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Higher dietary anthocyanin and flavonol intakes are associated with anti-inflammatory effects in a population of US adults[1]

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ABSTRACT

Background: Although growing evidence from trials and population-based studies has supported a protective role for flavonoids in relation to risk of certain chronic diseases, the underlying mechanisms remain unclear. Several previous studies focused on individual inflammatory biomarkers, but because of the limited specificity of any individual marker, an assessment of a combination of biomarkers may be more informative.

Objective: We used an inflammation score (IS) that integrated 12 individual inflammatory biomarkers for the examination of associations with intakes of different flavonoid classes.

Design: The study was a cross-sectional analysis of 2375 Framingham Heart Study Offspring Cohort participants. Intakes of total flavonoids and their classes (anthocyanins, flavonols, flavanones, flavan-3-ols, polymers, and flavones) were calculated from validated food-frequency questionnaires. Individual inflammatory biomarkers were ranked, standardized, and summed to derive an overall IS and subgroup scores of functionally related biomarkers.

Results: In multivariate analyses, an inverse association between higher anthocyanin and flavonol intakes and IS was observed with a mean ± SE difference between quintile categories 5 and 1 of −1.48 ± 0.32 (P-trend ≤ 0.001) and −0.72 ± 0.33 (P-trend = 0.01), respectively. Results remained significant after additional adjustment for physical activity, and vitamin C and fruit and vegetable intakes. Higher anthocyanin intake was inversely associated with all biomarker subgroups, whereas higher flavonol intake was associated only with lower cytokine and oxidative stress biomarker concentrations. In food-based analyses, higher intakes of apples and pears, red wine, and strawberries were associated with a lower IS with differences between quintiles 5 and 1 of −1.02 ± 0.43 (P = 0.006), −1.73 ± 0.39 (P < 0.001), and −0.44 ± 0.88 (P = 0.02), respectively. Although intakes of other classes were not associated with a reduction in overall IS, higher intakes of flavan-3-ols and their polymers were associated with a significant reduction in oxidative stress biomarkers.

Conclusion: These findings provide evidence to suggest that an anti-inflammatory effect may be a key component underlying the reduction in risk of certain chronic diseases associated with higher intakes of anthocyanins and flavonols. The Framingham Offspring Study was registered at clinicaltrials.gov as NCT00005121 (Framingham Heart Study). *Am J Clin Nutr* doi: 10.3945/ajcn.115.108555.

Keywords: anthocyanins, dietary intake, flavonoids, flavonols, inflammation

INTRODUCTION

Evidence from population-based studies and randomized controlled trials has supported a protective role for several dietary flavonoids in relation to a number of age-related chronic conditions including cardiovascular disease, diabetes, some cancers, Parkinson disease, and cognitive decline (1–8). One shared mechanism for these conditions is low-grade systemic chronic inflammation or metaflammation. A wealth of data from experimental, clinical, and epidemiologic research links inflammation and the biological networks integral to the inflammatory response to their pathophysiology (9–11). Many dietary factors influence various aspects of inflammation (11), and emerging data support the potential for several flavonoids to reduce a predisposition to chronic inflammation with particular interest in the anthocyanin, flavonol, and flavan-3-ol subclasses. Specifically, these bioactive compounds and their metabolites decrease inflammatory mediator production through effects on endogenous cell signaling pathways, gene expression, and gut microbiota and by exerting anti-inflammatory and neuroprotective effects (12–15). Inflammation plays a key role in a range of different diseases and conditions, and although resulting clinical signs and symptoms are different, many of the processes, cells, and molecules

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involved in the inflammatory response are remarkably similar and characterized by an increase in the number of circulating leukocytes and increasing concentrations of cytokines and chemokines (11).

Several recent prospective studies observed an inverse association between a higher intake of anthocyanins and risk of a range of chronic diseases including type 2 diabetes, Parkinson disease, myocardial infarction, and cancer (1, 2, 4–8). The limited data from previous population-based studies focused on individual biomarkers of inflammation and provided preliminary evidence to suggest that a higher anthocyanin intake is associated with lower pro-inflammatory cytokine levels [such as C-reactive protein (CRP)[7]] (16) and that flavanone and flavone intakes are inversely associated with IL-18 concentrations, whereas a higher flavonol intake is associated with reduced circulating concentrations of soluble vascular cell adhesion molecules (17). Although there remains no consensus on what biomarkers best represent low-grade chronic inflammation or differentiate between acute and chronic inflammation (or the various phases of the inflammatory response) (11), because of the limited specificity of any individual marker, the assessment of a wider range of biomarkers of inflammation might help elucidate the underlying mechanisms by which specific dietary flavonoids reduce risk of age-related chronic disease. Combinations or clusters of multiple biomarkers may be most informative, but to our knowledge, no previous population-based studies have derived an overall inflammation signature and examined associations with flavonoid intake.

Therefore, we derived an overall inflammation score (IS) and integrated markers that were functionally related to examine associations with flavonoid intake. It was hypothesized, on the basis of the available experimental and epidemiologic data, that higher intakes of anthocyanins, flavonols, and flavan-3-ols would be inversely associated with systemic markers of inflammation.

METHODS

Study design and population

The selection of study participants from the Framingham Heart Study Offspring cohort was described in detail previously (5). Briefly, participants were recruited in 1971, and the cohort underwent a repeat examination approximately every 3–4 y. For the current cross-sectional study, we used data derived from the seventh study examination, which spanned 3 y (1998–2001). Participants were excluded from the current study if they did not have valid dietary intake information (n = 509) or were missing data on inflammatory biomarkers (n = 655; excluding TNF-α and isoprostanes, which were measured on a subset of the cohort). Of the 3539 members of the cohort who participated in the seventh study examination, data on 2375 men and women were available for analysis.

The study was conducted according to the guidelines set forth in the Declaration of Helsinki, and all procedures involving human participants were approved by the Boston University Medical Center Institutional Review Board, and the current ancillary study was approved by the Tufts Medical Center Institutional Review Board.

Measurements

Assessment of flavonoid intakes

Dietary intakes were assessed by using a validated semiquantitative food-frequency questionnaire (FFQ) at the seventh examination (5). Dietary information was judged as unreliable and excluded from study if reported energy intakes were <600 or >4000 kcal/d for women and >4200 kcal/d for men or if >12 food items were left blank.

A database for the assessment of habitual intake of all flavonoid classes was used as previously described (18). Briefly, intakes of individual compounds were calculated as the sum of the consumption frequency of each food multiplied by the content of the specific flavonoid for the specified portion size. We derived intakes of classes commonly consumed in the US diet, specifically anthocyanins (cyanidin, delphinidin, malvidin, pelargonidin, petunidin, and peonidin), flavonols (quercetin, kaempferol, myricetin, and isorhamnetin), flavan-3-ols (catechins and epicatechins), flavanones (eriodictyol, hesperetin, and naringenin), flavones (luteolin and apigenin), and oligomer and polymer flavonoids (including proanthocyanidins, theaflavins, and thearubigins, which were classified as polymer flavonoids for this article). The validity and reproducibility of FFQs were reported previously, and correlations between major dietary sources of flavonoids (fruit, vegetables, tea, and wine) measured by diet records and an FFQ were 0.70, 0.50, 0.77, and 0.83, respectively (19, 20), which were correlations similar to those reported for an FFQ in a recent urinary flavonoid biomarker study (21).

Inflammatory biomarkers

Single measurements of plasma CRP were made by using a high-sensitivity assay, whereas the following inflammatory biomarkers were measured in duplicate from fasting blood samples taken during the seventh examination cycle (1998–2001) by using commercially available enzyme-linked immunoassay kits: plasma cluster of differentiation 40 ligand, plasma P-selectin, plasma osteoprotegerin, plasma TNF-α, plasma TNF receptor-2 (TNFR-2), serum soluble intercellular adhesion molecular-1, serum IL-6, serum monocyte chemotactic protein-1, serum myeloperoxidase, plasma lysosomal phospholipase-A2 (LPL-A2) mass and activity, and urinary isoprostanes indexed to urinary creatinine. Plasma fibrinogen was measured in duplicate by using the clot-time method of Clauss (22) with reagents (Diagnostica Stago).

With the use of this cluster of inflammatory biomarkers, we developed the following 2 types of scores to represent inflammation: a score representative of overall inflammation (the IS) and scores that were based on markers that are considered to be functionally interrelated, including available acute phase reactants, pro-inflammatory cytokines and receptors, and oxidative stress markers. This inflammation signature was previously used to examine associations between plasma pyridoxal-5-phosphate concentrations and inflammation (23).

Individual biomarker amounts were ranked, standardized as z scores, and summed to compute the different scores. The overall IS was the sum of standardized rank values of 12 of the

[7] Abbreviations used: CRP, C-reactive protein; FFQ, food-frequency questionnaire; IS, inflammation score; LPL-A2, lysosomal phospholipase-A2; TNFR-2, TNF receptor-2.

inflammatory biomarkers, including CRP, fibrinogen, IL-6, TNFR-2, osteoprotegerin, P-selectin, cluster of differentiation 40 ligand, intercellular adhesion molecular-1, monocyte chemoattractant peptide-1, myeloperoxidase, and LPL-A2 mass and activity. The subgroup acute phase reactants included the biomarkers CRP and fibrinogen. The other subgroups were designated as cytokines (which included IL-6, TNF-α, TNFR-2, and osteoprotegerin) and oxidative stress (which included myeloperoxidase, LPL-A2 and activity, and isoprostanes indexed to creatinine).

Covariates

Additional variables used in our analyses included age, sex, BMI, prevalent cardiovascular disease (yes or no), prevalent diabetes (yes or no), current smoker (yes or no), nonsteroidal anti-inflammatory drug use (yes or no), physical activity (metabolic equivalents), energy intake (kcal/d), and intakes of saturated and *trans* fat (g/d), fiber (g/d), potassium (mg/d), vitamin C (mg/d), and fruit and vegetables (servings/d). Criteria for the diagnoses of cardiovascular and type 2 diabetes events have been described elsewhere (24). BMI was calculated as body weight divided by the square of height (kg/m^2) by using examiner-assessed weight and height. A physical activity index, which was expressed in metabolic equivalents, was calculated by averaging the number of hours spent on specific activities (i.e., sleep, sedentary, slight activity, moderate activity, and heavy activity) during a typical day with each activity weighted by the oxygen consumption required to perform the activity (25).

Statistical methods

Our primary aim was to assess associations between flavonoid classes and the overall IS, and in secondary analyses, we examined the relative contribution of the different components and subgroups (acute inflammation, cytokines, and oxidative stress).

General linear models were used to examine associations between flavonoid intakes and the IS. Flavonoids were presented as quintile categories of intake. To assess trends across quintile categories, the median intake of each quintile category was assigned to individuals with intake in that category, and this quintile median variable was used as a continuous measure in our models.

We used the following 2 different models based on the inclusion of covariates, each by adding covariates to the previous model: *1*) an age-, sex-, smoking-, and energy-adjusted model (model 1) and *2*) model 1 with additional adjustment for BMI and nonsteroidal anti-inflammatory drug use, prevalent cardiovascular disease or diabetes, and saturated fat and *trans* fat intakes. In secondary analyses, we further adjusted for potassium, fiber, vitamin C, and fruit and vegetable intakes, both individually and in combination. Because many of the important sources of flavonoids also contribute these nutrients to the diet, it is important to consider them as potential confounders, but we included these sources in secondary analyses because there was also the concern of possible overadjustment. Physical activity was also considered in secondary analyses because of the relatively large number of participants with missing data for this variable.

To examine the relation between the top food sources of the specific flavonoids inversely associated with inflammation, we identified all foods that contributed ≥10% of the intake of each flavonoid class and related servings of these foods to the IS. We classified food intake into 4 categories (<1, 1–4, 5–6, and ≥7 servings/wk) and applied the same ANCOVA analysis approach

TABLE 1
Age-standardized baseline characteristics of 2375 participants of the Framingham Offspring Study seventh examination according to quintiles of total flavonoid intake[1]

	Quintile categories of total flavonoid intake					
Characteristic	1	2	3	4	5	*P*-trend
Total flavonoid intake,[2] mg/d	78 (8.7, 118)	150 (117, 189)	228 (189, 275)	343 (276, 430)	599 (430, 2323)	
Female,[3] %	53.2 (48.7, 57.6)	53.2 (48.8, 57.7)	47.4 (42.9, 51.8)	54.4 (50, 58.8)	66.8 (62.4, 71.3)	<0.001
Age, y[4]	60 (59.1, 60.8)	60.5 (59.7, 61.4)	61.2 (60.3, 62)	61.8 (60.9, 62.6)	61.7 (60.9, 62.6)	0.002
NSAID use,[3] %	23.3 (19.8, 26.9)	20.1 (16.5, 23.6)	19.8 (16.3, 23.4)	16.4 (12.8, 19.9)	17.8 (14.2, 21.3)	0.03
CVD,[3] %	15 (12.1, 17.8)	11.5 (8.6, 14.3)	11.7 (8.8, 14.5)	13.8 (11, 16.6)	10 (7.2, 12.9)	0.09
Diabetic,[3] %	12 (9.3, 14.8)	11.3 (8.6, 14.1)	12 (9.3, 14.8)	11.2 (8.5, 14)	7.5 (4.8, 10.3)	0.02
Current smoker,[3] %	20.3 (17.3, 23.3)	14.1 (11.1, 17.1)	11.8 (8.8, 14.8)	11.1 (8.1, 14.1)	8.2 (5.2, 11.2)	<0.001
BMI,[5] kg/m^2	28.4 (28.0, 28.9)	27.9 (27.5, 28.4)	27.4 (27.0, 27.9)	27.7 (27.3, 28.1)	26.9 (26.5, 27.3)	<0.001
Physical activity index[5]	36.8 (36.2, 37.4)	37.5 (36.9, 38.1)	37.2 (36.6, 37.8)	37.7 (37.1, 38.3)	37.7 (37.1, 38.4)	0.05
Calories,[5] kcal/d	1418 (1380, 1457)	1661 (1617, 1707)	1818 (1769, 1869)	1898 (1846, 1950)	1983 (1929, 2038)	<0.001
Fiber (AOAC),[5,6] g/d	13.4 (13.0, 13.7)	16.1 (15.7, 16.5)	17.7 (17.2, 18.2)	17.7 (17.3, 18.2)	19.4 (18.9, 19.9)	<0.001
Potassium intake,[5,6] mg/d	2498 (2450, 2547)	2768 (2717, 2821)	2974 (2919, 3030)	2964 (2909, 3020)	3247 (3186, 3310)	<0.001
Vitamin C intake,[5,6] mg/d	156 (144, 169)	225 (208, 243)	250 (231, 270)	276 (256, 299)	324 (299, 351)	<0.001
Saturated fat,[5,6] g/d	21.9 (21.4, 22.5)	20.9 (20.4, 21.5)	19.6 (19.1, 20.1)	19.1 (18.6, 19.5)	18.7 (18.2, 19.2)	<0.001
trans Fat,[5,6] g/d	2.65 (2.56, 2.75)	2.45 (2.37, 2.54)	2.24 (2.17, 2.32)	2.14 (2.07, 2.22)	2.12 (2.04, 2.19)	<0.001

[1] *P* values for the test of linear trend across quintile categories were based on linear regression models with the median intake of each quintile category assigned to individuals with intake in that category, and this quintile median variable was used as a continuous measure in regression models. AOAC, Association of Official Analytical Chemists; CVD, cardiovascular disease; NSAID, nonsteroidal anti-inflammatory drug.

[2] All values are medians; minimums and maximums in parentheses.

[3] All values are age- and sex-adjusted (least-squares) percentages; 95% CIs in parentheses.

[4] All values are age- and sex-adjusted (least-squares) means; 95% CIs in parentheses.

[5] All values are age- and sex-adjusted (least-squares) geometric means; 95% CIs in parentheses.

[6] Also adjusted for energy intake.

TABLE 2
Total flavonoid and class intakes in 2375 participants of the Framingham Offspring Study seventh examination

	Quintile categories of flavonoid intake				
	1	2	3	4	5
n	475	475	475	475	475
Flavonols, mg/d	4.8 (1.2, 6.3)[1]	7.8 (6.3, 9.4)	10.9 (9.4, 12.8)	15.1 (12.8, 18.4)	25.0 (18.4, 76.6)
Flavones, mg/d	0.41 (0.01, 0.70)	1.06 (0.71, 1.42)	1.95 (1.42, 2.26)	2.63 (2.26, 3.14)	4.12 (3.14, 16.3)
Flavanones, mg/d	4.5 (0.0, 8.5)	15.1 (8.5, 27.3)	37.8 (27.3, 53.9)	59.4 (53.9, 66.1)	87.3 (66.1, 527.8)
Flavan-3-ols, mg/d	6.8 (0.1, 10.6)	14.4 (10.6, 18.7)	23.6 (18.8, 32.1)	43.1 (32.1, 68.0)	86.8 (68.0, 449.0)
Anthocyanins, mg/d	1.8 (0.0, 3.5)	5.1 (3.5, 8.7)	13.1 (8.7, 15.3)	17.9 (15.3, 23.4)	32.0 (23.5, 364.8)
Polymer flavonoids, mg/d	35.4 (0.0, 54.4)	74.0 (54.5, 96.2)	124.8 (96.4, 155.6)	201.7 (155.8, 270.1)	377.5 (270, 1569)
Total flavonoids, mg/d	78.4 (8.7, 117.9)	150.3 (117.9, 188.7)	227.9 (188.8, 275.3)	343.1 (275.6, 429.7)	599.1 (430, 2323)

[1]Median; minimum and maximum in parentheses (all such values).

that was described for flavonoids. A test for trend across food-serving categories was based on assigning the median servings in each category to individuals in that category and treating that resulting variable as a continuous variable in regression models.

All analyses were performed with SAS version 9.3 software (SAS Institute). $P < 0.05$ was considered statistically significant.

RESULTS

Characteristics of participants (n = 2375) according to quintiles of total flavonoid intake are shown in **Table 1**. Participants with higher total flavonoid intakes were older, smoked less, and had higher habitual intakes of vitamin C, potassium, and fiber. The flavonoid polymer (proanthocyanidins) subclass, on average, contributed most to total flavonoid intake (0–1569 mg/d), whereas anthocyanin intakes ranged from 0 to 365 mg/d, and flavonol intakes ranged from 1 to 77 mg/d (**Table 2**).

In a multivariable-adjusted regression analysis, higher anthocyanin intake was associated with a 73% lower overall IS between highest and lowest quintile categories (between quintiles 5 and 1: −1.48; P-trend < 0.001) (**Table 3**). These results remained essentially unchanged after additional adjustment for physical activity or intakes of fiber, potassium, or vitamin C (data not shown). Even the addition of total fruit intake or total fruit and vegetable intake to the model did not substantially attenuate the observed inverse association for anthocyanins [between quintiles 5 and 1: −1.50 (P-trend = 0.001) and −1.45 (P-trend <0.001), respectively].

TABLE 3
Associations between different flavonoid subclass intakes and a combined inflammation score in 2375 participants of the Framingham Offspring Study[1]

	Quintile categories of flavonoid intake					
Inflammation score	1	2	3	4	5	*P*-trend
Flavonols						
Model 1	0.88 (0.42, 1.33)	0.50 (0.06, 0.93)	0.05 (−0.39, 0.49)	−0.40 (−0.84, 0.04)	−0.72 (−1.16, −0.27)	<0.001
Model 2	0.43 (−0.03, 0.88)	0.33 (−0.11, 0.76)	0.11 (−0.33, 0.55)	−0.43 (−0.87, 0.02)	−0.29 (−0.75, 0.16)	0.01
Flavones						
Model 1	0.87 (0.42, 1.32)	0.04 (−0.39, 0.48)	0.12 (−0.32, 0.56)	−0.25 (−0.69, 0.19)	−0.46 (−0.91, −0.01)	<0.001
Model 2	0.36 (−0.10, 0.82)	−0.05 (−0.48, 0.38)	0.12 (−0.32, 0.56)	−0.08 (−0.52, 0.35)	−0.11 (−0.57, 0.35)	0.24
Flavanones						
Model 1	0.50 (0.06, 0.95)	−0.19 (−0.63, 0.26)	0.25 (−0.19, 0.69)	−0.12 (−0.56, 0.32)	−0.12 (−0.58, 0.33)	0.16
Model 2	0.12 (−0.33, 0.56)	−0.27 (−0.70, 0.17)	0.13 (−0.31, 0.57)	0.11 (−0.33, 0.55)	0.15 (−0.31, 0.60)	0.46
Flavan-3-ols						
Model 1	1.19 (0.74, 1.64)	−0.38 (−0.82, 0.06)	−0.06 (−0.50, 0.38)	−0.20 (−0.64, 0.24)	−0.25 (−0.69, 0.20)	0.02
Model 2	0.70 (0.25, 1.15)	−0.38 (−0.82, 0.05)	0.01 (−0.43, 0.44)	−0.05 (−0.50, 0.39)	−0.08 (−0.52, 0.37)	0.31
Anthocyanins						
Model 1	0.98 (0.54, 1.43)	0.18 (−0.25, 0.62)	0.25 (−0.19, 0.68)	−0.27 (−0.72, 0.17)	−0.86 (−1.30, −0.41)	<0.001
Model 2	0.77 (0.33, 1.22)	0.09 (−0.34, 0.52)	0.30 (−0.13, 0.74)	−0.36 (−0.80, 0.09)	−0.71 (−1.17, −0.25)	<0.001
Polyflavonoids						
Model 1	0.63 (0.18, 1.09)	0.30 (−0.14, 0.74)	−0.18 (−0.62, 0.26)	0.02 (−0.42, 0.46)	−0.48 (−0.92, −0.03)	0.002
Model 2	0.29 (−0.16, 0.74)	0.17 (−0.26, 0.60)	−0.13 (−0.56, 0.31)	0.01 (−0.44, 0.45)	−0.14 (−0.59, 0.31)	0.24
Total flavonoids						
Model 1	0.67 (0.21, 1.12)	0.52 (0.08, 0.96)	−0.28 (−0.72, 0.16)	−0.22 (−0.66, 0.22)	−0.39 (−0.84, 0.06)	0.001
Model 2	0.22 (−0.24, 0.67)	0.37 (−0.06, 0.81)	−0.17 (−0.61, 0.26)	−0.21 (−0.66, 0.23)	−0.01 (−0.47, 0.44)	0.34

[1]All values are adjusted (least-squares) mean inflammation scores; 95% CIs in parentheses. Model 1 was adjusted for age, sex, smoking (yes or no), and energy intake. Model 2 was adjusted as for model 1 and for nonsteroidal anti-inflammatory drug use (yes or no), BMI, cardiovascular disease (yes or no), diabetes (yes or no), and saturated fat and *trans* fat intakes. *P* values for the test of linear trend across quintile categories were based on linear regression models with the median intake of each quintile category assigned to individuals with intake in that category, and this quintile median variable was used as a continuous measure in regression models.

TABLE 4
Relative contribution of different components of the inflammation score (subgroups of functionally related biomarker scores (acute phase reactants, cytokines, and oxidative stress) by quintiles of different flavonoid class intake in participants of the Framingham Offspring Study[1]

	Quintile categories of flavonoid intake					
	1	2	3	4	5	*P*-trend
Flavonols						
Acute inflammation						
Model 1	0.15 (0.00, 0.31)	0.06 (−0.10, 0.21)	0.00 (−0.15, 0.15)	−0.05 (−0.20, 0.10)	−0.25 (−0.40, −0.10)	<0.001
Model 2	−0.03 (−0.19, 0.12)	−0.03 (−0.18, 0.11)	−0.01 (−0.16, 0.13)	−0.12 (−0.27, 0.03)	−0.12 (−0.27, 0.03)	0.30
Cytokines						
Model 1	0.37 (0.14, 0.61)	0.12 (−0.11, 0.35)	0.14 (−0.10, 0.37)	0.03 (−0.21, 0.27)	−0.39 (−0.63, −0.15)	<0.001
Model 2	0.29 (0.05, 0.53)	0.10 (−0.13, 0.33)	0.21 (−0.03, 0.44)	0.00 (−0.24, 0.25)	−0.25 (−0.50, 0.00)	0.001
Oxidative stress						
Model 1	0.46 (0.26, 0.67)	0.24 (0.05, 0.44)	0.04 (−0.16, 0.23)	−0.23 (−0.42, −0.03)	−0.26 (−0.46, −0.06)	<0.001
Model 2	0.32 (0.10, 0.53)	0.20 (0.00, 0.40)	0.03 (−0.17, 0.24)	−0.21 (−0.42, 0.00)	−0.16 (−0.38, 0.05)	0.001
Flavones						
Acute inflammation						
Model 1	0.22 (0.07, 0.37)	0.02 (−0.13, 0.17)	−0.03 (−0.18, 0.12)	−0.10 (−0.25, 0.05)	−0.19 (−0.35, −0.04)	<0.001
Model 2	0.02 (−0.13, 0.18)	−0.03 (−0.17, 0.11)	−0.07 (−0.21, 0.08)	−0.09 (−0.24, 0.05)	−0.15 (−0.30, 0.01)	0.11
Cytokines						
Model 1	0.30 (0.06, 0.54)	0.04 (−0.19, 0.27)	0.04 (−0.20, 0.27)	−0.01 (−0.25, 0.23)	−0.07 (−0.31, 0.17)	0.07
Model 2	0.15 (−0.09, 0.40)	0.05 (−0.18, 0.29)	0.08 (−0.16, 0.32)	0.07 (−0.17, 0.31)	0.04 (−0.21, 0.29)	0.63
Oxidative stress						
Model 1	0.36 (0.16, 0.56)	0.05 (−0.15, 0.24)	0.03 (−0.16, 0.23)	0.04 (−0.16, 0.23)	−0.21 (−0.41, −0.01)	0.001
Model 2	0.18 (−0.04, 0.39)	0.00 (−0.20, 0.20)	0.02 (−0.19, 0.23)	0.09 (−0.12, 0.29)	−0.06 (−0.28, 0.15)	0.26
Flavanones						
Acute inflammation						
Model 1	0.07 (−0.08, 0.22)	−0.03 (−0.18, 0.12)	−0.02 (−0.17, 0.13)	−0.04 (−0.19, 0.11)	−0.06 (−0.21, 0.10)	0.35
Model 2	−0.08 (−0.23, 0.07)	−0.09 (−0.24, 0.06)	−0.11 (−0.25, 0.04)	0.00 (−0.14, 0.15)	−0.03 (−0.18, 0.12)	0.42
Cytokines						
Model 1	0.17 (−0.07, 0.40)	0.02 (−0.21, 0.25)	0.09 (−0.14, 0.32)	−0.05 (−0.29, 0.19)	0.06 (−0.18, 0.31)	0.56
Model 2	0.08 (−0.16, 0.31)	0.02 (−0.21, 0.26)	0.08 (−0.16, 0.31)	0.06 (−0.18, 0.30)	0.17 (−0.09, 0.42)	0.53
Oxidative stress						
Model 1	0.32 (0.12, 0.52)	0.03 (−0.17, 0.23)	0.15 (−0.04, 0.35)	−0.11 (−0.30, 0.09)	−0.13 (−0.33, 0.07)	0.003
Model 2	0.19 (−0.02, 0.40)	−0.01 (−0.22, 0.19)	0.12 (−0.08, 0.33)	−0.06 (−0.27, 0.15)	−0.02 (−0.23, 0.20)	0.23
Flavan-3-ols						
Acute inflammation						
Model 1	0.35 (0.19, 0.50)	−0.11 (−0.26, 0.04)	−0.08 (−0.23, 0.07)	−0.14 (−0.29, 0.01)	−0.10 (−0.26, 0.05)	0.02
Model 2	0.14 (−0.01, 0.29)	−0.15 (−0.29, 0.00)	−0.10 (−0.25, 0.04)	−0.13 (−0.28, 0.02)	−0.08 (−0.23, 0.07)	0.37
Cytokines						
Model 1	0.43 (0.19, 0.67)	−0.15 (−0.38, 0.09)	0.08 (−0.15, 0.31)	0.08 (−0.16, 0.32)	−0.16 (−0.39, 0.08)	0.04
Model 2	0.31 (0.07, 0.55)	−0.11 (−0.34, 0.13)	0.13 (−0.10, 0.37)	0.12 (−0.13, 0.36)	−0.09 (−0.34, 0.15)	0.12
Oxidative stress						
Model 1	0.52 (0.32, 0.72)	−0.05 (−0.25, 0.14)	0.04 (−0.16, 0.23)	−0.09 (−0.29, 0.10)	−0.16 (−0.35, 0.04)	0.001
Model 2	0.37 (0.16, 0.58)	−0.07 (−0.28, 0.13)	0.07 (−0.14, 0.27)	−0.03 (−0.24, 0.18)	−0.14 (−0.35, 0.07)	0.02
Anthocyanins						
Acute inflammation						
Model 1	0.23 (0.08, 0.39)	−0.04 (−0.19, 0.11)	0.06 (−0.09, 0.21)	−0.03 (−0.18, 0.13)	−0.33 (−0.48, −0.18)	<0.001
Model 2	0.14 (−0.01, 0.28)	−0.10 (−0.24, 0.04)	0.06 (−0.08, 0.20)	−0.09 (−0.24, 0.05)	−0.34 (−0.49, −0.19)	<0.001
Cytokines						
Model 1	0.29 (0.06, 0.53)	0.15 (−0.09, 0.38)	0.07 (−0.17, 0.30)	−0.05 (−0.29, 0.19)	−0.17 (−0.40, 0.07)	0.006
Model 2	0.27 (0.03, 0.51)	0.13 (−0.10, 0.37)	0.14 (−0.10, 0.37)	−0.05 (−0.30, 0.19)	−0.13 (−0.38, 0.11)	0.02
Oxidative stress						
Model 1	0.42 (0.22, 0.62)	0.13 (−0.06, 0.33)	0.07 (−0.13, 0.26)	−0.13 (−0.33, 0.07)	−0.24 (−0.44, −0.04)	<0.001
Model 2	0.31 (0.10, 0.52)	0.10 (−0.11, 0.30)	0.04 (−0.16, 0.25)	−0.14 (−0.34, 0.07)	−0.13 (−0.34, 0.09)	0.004
Polymer flavonoids						
Acute inflammation						
Model 1	0.19 (0.04, 0.35)	0.04 (−0.11, 0.19)	−0.12 (−0.27, 0.03)	−0.02 (−0.17, 0.14)	−0.19 (−0.34, −0.04)	0.003
Model 2	0.06 (−0.09, 0.21)	−0.05 (−0.20, 0.09)	−0.15 (−0.29, 0.00)	−0.07 (−0.21, 0.08)	−0.11 (−0.26, 0.04)	0.30
Cytokines						
Model 1	0.26 (0.03, 0.50)	0.18 (−0.05, 0.42)	−0.01 (−0.25, 0.22)	0.09 (−0.15, 0.33)	−0.25 (−0.49, −0.01)	0.003
Model 2	0.19 (−0.04, 0.43)	0.19 (−0.04, 0.43)	0.03 (−0.20, 0.27)	0.08 (−0.16, 0.32)	−0.14 (−0.39, 0.10)	0.04

(Continued)

TABLE 4 (*Continued*)

	Quintile categories of flavonoid intake					
	1	2	3	4	5	*P*-trend
Oxidative stress						
Model 1	0.35 (0.14, 0.55)	0.19 (−0.01, 0.38)	−0.02 (−0.21, 0.18)	−0.07 (−0.26, 0.13)	−0.20 (−0.40, 0.00)	<0.001
Model 2	0.22 (0.01, 0.43)	0.16 (−0.05, 0.36)	0.00 (−0.20, 0.21)	−0.05 (−0.26, 0.15)	−0.13 (−0.35, 0.08)	0.02
Total flavonoids						
Acute inflammation						
Model 1	0.23 (0.07, 0.38)	0.05 (−0.10, 0.20)	−0.11 (−0.26, 0.04)	−0.08 (−0.23, 0.07)	−0.18 (−0.34, −0.03)	0.001
Model 2	0.05 (−0.10, 0.19)	−0.03 (−0.18, 0.11)	−0.11 (−0.26, 0.03)	−0.13 (−0.28, 0.01)	−0.09 (−0.24, 0.06)	0.28
Cytokines						
Model 1	0.28 (0.04, 0.51)	0.23 (0.00, 0.46)	−0.08 (−0.32, 0.15)	0.07 (−0.16, 0.31)	−0.22 (−0.46, 0.02)	0.005
Model 2	0.19 (−0.05, 0.43)	0.22 (−0.02, 0.45)	−0.01 (−0.24, 0.23)	0.05 (−0.19, 0.29)	−0.09 (−0.34, 0.15)	0.07
Oxidative stress						
Model 1	0.36 (0.16, 0.57)	0.32 (0.13, 0.52)	−0.10 (−0.29, 0.10)	−0.15 (−0.35, 0.05)	−0.19 (−0.39, 0.02)	<0.001
Model 2	0.22 (0.01, 0.43)	0.25 (0.05, 0.46)	−0.06 (−0.26, 0.14)	−0.13 (−0.34, 0.08)	−0.10 (−0.31, 0.11)	0.02

[1]All values are adjusted (least-squares) mean inflammation scores; 95% CIs in parentheses. Model 1 was adjusted for age, sex, smoking (yes or no), and energy intake. Model 2 was adjusted as for model 1 and for nonsteroidal anti-inflammatory drug use (yes or no), BMI, cardiovascular disease (yes or no), diabetes (yes or no), and saturated fat and *trans* fat intakes. *P* values for the test of linear trend across quintile categories were based on linear regression models with the median intake of each quintile category assigned to individuals with intake in that category, and this quintile median variable was used as a continuous measure in regression models.

A higher flavonol intake was also associated with a 42% lower overall IS (between quintiles 5 and 1: −0.72, *P*-trend = 0.01) (Table 3). Additional adjustment for total fruit intake, total vegetable intake, vitamin C intake, or physical activity did not substantially alter the association (data not shown). However, the addition of fiber or potassium intake attenuated the relationship, and the trend across quintile categories was no longer significant [e.g., after adjustment for fiber intake, there was a difference between quintiles 5 and 1 of −0.52 (a 33% decrease); *P*-trend = 0.06].

When we examined the relative impact of the different clusters of the IS (acute phase reactants, cytokines, and oxidative stress), anthocyanin intake was inversely associated with all subgroups, with a 100% decrease across anthocyanin intake quintile categories for the acute inflammation (acute phase reactants) score (*P*-trend <0.001), a 75% decrease for the cytokine score (*P*-trend = 0.02), and a 52% decrease for the oxidative stress score (*P*-trend = 0.004). A higher flavonol intake was related to a reduction in both the cytokine score (an 81% decrease; *P*-trend = 0.001) and the oxidative stress score (a 56% decrease; *P*-trend = 0.001) (**Table 4**).

Although intakes of other flavonoid classes were not significantly associated with a reduction in overall IS, higher intakes of flavan-3-ols, their polymers, and total flavonoids were consistently associated with a significant reduction in concentrations of oxidative stress biomarkers across quintile categories [58% (*P*-trend = 0.02), 46% (*P*-trend = 0.02), and 43% (*P*-trend = 0.02), respectively] (Table 4). In addition, flavan-3-ol polymer intakes were inversely associated with the cytokine score, exhibiting a 60% decrease across quintile categories (*P*-trend = 0.04).

To confirm these findings and relate the observations to public health and dietary recommendations, we conducted food-based analyses for the main dietary sources (those that contributed >10% of intake) of anthocyanins (blueberries, strawberries, red wine, and apples and pears) and flavonols (tea and apples and pears) (**Table 5**). Higher intakes of apples and pears, red wine, and strawberries were associated with a lower overall IS with across quintile category differences of −1.02 (65% decrease; $P = 0.006$), −1.73 (89% decrease; $P < 0.001$), and −0.44 (27% decrease; $P = 0.02$), respectively. Daily red wine consumption was associated with a decrease in all biomarker subscores, whereas strawberry intake was associated with a reduction in acute IS (acute phase reactants) (Table 5). Blueberries and tea were not associated with the overall IS.

DISCUSSION

To our knowledge, this is the first study to integrate a range of inflammatory biomarkers into a combined score to examine associations with flavonoid intake. Because of the limited specificity of any individual biomarker of inflammation, it has not been possible to identify a single marker or even a small number of biomarkers to define inflammation for evaluating the impact of diet (11). This difficulty highlights the importance of our integrated approach, whereby we combined multiple inflammatory factors to identify a pattern or cluster of markers in an IS.

We observed an inverse association between higher intakes of anthocyanins (median intake in quintile 5: 32 mg/d) and flavonols (median intake in quintile 5: 25 mg/d) and IS with reductions of 73% and 42%, respectively, when extreme quintiles of intake were compared. Higher anthocyanin intake was consistently associated with lower inflammation scores across all subgroups of the IS, whereas higher flavonol intake was associated with lower cytokine and oxidative stress biomarker concentrations.

To date, there have been few long-term trials that investigated the impact of anthocyanin intake on inflammatory biomarkers. However, Zhu et al. (26) observed a significant decrease in CRP, soluble vascular cell adhesion molecule-1, and plasma interleukin-1β concentrations after 6-mo intake of a purified mixture of anthocyanins (320 mg/d) in hypercholesterolemic patients. Acute intake of a strawberry beverage (that contained 39 mg anthocyanins) attenuated the 6-h postprandial inflammatory response in overweight dyslipidemic participants (27),

TABLE 5
Associations between top food sources of anthocyanins and flavonols (>10% of intake) and a combined inflammation score (and relative contribution of different components of the score) in 2375 participants of the Framingham Offspring Study[1]

Food	<1 servings/wk	1–4 servings/wk	5–6 servings/wk	≥7 servings/wk	*P*-trend
Apple and pears, g/d	<19.7	19.7–78.9	98.6–118.3	≥138	
Inflammation score					
Model 1	0.27 (−0.16, 0.71)[2]	0.21 (−0.03, 0.45)	−1.08 (−2.00, −0.17)	−1.02 (−1.80, −0.24)	0.001
Model 2	0.28 (−0.16, 0.72)	0.15 (−0.11, 0.42)	−0.74 (−1.61, 0.13)	−0.74 (−1.49, 0.01)	0.006
Acute inflammation subscore					
Model 1	−0.04 (−0.19, 0.11)	0.04 (−0.04, 0.12)	−0.34 (−0.65, −0.02)	−0.24 (−0.51, 0.02)	0.08
Model 2	−0.06 (−0.21, 0.08)	−0.03 (−0.12, 0.06)	−0.26 (−0.55, 0.03)	−0.20 (−0.45, 0.05)	0.20
Cytokine subscore					
Model 1	0.03 (−0.20, 0.26)	0.15 (0.02, 0.27)	−0.64 (−1.11, −0.17)	−0.09 (−0.52, 0.34)	0.19
Model 2	0.10 (−0.14, 0.34)	0.15 (0.01, 0.29)	−0.50 (−0.96, −0.04)	−0.03 (−0.45, 0.39)	0.21
Oxidative stress subscore					
Model 1	0.26 (0.06, 0.45)	0.05 (−0.06, 0.16)	−0.29 (−0.70, 0.12)	−0.21 (−0.55, 0.14)	0.007
Model 2	0.19 (−0.01, 0.40)	0.04 (−0.08, 0.17)	−0.19 (−0.60, 0.22)	−0.06 (−0.41, 0.29)	0.12
Tea, mL/d	<33.9	33.9–135.4	169.3–203.1	≥237	
Inflammation score					
Model 1	0.17 (−0.12, 0.46)	0.05 (−0.30, 0.40)	0.14 (−0.96, 1.24)	−0.10 (−0.57, 0.38)	0.37
Model 2	0.06 (−0.25, 0.37)	0.02 (−0.34, 0.38)	0.39 (−0.66, 1.43)	0.08 (−0.38, 0.55)	0.86
Acute inflammation subscore					
Model 1	−0.01 (−0.11, 0.09)	0.01 (−0.11, 0.13)	−0.06 (−0.44, 0.31)	−0.09 (−0.25, 0.07)	0.40
Model 2	−0.09 (−0.19, 0.01)	−0.04 (−0.16, 0.08)	0.02 (−0.33, 0.36)	−0.06 (−0.21, 0.10)	0.68
Cytokine subscore					
Model 1	0.15 (0.00, 0.30)	0.02 (−0.16, 0.21)	0.40 (−0.19, 1.00)	−0.13 (−0.39, 0.12)	0.10
Model 2	0.15 (−0.01, 0.31)	0.05 (−0.15, 0.24)	0.45 (−0.13, 1.02)	−0.05 (−0.30, 0.21)	0.25
Oxidative stress subscore					
Model 1	0.17 (0.04, 0.30)	0.00 (−0.16, 0.15)	−0.16 (−0.65, 0.33)	−0.08 (−0.29, 0.13)	0.04
Model 2	0.14 (0.00, 0.28)	0.00 (−0.17, 0.17)	−0.11 (−0.60, 0.38)	−0.08 (−0.30, 0.14)	0.08
Red wine, mL/d	<16.9	16.9–67.4	84.3–101.1	≥118	
Inflammation score					
Model 1	0.85 (0.59, 1.11)	−0.79 (−1.11, −0.46)	−1.45 (−2.77, −0.13)	−1.35 (−2.11, −0.59)	<0.001
Model 2	0.69 (0.40, 0.97)	−0.70 (−1.04, −0.36)	−1.27 (−2.51, −0.02)	−1.04 (−1.78, −0.31)	<0.001
Acute inflammation subscore					
Model 1	0.12 (0.03, 0.21)	−0.14 (−0.25, −0.03)	−0.29 (−0.75, 0.16)	−0.45 (−0.72, −0.19)	<0.001
Model 2	0.03 (−0.06, 0.13)	−0.15 (−0.27, −0.04)	−0.26 (−0.67, 0.16)	−0.39 (−0.64, −0.15)	<0.001
Cytokine subscore					
Model 1	0.36 (0.22, 0.49)	−0.30 (−0.48, −0.12)	−0.87 (−1.59, −0.15)	−0.15 (−0.55, 0.25)	<0.001
Model 2	0.34 (0.18, 0.49)	−0.26 (−0.44, −0.07)	−0.79 (−1.48, −0.10)	−0.05 (−0.44, 0.35)	<0.001
Oxidative stress subscore					
Model 1	0.24 (0.12, 0.36)	−0.15 (−0.29, 0.00)	−0.14 (−0.73, 0.45)	−0.34 (−0.68, 0.00)	<0.001
Model 2	0.19 (0.05, 0.32)	−0.12 (−0.28, 0.04)	−0.10 (−0.69, 0.49)	−0.23 (−0.57, 0.12)	0.003
Strawberries, g/d	<10.7	10.7–42.9	53.6–64.3	≥75	
Inflammation score					
Model 1	0.36 (0.04, 0.69)	−0.07 (−0.32, 0.19)	−2.11 (−3.73, −0.49)	−0.31 (−2.12, 1.49)	0.01
Model 2	0.33 (−0.01, 0.67)	−0.08 (−0.36, 0.20)	−1.86 (−3.39, −0.34)	−0.11 (−1.80, 1.59)	0.02
Acute inflammation subscore					
Model 1	0.08 (−0.03, 0.19)	−0.06 (−0.15, 0.02)	−0.47 (−1.02, 0.08)	−0.30 (−0.92, 0.32)	0.01
Model 2	0.04 (−0.07, 0.15)	−0.11 (−0.20, −0.01)	−0.44 (−0.95, 0.07)	−0.29 (−0.85, 0.28)	0.01
Cytokine subscore					
Model 1	0.14 (−0.04, 0.31)	0.01 (−0.13, 0.15)	−0.30 (−1.16, 0.55)	0.44 (−0.47, 1.36)	0.55
Model 2	0.18 (0.00, 0.36)	0.02 (−0.13, 0.18)	−0.31 (−1.13, 0.51)	0.52 (−0.35, 1.40)	0.46
Oxidative stress subscore					
Model 1	0.22 (0.08, 0.37)	−0.02 (−0.14, 0.09)	−1.12 (−1.84, −0.40)	0.13 (−0.67, 0.94)	0.005
Model 2	0.18 (0.02, 0.34)	−0.01 (−0.15, 0.12)	−0.92 (−1.63, −0.20)	0.29 (−0.51, 1.09)	0.05
Blueberries, g/d	<10.4	10.4–41.7	52.1–62.6	≥73	
Inflammation score					
Model 1	0.32 (0.05, 0.59)	−0.23 (−0.53, 0.07)	−2.40 (−4.72, −0.09)	0.99 (−1.56, 3.54)	0.02
Model 2	0.22 (−0.07, 0.51)	−0.16 (−0.48, 0.16)	−2.28 (−4.45, −0.11)	1.37 (−1.02, 3.76)	0.10
Acute inflammation subscore					
Model 1	0.04 (−0.05, 0.13)	−0.07 (−0.17, 0.03)	−0.57 (−1.37, 0.22)	−0.44 (−1.31, 0.43)	0.04
Model 2	−0.03 (−0.12, 0.07)	−0.09 (−0.20, 0.01)	−0.63 (−1.35, 0.09)	−0.36 (−1.15, 0.44)	0.13

(Continued)

TABLE 5 (*Continued*)

Food	<1 servings/wk	1–4 servings/wk	5–6 servings/wk	≥7 servings/wk	*P*-trend
Cytokine subscore					
Model 1	0.15 (0.00, 0.29)	−0.05 (−0.21, 0.11)	−0.97 (−2.21, 0.27)	0.85 (−0.55, 2.25)	0.15
Model 2	0.14 (−0.02, 0.29)	0.00 (−0.17, 0.17)	−0.98 (−2.17, 0.20)	0.97 (−0.37, 2.30)	0.35
Oxidative stress subscore					
Model 1	0.19 (0.07, 0.31)	−0.12 (−0.25, 0.02)	−0.70 (−1.73, 0.33)	0.92 (−0.22, 2.05)	0.01
Model 2	0.14 (0.01, 0.28)	−0.08 (−0.23, 0.07)	−0.53 (−1.55, 0.49)	1.01 (−0.11, 2.13)	0.11

[1]Model 1 was adjusted for age, sex, smoking (yes or no), and energy intake. Model 2 was adjusted as for model 1 and for nonsteroidal anti-inflammatory drug use (yes or no), BMI, cardiovascular disease (yes or no), diabetes (yes or no), and saturated fat and *trans* fat intakes. *P* values for the test of linear trend across quintile categories were based on linear regression models with the median intake of each quintile category assigned to individuals with intake in that category, and this quintile median variable was used as a continuous measure in regression models.

[2]Adjusted (least-squares) mean inflammation score; 95% CI in parentheses (all such values).

and a 6-wk intervention with freeze-dried strawberries (equivalent to 500 g fresh strawberries) decreased CRP concentrations in type 2 diabetic patients (28). In contrast, several other studies failed to show significant effects on inflammatory biomarkers (29, 30), including a recent 3-mo dose-response study [feeding a strawberry beverage (equivalent to 250 and 500 g/d fresh strawberries) that contained 78 and 155 mg anthocyanins/d) in which no effect on CRP was observed despite observed beneficial effects on total and LDL cholesterol concentrations in the high-intake group (31). Some of these observed differences may be explained by the sample size, study duration, dose, or form of the intervention (supplement compared with food-based intervention) and the narrow range of inflammatory biomarkers that were assessed. Additional long-term studies are needed that use combinations or clusters of biomarkers of inflammation as were used in this study. In this cross-sectional study, in which habitual intakes of anthocyanins ranged from 24 to 365 mg/d (median intake: 32 mg/d), we observed anti-inflammatory associations that were based on these relatively low median habitual intakes but within the range used in 2 of the trials that observed beneficial effects (26, 27). These effects are readily achievable by relatively small changes to the habitual diet; a serving (0.5 cups) of strawberries, raspberries, blueberries, or red grapes or a 5-oz glass of red wine would provide ~21, 30, 121, 37, and 28 mg of anthocyanins, respectively. In our food-based analyses, the findings were not driven by one particular food source of anthocyanins but were observed for several of its main sources; higher intakes of apples and pears, red wine, and strawberries were associated with a lower overall IS (Table 5).

Our study suggests that an anti-inflammatory effect may be one of the key mechanisms involved in explaining reductions in risk of certain chronic diseases. Our data are supported by a previous cross-sectional study, which used a single biomarker approach and observed an inverse association between higher anthocyanin intake and circulating concentrations of CRP (−0.3-mg/L difference between extreme intake quintile categories) (16), although in another study, no association was observed (17). Reductions in individual inflammatory biomarkers were also observed in short-term studies in which anthocyanin-rich bilberry or strawberry products were fed (27, 32–34).

Our findings are also supported by mechanistic data that suggested that anthocyanins may modulate the expression of key inflammatory mediators (35–38). After ingestion, anthocyanins are extensively metabolized (39), and their degradation products and colonic metabolites were also shown to suppress pro-inflammatory cytokine production (40) and exert anti-inflammatory effects in vitro (41–44).

The flavonol subclass was also inversely associated with the IS score although the magnitude of association was substantially less than that observed for anthocyanins. These data may provide a mechanistic insight to explain the inverse association between habitual flavonol intake and risk of type 2 diabetes observed in 2 previous prospective studies (5, 7). In addition, in mice, a high-flavonoid apple, rich in flavonols, altered gut microbiota that resulted in a decrease in the transcription of a number of inflammatory genes (45). Like anthocyanins, metabolic transformation by the microbiota after flavonol ingestion also has profound effects on the anti-inflammatory activity (46). In our food-based analyses, apples and pears, which are a major source of flavonols in the habitual diet, were associated with a significant reduction in IS. Tea intake was not associated with IS overall but was associated with a reduction in oxidative stress biomarkers.

We also hypothesized that higher intake of flavan-3-ols would be associated with an anti-inflammatory effect, but we did not observe an association in this study. The reported doses of flavan-3-ols that showed vascular benefits in intervention studies were ≤112 mg/d (47), whereas median intake in our population only started to reach this amount in the top quintile of intake (median: 86.8 mg/d; range: 68.0–449 mg/d), and median intake in the third quintile category was only 23.6 mg/d. This large differential in intake may explain the lack of association. Higher flavan-3-ol intake, together with its polymers, was associated with a significant reduction in concentrations of oxidative stress biomarkers (which included myeloperoxidase, LPL-A2 mass, LPL-A2 activity, and isoprostanes), which suggested that this may be a major pathway by which these flavonoid classes reduce disease risk.

The strengths of our study included the large sample size, detailed data on important risk factors and confounders, comprehensive assessment of the range of flavonoid classes present in the habitual diet, and assessment of multiple inflammatory biomarkers as a combined inflammation score. Limitations of our study also warrant discussion. Although we adjusted for a range of confounders (including BMI, smoking, and family history), there was still the possibility of residual or unmeasured confounding from additional unmeasured factors, and this possibility may have been greatest when we compared subjects with the highest intakes with

those with the lowest intakes. However, because of our detailed and updated adjustment for potential confounders, it was unlikely that these factors would have accounted fully for the observed results. Of all flavonoid classes assessed, only anthocyanin and flavonol intakes were associated with the IS, which suggested something specific about these classes. The flavonoid content of foods varies depending on growing conditions and manufacturing processes, but despite this variation, the data allowed us to rank order intakes and compare high and low intakes in a large population-based cohort. To our knowledge, there are no specific biomarkers for anthocyanin or flavonol intake, and there is limited understanding of flavonoids degradation and metabolism after ingestion. Our findings might have been due to other constituents in the foods that contribute most to this subclass; however, the addition of other confounders, including vitamin C and total fruit intakes, to our multivariate model did not substantially attenuate the associations. However, in a population-based study like ours, it is impossible to disentangle the relative influence of all constituents of fruit and vegetables. Finally, it remains to be seen if the IS or subscores are associated with chronic disease risk.

In conclusion, by using multiple inflammatory biomarkers assessed in combination as an IS, we provide evidence to suggest that an anti-inflammatory effect that is due to intakes of anthocyanins and flavonols may contribute to the reduction in risk of certain chronic diseases. Dose-response intervention studies are needed to determine the optimal dose and source for reducing inflammation on the basis of multiple biomarkers of inflammation and, ultimately, indicators of disease risk.

The authors' responsibilities were as follows—PFJ, JTD, JJP, and AC: conducted the dietary analyses and developed the database; PFJ and GR: set up and coordinated the collection of dietary data; GR: performed the statistical analysis after discussion with PFJ and AC; AC and PFJ: wrote the manuscript; JTD and HL: provided critical review of the manuscript; and all authors: read and approved the final manuscript. None of the authors reported a conflict of interest related to the study.

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