0	13.7	24.3	
Cases/person-years	898/270,677	702/277,111	513/281,465	515/281,334	456/282,225	
Model 1[2]	1.00	0.87 (0.79, 0.96)	0.72 (0.64, 0.80)	0.74 (0.66, 0.82)	0.68 (0.61, 0.76)	<0.001
Model 2[3]	1.00	0.98 (0.88, 1.08)	0.84 (0.75, 0.94)	0.88 (0.79, 0.99)	0.83 (0.73, 0.94)	0.002
HPFS						
Median value (mg/d)	2.3	4.9	8.3	14.0	24.2	
Cases/person-years	621/144,223	541/144,956	519/145,403	508/145,413	460/145,183	
Model 1[2]	1.00	0.90 (0.80, 1.01)	0.88 (0.78, 0.99)	0.87 (0.77, 0.98)	0.80 (0.70, 0.90)	<0.001
Model 2[3]	1.00	0.95 (0.84, 1.06)	0.96 (0.85, 1.08)	0.95 (0.84, 1.07)	0.93 (0.81, 1.05)	0.34
Pooled results[4]						
Random-effects model	1.00	0.94 (0.89, 0.99)	0.87 (0.80, 0.94)	0.88 (0.83, 0.94)	0.85 (0.80, 0.91)	<0.001
P for heterogeneity	—	0.69	0.15	0.33	0.34	0.20
Total flavonoids						
NHS						
Median value (mg/d)	105.2	174.8	249.2	369.1	718.1	
Cases/person-years	1580/295,862	1377/306,228	1309/311,654	1348/307,160	1264/306,694	
Model 1[2]	1.00	0.87 (0.81, 0.94)	0.84 (0.78, 0.91)	0.88 (0.82, 0.95)	0.82 (0.76, 0.89)	<0.001
Model 2[3]	1.00	0.95 (0.88, 1.02)	0.93 (0.86, 1.01)	0.96 (0.89, 1.03)	0.85 (0.79, 0.92)	<0.001
NHS II						
Median value (mg/d)	112.1	182.5	256.1	378.4	770.3	
Cases/person-years	754/274,738	554/280,370	506/280,556	582/279,499	688/277,648	
Model 1[2]	1.00	0.85 (0.76, 0.95)	0.82 (0.73, 0.92)	0.92 (0.82, 1.02)	0.98 (0.89, 1.09)	0.20
Model 2[3]	1.00	0.94 (0.84, 1.05)	0.92 (0.82, 1.03)	1.00 (0.89, 1.12)	0.99 (0.89, 1.11)	0.56
HPFS						
Median value (mg/d)	112.5	182.2	251.7	352.9	624.3	
Cases/person-years	600/144,345	540/145,059	505/145,303	501/145,241	503/145,228	
Model 1[2]	1.00	0.93 (0.83, 1.05)	0.92 (0.82, 1.03)	0.90 (0.80, 1.02)	0.88 (0.79, 1.00)	0.07
Model 2[3]	1.00	0.98 (0.87, 1.11)	1.00 (0.89, 1.13)	0.97 (0.86, 1.10)	0.92 (0.81, 1.04)	0.15
Pooled results[4]						
Random-effects model	1.00	0.95 (0.90, 1.01)	0.94 (0.89, 1.00)	0.97 (0.92, 1.03)	0.92 (0.83, 1.01)	0.21
P-heterogeneity	—	0.84	0.53	0.83	0.07	0.02

[1] HPFS, Health Professionals Follow-Up Study; NHS, Nurses' Health Study; Q, quintile.

[2] Adjusted for age (continuous) and BMI category (in kg/m^2; <23, 23.0–24.9, 25.0–26.9, 27.0–28.9, 29.0–30.9, 31.0–32.9, 33.0–34.9, 35.0–36.9, 37.0–38.9, 39.0–40.9, 41.0–42.9, 43.0–44.9, or ≥45.0).

[3] Further adjusted for variables in model 1 plus smoking status [never smoker, past smoker, or current smoker (1–14, 15–24, or ≥25 cigarettes/d)], alcohol intake (0, 0.1–4.9, 5.0–9.9, 10.0–14.9, or ≥15 g/d in women; 0, 0.1–4.9, 5.0–29.9, or ≥30 g/d in men), multivitamin use (yes or no), physical activity (quintiles of hours of metabolic equivalent tasks per week), a family history of diabetes, postmenopausal status and hormone use (NHS and NHS II), oral contraceptive use (NHS II), ethnicity (white, African American, Hispanic, or Asian), total energy (kcal/d), and polyunsaturated:saturated fat ratio and intakes of red meat, fish, whole grains, coffee, high-calorie sodas (including punch), and *trans* fat (all in quintiles).

[4] Data were pooled by using random-effects model of results from model 2.

anthocyanin intake was associated with a significant lower risk of T2DM. Compared with the lowest quintile, the pooled HR for the highest quintile was 0.85 (95% CI: 0.80, 0.90; *P*-trend < 0.001; *P*-heterogeneity = 0.20). In a sensitivity analysis using only the baseline anthocyanin information, the association was attenuated (pooled HR: 0.93; 95% CI: 0.88, 0.98 for highest compared with the lowest quintile; *P*-trend = 0.04). We also evaluated whether the abovementioned associations were modified by physical activity or obesity status and found no significant interactions (ie, all *P* values > 0.10 from likelihood-ratio tests).

For other flavonoid subclasses, higher intakes of flavones and flavanones were associated with a slightly higher risk of T2DM. However, citrus juices are major sources of flavones and flavanones in these populations, and we previously reported that juice intake is associated with a higher risk of T2DM (27). We therefore additionally adjusted for quintiles of juice intake (fruit juices and fruit punch) to evaluate potential positive confounding. After adjustment for juice intake, associations were attenuated and were no longer significant for either flavones (pooled HR: 1.00; 95% CI: 0.93, 1.07 for highest compared with lowest quintile; *P*-trend = 0.78) or flavanones (pooled HR: 1.01; 95% CI: 0.91, 1.11; *P*-trend = 0.49).

Given the strength of the associations for anthocyanins and the consistency of results across the 3 cohorts, the remaining analyses were focused on the associations for specific anthocyanin compounds and anthocyanin-rich foods. Baseline characteristics of the participants according to quintiles of anthocyanin intake are shown elsewhere (*see* Supplemental Table 1 under "Supplemental data" in the online issue). We also noted a low or moderate correlation between anthocyanin with total and other subclasses of flavonoids (*see* Supplemental Table 2 under "Supplemental data" in the online issue).

In secondary analyses of individual anthocyanins, the strongest association was observed for cyanidin (pooled HR: 0.79; 95% CI: 0.72, 0.85) from a comparison of the highest with the lowest quintile (*P*-trend < 0.001; *P*-heterogeneity = 0.06). Associations were weaker for delphinidin (pooled HR: 0.87; 95% CI: 0.80, 0.96), malvidin (pooled HR: 0.82; 95% CI: 0.72, 0.94), peonidin (pooled HR: 0.87; 95% CI: 0.78, 0.96), and petunidin (pooled HR: 0.88; 95% CI: 0.81, 0.97) for the highest compared with the lowest quintile [*P*-trend < 0.001 for all; *P*-heterogeneity > 0.10 for all except for malvidin (*P*-heterogeneity = 0.04)]. Pelargonidin was not significantly associated with diabetes risk (pooled HR: 0.97; 95% CI: 0.92, 1.03; *P*-trend = 0.79; *P*-heterogeneity = 0.71).

We also evaluated the major anthocyanin-rich foods consumed in these cohorts: blueberries, strawberries, and apples/pears (**Table 3**). For the pooled analyses, we observed a significantly lower risk of T2DM for all of these foods (*P*-trend < 0.01 for all), and the strongest and most consistent associations were for apples/pears (HR: 0.77; 95% CI: 0.65, 0.93 from a comparison of ≥5/wk with <1/mo) and blueberries (HR: 0.77; 95% CI: 0.68, 0.87 from a comparison of ≥2/wk with <1/mo). In a secondary analysis, we examined the association of a combination of all other fruit with diabetes risk and found a pooled HR of 0.97 (95% CI: 0.79, 1.19; *P*-trend = 0.61) from a comparison of ≥5/wk with <1/mo.

DISCUSSION

In these 3 prospective cohort studies including ~200,000 US men and women, a higher intake of anthocyanins was consistently associated with a significantly lower risk of T2DM. Consumption of foods rich in anthocyanins, particularly blueberries and apples/pears, was also inversely associated with the risk of T2DM. Although flavonols, flavan-3-ols, and total flavonoids were also inversely associated with diabetes risk in individual cohorts, results for these compounds were not consistent across all cohorts.

Our findings for apples/pears and berries are consistent with a Finnish study of >10,000 men and women with 526 cases of T2DM, which also reported significant inverse associations with risk of T2DM: apples (HR: 0.73; 95% CI: 0.57, 0.92 from a comparison of the top with the bottom quartile) and berries (HR: 0.74; 95% CI: 0.58, 0.95 from a comparison of the top with the bottom quartile) (10). In that study, a nonsignificant trend toward a lower risk of T2DM was observed for flavonols but not for the other 2 flavonoids that were also examined (ie, flavones and flavanones) (10). In contrast, in the Women's Health Study of US women aged ≥45 y, no significant association was observed for intakes of total flavonoids, the 2 flavonoid subclasses studied (flavonols and flavones), or flavonoid-rich foods, except for red wine, for which an inverse trend was reported (12). In the Iowa Women's Health Study, no significant inverse associations with risk of T2DM were observed for total flavonoid intake or any of the flavonoid subclasses, including anthocyanins (11). Inconsistent findings may be due in part to the older less complete versions of the USDA database used in previous studies conducted in the United States (11, 12) and differences in food items ascertained on dietary questionnaires. It is also plausible that the use of only baseline questionnaires may have introduced misclassification, because dietary intakes may have changed during follow-up. In a sensitivity analysis in which we used only baseline anthocyanin intake to predict T2DM risk, the association was indeed weaker. This finding suggests that more recent usual flavonoid intakes may be more related to the etiology of T2DM than to intakes further in the past.

Several mechanisms have been proposed by which specific flavonoid constituents can reduce biological pathways related to the development of T2DM. Cellular and physiologic data support a correlative relation between insulin resistance and endothelial dysfunction (28). Some subclasses of flavonoids have been shown to improve endothelial function; specifically, dark chocolate rich in flavan-3-ols (primarily the epicatechin compound) was shown to improve flow-mediated dilatation and decreased blood pressure (29). In a trial of hypertensive subjects, dark chocolate consumption resulted in significantly decreased blood pressure and improvements in measurements of insulin sensitivity compared with white chocolate (30). Flavonoids also interact with molecular targets and affect signaling pathways with evidence in vitro that both the nuclear factor κ-B and mitogen-activated protein kinase signaling pathways are modified (2). In an animal model of T2DM, anthocyanins (ie, cyanidin 3-glucoside) significantly decreased blood glucose conentrations and improved insulin sensitivity after an insulin tolerance test in male mice (31). In addition, gene expression of the glucose transporter GLUT4 was upregulated in white adipose tissue, whereas expression of retinol binding protein 4 was downregulated, which resulted in suppression of gluconeogenesis and improved glycemia. Similarly, an anthocyanin-rich bilberry extract improved glycemia and insulin sensitivity in male mice with T2DM accompanied by increased activation of AMP-activated protein

TABLE 3
HRs (and 95% CIs) for incident type 2 diabetes in women by dietary intake of anthocyanin-rich foods[1]

	Intake of anthocyanin-rich foods					
	<1 time/mo	1–3 times/mo	1 time/wk	2–4 times/wk	≥5 times/wk	*P*-trend
Strawberries						
NHS						
Cases/person-years	1746/372,692	2842/643,347	1808/400,048	404/92,670	78/18,840	
Model 1[2]	1.00	0.95 (0.90, 1.01)	0.94 (0.88, 1.00)	0.84 (0.76, 0.94)	0.83 (0.66, 1.04)	0.001
Model 2[3]	1.00	0.97 (0.92, 1.03)	0.97 (0.91, 1.04)	0.88 (0.79, 0.98)	0.89 (0.71, 1.11)	0.02
NHS II						
Cases/person-years	567/213,115	1210/556,146	841/406,709	395/184,982	71/31,859	
Model 1[2]	1.00	0.81 (0.73, 0.90)	0.80 (0.72, 0.89)	0.75 (0.66, 0.86)	0.81 (0.63, 1.04)	0.008
Model 2[3]	1.00	0.87 (0.78, 0.96)	0.89 (0.79, 0.99)	0.83 (0.73, 0.95)	0.88 (0.68, 1.13)	0.13
HPFS						
Cases/person-years	924/227,757	1155/333,650	402/114,418	147/43,417	21/5935	
Model 1[2]	1.00	0.87 (0.80, 0.95)	0.88 (0.79, 0.99)	0.81 (0.68, 0.97)	0.88 (0.57, 1.36)	0.03
Model 2[3]	1.00	0.91 (0.83, 0.99)	0.93 (0.83, 1.05)	0.88 (0.73, 1.05)	0.90 (0.58, 1.39)	0.19
Pooled results[4]						
Random-effects model	1.00	0.92 (0.86, 0.99)	0.94 (0.90, 0.99)	0.86 (0.80, 0.93)	0.89 (0.76, 1.04)	0.003
P-heterogeneity	—	0.12	0.40	0.80	0.99	0.95
Blueberries[5]						
NHS						
Cases/person-years	4545/954,221	1575/383,866	641/157,315	117/32,196		
Model 1[2]	1.00	0.90 (0.85, 0.95)	0.86 (0.79, 0.93)	0.78 (0.65, 0.94)		<0.001
Model 2[3]	1.00	0.93 (0.88, 0.99)	0.91 (0.84, 0.99)	0.83 (0.69, 1.00)		0.002
NHS II						
Cases/person-years	1942/760,223	749/406,799	294/165,249	99/60,541		
Model 1[2]	1.00	0.77 (0.70, 0.83)	0.77 (0.68, 0.87)	0.65 (0.53, 0.79)		<0.001
Model 2[3]	1.00	0.83 (0.76, 0.90)	0.85 (0.75, 0.96)	0.68 (0.56, 0.84)		<0.001
HPFS						
Cases/person-years	1698/436,603	748/222,924	147/45,768	56/19,883		
Model 1[2]	1.00	0.88 (0.81, 0.96)	0.87 (0.74, 1.03)	0.74 (0.57, 0.96)		0.002
Model 2[3]	1.00	0.92 (0.84, 1.00)	0.94 (0.79, 1.12)	0.79 (0.61, 1.04)		0.03
Pooled results[4]						
Random-effects model	1.00	0.89 (0.83, 0.96)	0.90 (0.84, 0.96)	0.77 (0.68, 0.87)		<0.001
P-heterogeneity	—	0.07	0.53	0.38		0.26
Apples and pears						
NHS						
Cases/person-years	887/183,788	1739/374,745	1609/349,603	1740/405,921	903/213,541	
Model 1[2]	1.00	0.93 (0.86, 1.01)	0.90 (0.83, 0.97)	0.82 (0.75, 0.88)	0.79 (0.72, 0.87)	<0.001
Model 2[3]	1.00	0.97 (0.89, 1.05)	0.97 (0.89, 1.05)	0.91 (0.83, 0.99)	0.88 (0.80, 0.97)	0.002
NHS II						
Cases/person-years	330/101,058	784/315,976	675/297,235	879/454,119	416/224,424	
Model 1[2]	1.00	0.71 (0.63, 0.81)	0.68 (0.60, 0.78)	0.60 (0.53, 0.68)	0.57 (0.49, 0.66)	<0.001
Model 2[3]	1.00	0.75 (0.66, 0.85)	0.75 (0.65, 0.86)	0.68 (0.59, 0.78)	0.65 (0.56, 0.76)	<0.001
HPFS						
Cases/person-years	292/65,996	606/160,617	493/131,919	779/222,097	479/144,548	
Model 1[2]	1.00	0.85 (0.74, 0.98)	0.86 (0.74, 0.99)	0.78 (0.68, 0.89)	0.73 (0.63, 0.85)	<0.001
Model 2[3]	1.00	0.85 (0.74, 0.98)	0.90 (0.78, 1.05)	0.82 (0.72, 0.95)	0.79 (0.68, 0.93)	0.01
Pooled results[4]						
Random-effects model	1.00	0.86 (0.73, 1.01)	0.87 (0.75, 1.02)	0.80 (0.67, 0.95)	0.77 (0.65, 0.93)	<0.001
P-heterogeneity	—	0.003	0.008	0.002	0.005	0.20

[1] HPFS, Health Professionals Follow-Up Study; NHS, Nurses' Health Study.

[2] Adjusted for age (continuous) and BMI category (in kg/m^2; <23, 23.0–24.9, 25.0–26.9, 27.0–28.9, 29.0–30.9, 31.0–32.9, 33.0–34.9, 35.0–36.9, 37.0–38.9, 39.0–40.9, 41.0–42.9, 43.0–44.9, or ≥45.0).

[3] Further adjusted for variables in model 1 plus smoking status [never smoker, past smoker, or current smoker (1–14, 15–24, or ≥25 cigarettes/d)], alcohol intake (0, 0.1–4.9, 5.0–9.9, 10.0–14.9, or ≥15 g/d in women; 0, 0.1–4.9, 5.0–29.9, or ≥30 g/d in men), multivitamin use (yes or no), physical activity (quintiles of hours of metabolic equivalent tasks per week), a family history of diabetes, postmenopausal status and hormone use (NHS and NHS II), oral contraceptive use (NHS II), ethnicity (white, African American, Hispanic, or Asian), total energy (kcal/d), and polyunsaturated:saturated fat ratio and intakes of red meat, fish, whole grains, coffee, high-calorie sodas (including punch), and *trans* fat (all in quintiles).

[4] Data were pooled by using random-effects model of results from model 2.

[5] Because of the low number of type 2 diabetes cases, the 2 highest categories for blueberry intake were combined to yield more stable estimates.

kinase and resulted in upregulation of GLUT4 (32). In human intervention trials, berries have been shown to significantly improve insulin sensitivity (33), reduce fasting plasma glucose (34), and reduce the postprandial glucose response to a sucrose load (35). These trials evaluated blueberry bioactives, blueberry leave extracts, and a berry purée (ie, blend of bilberries, black currants, cranberries, and strawberries), respectively. In contrast, 2 trials using freeze-dried strawberry powder found no evidence of improved glycemia, although LDL and total cholesterol were significantly decreased (36, 37). Given the variability in specific anthocyanin compounds for particular berry substances, it will be important in future trials to characterize which specific compounds confer the most benefit to glucose homeostasis.

Our study had several strengths, including the prospective design, large sample size, and low attrition of participants in all 3 cohorts. In addition, we used repeated measurements of dietary intake, which enabled the use of the cumulative average of dietary exposure before disease onset to better represent long-term consumption, which may be more relevant for the pathogenesis of T2DM (25). Finally, the recent integration of the range of flavonoid subclasses into our food composition databases provided an opportunity to expand beyond previous investigations to evaluate these compounds in relation to T2DM risk. However, we cannot conclude causation based on the observational study design. Although we were able to control for many potential covariates in multivariate models, it is plausible that residual confounding may still exist. Some misclassification of flavonoid intakes is inevitable, although our validation studies indicated that intakes reported on the FFQ were reasonably reproducible and valid for many of the flavonoid-rich foods evaluated in these cohorts (16, 17, 38). Certain flavonoid-rich foods may not be captured on the FFQ, for example, whereas the FFQ did ascertain strawberry and blueberry intakes, inquiry for other berry sources (eg, blackberries, raspberries, and currants) was not included in the questionnaires for this study. Given that flavonoid exposure was ascertained before diagnosis of disease, misclassification would tend to bias estimates toward the null and underestimate true associations. The results from our studies may not be generalizable to other populations, such as those of different ethnic composition.

In conclusion, our data suggest an inverse association between intake of anthocyanins and anthocyanin-rich foods (eg, blueberries and apples/pears) and T2DM in US men and women. It is possible that these findings reflect other dietary components that co-exist in anthocyanin-rich foods, and randomized trials will be needed to establish the effects that can be specifically attributed to anthocyanins. Further research on anthocyanin-rich foods may lead to more specific recommendations on consumption of fruit, which may contribute to the prevention of T2DM.

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REFERENCES

1. Schewe T, Steffen Y, Sies H. How do dietary flavanols improve vascular function? A position paper. Arch Biochem Biophys 2008;476:102–6.
2. Crozier A, Jaganath IB, Clifford MN. Dietary phenolics: chemistry, bioavailability and effects on health. Nat Prod Rep 2009;26:1001–43.
3. Clifford MN. Chlorogenic acids and other cinnamates—nature, occurrence and dietary burden. J Sci Food Agric 1999;79:362–72.
4. Richelle M, Tavazzi I, Offord E. Comparison of the antioxidant activity of commonly consumed polyphenolic beverages (coffee, cocoa, and tea) prepared per cup serving. J Agric Food Chem 2001;49:3438–42.
5. Hanhineva K, Torronen R, Bondia-Pons I, Pekkinen J, Kolehmainen M, Mykkanen H, Poutanen K. Impact of dietary polyphenols on carbohydrate metabolism. Int J Mol Sci 2010;11:1365–402.
6. Prior RL, Wu X. Anthocyanins: structural characteristics that result in unique metabolic patterns and biological activities. Free Radic Res 2006;40:1014–28.
7. Zunino S. Type 2 diabetes and glycemic response to grapes or grape products. J Nutr 2009;139:1794S–800S.
8. Del Rio D, Borges G, Crozier A. Berry flavonoids and phenolics: bioavailability and evidence of protective effects. Br J Nutr 2010;104 (suppl 3):S67–90.
9. Nizamutdinova IT, Jin YC, Chung JI, Shin SC, Lee SJ, Seo HG, Lee JH, Chang KC, Kim HJ. The anti-diabetic effect of anthocyanins in streptozotocin-induced diabetic rats through glucose transporter 4 regulation and prevention of insulin resistance and pancreatic apoptosis. Mol Nutr Food Res 2009;53:1419–29.
10. Knekt P, Kumpulainen J, Järvinen R, Rissanen H, Heliövaara M, Reunanen A, Hakulinen T, Aromaa A. Flavonoid intake and risk of chronic diseases. Am J Clin Nutr 2002;76:560–8.
11. Nettleton JA, Harnack LJ, Scrafford CG, Mink PJ, Barraj LM, Jacobs DR Jr. Dietary flavonoids and flavonoid-rich foods are not associated with risk of type 2 diabetes in postmenopausal women. J Nutr 2006;136:3039–45.
12. Song Y, Manson JE, Buring JE, Sesso HD, Liu S. 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. J Am Coll Nutr 2005;24:376–84.
13. van Dam RM, Willett WC, Rimm EB, Stampfer MJ, Hu FB. Dietary fat and meat intake in relation to risk of type 2 diabetes in men. Diabetes Care 2002;25:417–24.
14. Fung TT, Schulze M, Manson JE, Willett WC, Hu FB. Dietary patterns, meat intake, and the risk of type 2 diabetes in women. Arch Intern Med 2004;164:2235–40.
15. Schulze MB, Manson JE, Willett WC, Hu FB. Processed meat intake and incidence of Type 2 diabetes in younger and middle-aged women. Diabetologia 2003;46:1465–73.
16. Feskanich D, Rimm EB, Giovannucci EL, Colditz GA, Stampfer MJ, Litin LB, Willett WC. Reproducibility and validity of food intake measurements from a semiquantitative food frequency questionnaire. J Am Diet Assoc 1993;93:790–6.
17. Salvini S, Hunter DJ, Sampson L, Stampfer MJ, Colditz GA, Rosner B, Willett WC. Food-based validation of a dietary questionnaire: the effects of week-to-week variation in food consumption. Int J Epidemiol 1989;18:858–67.
18. Cassidy A, O'Reilly EJ, Kay C, Sampson L, Franz M, Forman J, Curhan G, Rimm EB. Habitual intake of flavonoid subclasses and incident hypertension in adults. Am J Clin Nutr 2011;93:338–47.
19. USDA. USDA database for the flavonoid content of selected foods. Release 2.1. Washington, DC: USDA, 2007.
20. National Diabetes Data Group. Classification and diagnosis of diabetes mellitus and other categories of glucose intolerance. Diabetes 1979;28:1039–57.
21. Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Diabetes Care 1997;20:1183–97.
22. Manson JE, Colditz GA, Stampfer MJ, Willett WC, Krolewski AS, Rosner B, Arky RA, Speizer FE, Hennekens CH. A prospective study of maturity-onset diabetes mellitus and risk of coronary heart disease and stroke in women. Arch Intern Med 1991;151:1141–7.

23. Hu FB, Leitzmann MF, Stampfer MJ, Colditz GA, Willett WC, Rimm EB. Physical activity and television watching in relation to risk for type 2 diabetes mellitus in men. Arch Intern Med 2001;161:1542–8.
24. Cox DR. Regression models and life tables. J R Stat Soc B 1972;34: 187–220.
25. Hu FB, Stampfer MJ, Rimm E, Ascherio A, Rosner BA, Spiegelman D, Willett WC. Dietary fat and coronary heart disease: a comparison of approaches for adjusting for total energy intake and modeling repeated dietary measurements. Am J Epidemiol 1999;149:531–40.
26. Shekelle RB, Stamler J, Paul O, Shryock AM, Liu S, Lepper M. Dietary lipids and serum cholesterol level: change in diet confounds the cross-sectional association. Am J Epidemiol 1982;115:506–14.
27. Bazzano LA, Li TY, Joshipura KJ, Hu FB. Intake of fruit, vegetables, and fruit juices and risk of diabetes in women. Diabetes Care 2008;31:1311–7.
28. Kim JA, Montagnani M, Koh KK, Quon MJ. Reciprocal relationships between insulin resistance and endothelial dysfunction: molecular and pathophysiological mechanisms. Circulation 2006;113:1888–904.
29. Hooper L, Kroon PA, Rimm EB, Cohn JS, Harvey I, Le Cornu KA, Ryder JJ, Hall WL, Cassidy A. Flavonoids, flavonoid-rich foods, and cardiovascular risk: a meta-analysis of randomized controlled trials. Am J Clin Nutr 2008;88:38–50.
30. Grassi D, Necozione S, Lippi C, Croce G, Valeri L, Pasqualetti P, Desideri G, Blumberg JB, Ferri C. Cocoa reduces blood pressure and insulin resistance and improves endothelium-dependent vasodilation in hypertensives. Hypertension 2005;46:398–405.
31. Sasaki R, Nishimura N, Hoshino H, Isa Y, Kadowaki M, Ichi T, Tanaka A, Nishiumi S, Fukuda I, Ashida H, et al. Cyanidin 3-glucoside ameliorates hyperglycemia and insulin sensitivity due to down-regulation of retinol binding protein 4 expression in diabetic mice. Biochem Pharmacol 2007;74:1619–27.
32. Takikawa M, Inoue S, Horio F, Tsuda T. Dietary anthocyanin-rich bilberry extract ameliorates hyperglycemia and insulin sensitivity via activation of AMP-activated protein kinase in diabetic mice. J Nutr 2010;140:527–33.
33. Stull AJ, Cash KC, Johnson WD, Champagne CM, Cefalu WT. Bioactives in blueberries improve insulin sensitivity in obese, insulin-resistant men and women. J Nutr 2010;140:1764–8.
34. Abidov M, Ramazanov A, Jimenez Del Rio M, Chkhikvishvili I. Effect of Blueberin on fasting glucose, C-reactive protein and plasma aminotransferases, in female volunteers with diabetes type 2: double-blind, placebo controlled clinical study. Georgian Med News 2006;Dec: 66–72.
35. Törrönen R, Sarkkinen E, Tapola N, Hautaniemi E, Kilpi K, Niskanen L. Berries modify the postprandial plasma glucose response to sucrose in healthy subjects. Br J Nutr 2010;103:1094–7.
36. Basu A, Wilkinson M, Penugonda K, Simmons B, Betts NM, Lyons TJ. Freeze-dried strawberry powder improves lipid profile and lipid peroxidation in women with metabolic syndrome: baseline and post intervention effects. Nutr J 2009;8:43.
37. Basu A, Fu DX, Wilkinson M, Simmons B, Wu M, Betts NM, Du M, Lyons TJ. Strawberries decrease atherosclerotic markers in subjects with metabolic syndrome. Nutr Res 2010;30:462–9.
38. Willett WC, Sampson L, Stampfer MJ, Rosner B, Bain C, Witschi J, Hennekens CH, Speizer FE. Reproducibility and validity of a semiquantitative food frequency questionnaire. Am J Epidemiol 1985;122: 51–65.