High Anthocyanin Intake Is Associated With a Reduced Risk of Myocardial Infarction in Young and Middle-Aged Women

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Background—Our current knowledge of modifiable risk factors to prevent myocardial infarction (MI) in young and middle-aged women is limited, and the impact of diet is largely unknown. Dietary flavonoids exert potential beneficial effects on endothelial function in short-term trials; however, the relationship between habitual intake and risk of MI in women is unknown.

Methods and Results—We followed up 93 600 women 25 to 42 years of age from the Nurses' Health Study (NHS) II who were healthy at baseline (1989) to examine the relationship between anthocyanins and other flavonoids and the risk of MI. Intake of flavonoid subclasses was calculated from validated food-frequency questionnaires collected every 4 years using an updated and extended US Department of Agriculture database. During 18 years of follow-up, 405 cases of MI were reported. An inverse association between higher intake of anthocyanins and risk of MI was observed (hazard ratio, 0.68; 95% confidence interval, 0.49–0.96; *P*=0.03, highest versus lowest quintiles) after multivariate adjustment. The addition of intermediate conditions, including history of hypertension, did not significantly attenuate the relationship (hazard ratio, 0.70; 95% confidence interval,0.50–0.97; *P*=0.03). Combined intake of 2 anthocyanin-rich foods, blueberries and strawberries, tended to be associated with a decreased risk of MI (hazard ratio, 0.66; 95% confidence interval, 0.40–1.08) in a comparison of those consuming >3 servings a week and those with lower intake. Intakes of other flavonoid subclasses were not significantly associated with MI risk.

Conclusions—A high intake of anthocyanins may reduce MI risk in predominantly young women. Intervention trials are needed to further examine the health impact of increasing intakes of commonly consumed anthocyanin-rich foods. **(*Circulation.* 2013;127:188-196.)**

Key Words: anthocyanins ■ diet ■ epidemiology ■ myocardial ■ infarction ■ women

Coronary heart disease (CHD), a leading cause of death and disability worldwide, occurs predominantly in older age groups with a lower prevalence in women than men at middle-age.[1] To date, most epidemiological studies on CHD have concentrated on older men and women, but risk factors may vary with age, particularly in women, in whom menopause leads to several metabolic changes. For young and middle-aged women, previous studies have suggested the use of oral contraceptives and smoking as factors that increase risk,[2,3] but to the best of our knowledge, no prospective studies have examined the impact of dietary factors on MI in a large sample of well-characterized middle-aged women with long-term follow-up and repeated measures of dietary intake. The mechanisms underlying CHD in young and middle-aged women may also differ from those in older women because coronary vasospasm, a consequence of endothelial dysfunction, may play a particularly important role.[4,5]

Clinical Perspective on p 196

From a dietary perspective, growing evidence supports the beneficial effects of dietary flavonoids on endothelial function and blood pressure,[6–8] suggesting that flavonoids might be more likely than other dietary factors to lower the risk of CHD in predominantly young women.[6–8] Specific flavonoids appear to improve endothelial function by exerting antiinflammatory effects, inhibiting low-density lipoprotein oxidation and endothelial NADPH oxidase, modulating nitric oxide synthase activity/expression, and augmenting nitric oxide status.[6,7] Flavonoids are widely distributed in many plant-based foods and beverages, including fruits, vegetables, tea, and wine, and the subclasses commonly consumed in the US diet include flavanones, anthocyanins, flavan-3-ols, flavonols, flavones, and polymeric flavonoids.

Which classes of flavonoids might be associated with risk of CHD, if any, is uncertain. The limited available data from

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older adults suggest that the flavonoid subclasses anthocyanins, flavonols, and flavanones are associated with a reduction in CHD mortality.[9,10] In middle-aged and older women, the beneficial effects of anthocyanins on blood pressure were greatest in young and middle-aged women with a 12% reduction in incident hypertension when the extreme quintiles of intake were compared,[11] supporting a role for flavonoids in lowering the risk of CHD in this age group. A recent meta-analysis of intervention trials highlighted the cardioprotective effects of the flavan-3-ol subclass on biomarkers of CHD risk, including blood pressure, vascular function, and insulin resistance.[8,12] From these data, we hypothesized that a higher intake of anthocyanins and flavan-3-ols would be associated with a reduced risk of MI in young and middle-aged women.

Methods

Study Population

In 1989, 116 430 women 25 to 42 years of age were enrolled in the Nurses' Health Study II (NHSII). Each participant returned a questionnaire by mail on lifestyle and medical history and received follow-up questionnaires biennially to record newly diagnosed illnesses and to update lifestyle factors. Beginning in 1991, they received semiquantitative food-frequency questionnaires (FFQs) every 4 years.[13,14] Participants who reported a history of myocardial infarction (MI), stroke, angina, other cardiovascular diseases (CVDs), coronary bypass surgery, or cancer (except nonmelanoma skin cancer) at baseline were excluded. Participants who were missing dietary data at baseline or had implausible values for total caloric intake (<500 or >3500 kcal/d) were also excluded, resulting in the inclusion of 93 600 women in these analyses. The institutional review board at Brigham and Women's Hospital reviewed and approved this study, and participants provided implied consent by virtue of returning their questionnaires.

Outcome Assessment

The outcome was incident MI, which included nonfatal MI and fatal CHD that occurred after the return of the 1991 questionnaire and before 2009. Nonfatal MI was confirmed if data in the medical records met World Health Organization criteria based on symptoms plus either diagnostic ECG changes or elevated cardiac enzyme concentrations.[15] Fatal CHD was defined as a fatal MI if confirmed by hospital records or autopsy or if CHD was listed on the death certificate as cause of death and evidence of previous CHD was available.

Dietary Assessment

Dietary intake data were collected from NHSII participants in 1991 and subsequently every 4 years. A database for assessment of intake of the different flavonoid subclasses was constructed as previously described[11] and was compiled before the release of the more recent flavonoid database, the phenol-explorer database. 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 the subclasses commonly consumed in the US diet, specifically flavanones (eriodictyol, hesperetin, naringenin), anthocyanins (cyanidin, delphinidin, malvidin, pelargonidin, petunidin, peonidin), flavan-3-ols (catechins, epicatachin), flavonols (quercetin, kaempferol, myricetin, isohamnetin), flavones (luteolin, apigenin), and polymers (including proanthocyanidins, theaflavins, and thearubigins). Cumulative intakes (energy adjusted) were calculated for a given questionnaire cycle by averaging the intake for the current and preceding FFQs. The validity and reproducibility of the FFQs have been reported previously, and correlations between major dietary sources of flavonoids (fruits, vegetables, tea, and wine) measured by diet records and the FFQ were 0.70, 0.50, 0.77, and 0.83 respectively.[16–18]

Statistical Methods

Participants contributed person-time of follow-up from the date of return of the 1991 questionnaire to the date of MI diagnosis, death, or end of follow-up (June 2009). We used a left-truncated Cox proportional hazard regression for time-varying covariates, with a counting process data structure and age in months as the time scale, stratifying additionally on calendar year,[19] to estimate the hazard ratio (HR) for flavonoid subclass intake in relation to the risk of MI with the lowest intake quintile as the referent group. Covariates were updated biennially.[20] We controlled for body mass index (<25, 25–29.9, or ≥30 kg/m^2); physical activity (metabolic equivalents per week, in quintiles); alcohol consumption (0, 0.1–4.9, 5–14.9, 15–29.9, ≥30 g/d); energy intake (kcal/d, in quintiles); cereal fiber intake (g/d, in quintiles); saturated, *trans*, polyunsaturated, and monounsaturated fat intake (g/d, in quintiles); caffeine intake (mg/d, in quintiles); use of aspirin (nonuser or <6 or ≥6 per week); menopausal status (premenopausal, unknown menopause, postmenopausal); postmenopausal hormone use (never, past, or current hormone use); oral contraceptive use (never, past, or current hormone use); smoking (never, past and current [1–14 or ≥15 cigarettes per day]); and family history of MI. All analyses were conducted with SAS software, version 9 (SAS Institute, Inc, Cary, NC). All *P* values were 2 sided. We examined the possible nonlinear relationship between anthocyanin intake and risk of MI nonparametrically using stepwise restricted cubic splines.[21,22] Tests for nonlinearity used the likelihood ratio test, comparing the model with only the linear term and the model with the linear and cubic spline terms that were selected.

In secondary analyses, we additionally adjusted for potassium, folate, and fruit and vegetable intake, both individually and in combination. We created several Cox proportional hazards regression models to measure the associations between flavonoid subclasses intake and MI in the presence of intermediate outcome measures, including history of hypertension, diabetes mellitus, angina, and hypercholesterolemia.

To identify risk factors that may modify the relationship between flavonoid subclasses that were associated with MI risk, we examined the associations among strata of smoking, physical activity, prevalent hypertension, alcohol, and body mass index. We also conducted food-based analyses of the main sources of anthocyanins, flavonols, and flavonoid polymers: tea, onions, apples, strawberries, and blueberries.

Results

During 18 years of follow-up, among the 93|600 participants, we documented 405 cases of incident MI. Baseline characteristics of the participants according to quintiles of anthocyanin intake are shown in Table 1. The median age of cases at diagnosis was 48.9 years, with an age range of 33.8 to 60.8 years. Women with higher anthocyanin intake smoked less, exercised more, and had lower total fat and energy intakes and higher whole grain and fiber intakes. The flavonoid polymer subclass contributed most to total flavonoid intake (58–643 mg/d), whereas anthocyanin intakes ranged from 2 to 35 mg/d.

After multivariate adjustment, we observed an inverse association between anthocyanin intake and MI risk (*P* for trend=0.047), and the greatest reduction in risk was 32% when participants in the highest and lowest quintiles of anthocyanin intake were compared (HR, 0.68; 95% confidence interval [CI], 0.49–0.96; *P* for trend=0.047; Table 2). We tested for deviation from linearity and did not detect any significant deviations from linearity (*P* for deviation=0.41).The addition of intermediate conditions, including history of hypertension, diabetes mellitus, angina, or hypercholesterolemia, to the model did not significantly attenuate the relationship (HR, 0.70; 95% CI, 0.50–0.97). For every 15-mg increase in intake of anthocyanins, the relative risk of MI decreased by 17%

Table 1. Characteristics of the Women From the Nurses' Health Study II by Quintile of Anthocyanin Intake at Baseline

	Anthocyanin Intake, mg/d					
	Quintile 1 (n=18 676)	Quintile 2 (n=18 736)	Quintile 3 (n=18 727)	Quintile 4 (n=18 729)	Quintile 5 (n=18 732)	P for Trend
Age, y	36.4 (4.7)	36.4 (4.7)	36.6 (4.7)	36.7 (4.6)	36.9 (4.6)	—
BMI, kg/m^2	25.1 (5.8)	24.8 (5.4)	24.5 (5.1)	24.4 (5.0)	24.2 (4.8)	<0.0001
Premenopausal, %	96.2	96.2	96.0	96.5	96.6	<0.001
Oral contraceptive use, %						
Never	16	15	15	16	16	0.82
Past	74	74	73	74	73	0.12
Current	10	11	11	10	11	0.045
Smoking, %						
Never	61	66	67	68	66	<0.0001
Former	20	21	22	23	24	<0.0001
Current, 1–14 per day	7	5	5	5	5	<0.0001
Current, ≥15 per day	12	7	6	5	4	<0.0001
Physical activity, METs/wk	16.4 (24.2)	18.3 (24.2)	20.7 (26.2)	23.2 (29.3)	25.8 (30.7)	<0.0001
Family history of MI, %	23	21	22	21	21	0.0006
History of hypertension, %	7.3	6.3	6.2	5.9	5.9	<0.0001
History of diabetes mellitus, %	0.9	0.9	1.1	0.9	1.0	0.64
History of hypercholesterolemia, %	16.1	15.0	14.1	13.9	13.7	<0.0001
Energy intake, kcal/d	1847 (594)	1831 (486)	1740 (606)	1922 (523)	1606 (461)	<0.0001
Alcohol, g/d	3.0 (7.1)	2.8 (5.5)	3.0 (5.5)	3.4 (6.0)	3.3 (6.1)	<0.0001
Total fat, g/d	68.3 (25.2)	65.7 (20.2)	60.7 (23.7)	66.0 (21.1)	52.9 (17.3)	<0.0001
Saturated fat, g/d	24.7 (9.7)	23.4 (7.7)	21.5 (8.9)	23.3 (8.0)	18.5 (6.5)	<0.0001
Monounsaturated fat, g/d	26.1 (9.9)	25.0 (8.1)	23.0 (9.4)	25.0 (8.4)	19.9 (6.8)	<0.0001
Polyunsaturated fat, g/d	11.6 (4.8)	11.6 (4.1)	10.9 (4.6)	12.0 (4.2)	9.8 (3.6)	<0.0001
Trans fat, g/d	3.8 (1.8)	3.5 (1.5)	3.1 (1.6)	3.3 (1.5)	2.5 (1.1)	<0.0001
Dietary fiber, g/d	15.7 (6.6)	17.6 (6.8)	17.9 (7.7)	20.6 (7.8)	18.9 (8.0)	<0.0001
Whole grain, g/d	16.8 (15.4)	19.6 (15.5)	20.0 (16.3)	23.1 (17.2)	20.7 (16.3)	<0.0001
Potassium, mg/d	2734 (924)	2890 (860)	2837 (995)	3199 (961)	2857 (929)	<0.0001
Total flavonoids, mg/d	351 (541)	366 (451)	422 (502)	402 (423)	520 (519)	<0.0001
Flavanones, mg/d	26.8 (34.3)	30.4 (31.1)	34.4 (35.4)	34.4 (32.8)	41.2 (38.5)	<0.0001
Flavonols, mg/d	16.0 (13.7)	17.2 (12.8)	18.6 (13.2)	19.3 (12.5)	21.6 (13.9)	<0.0001
Flavones, mg/d	1.2 (1.1)	1.4 (1.0)	1.6 (1.0)	1.7 (1.0)	1.9 (1.2)	<0.0001
Flavan-3-ols, mg/d	58.1 (93.8)	58.4 (83.7)	62.6 (89.2)	60.2 (77.6)	71.1 (90.5)	<0.0001
Polymers, mg/d	246.8 (439)	254.0 (359)	297.4 (403)	273.3 (336)	351.4 (415)	<0.0001

BMI indicates body mass index; METs, metabolic equivalents; and MI, myocardial infarction. All values (except age) are age-adjusted mean ± SD.

(HR, 0.83; 95% CI, 0.68–1.00) in the multivariate model. We also examined the association between deciles of anthocyanin intake and risk of MI, and in a comparison of the top and bottom 10% of intake (median intake, 34.3 mg in the top decile), the relative risk was 0.53 (95% CI, 0.33–0.86), suggesting continual dose-response at higher levels of habitual intake.

Although intakes of other subclasses were not significantly associated with a reduction in MI, in a comparison of the highest and lowest quintiles, there was a trend toward a reduction in risk with a higher intake of flavonols and flavonoid polymers (Table 2). The results remained essentially unchanged after additional adjustment for potassium, folate, or total fruit and vegetable intake either individually or when added together to our final multivariate model (HR, 0.71; 95% CI, 0.50–1.00).

In stratified analyses, the inverse association between anthocyanins and MI was stronger among women who never smoked compared with those who currently smoked, although this interaction was not significant (P for heterogeneity=0.73; Table 3). In other stratified analyses, we found that the inverse association was similar in alcohol drinkers and nondrinkers and in those without diabetes mellitus and those with a history of the disease, although these interactions were also not significant (Table 3).

To confirm these findings and to relate the effects to public health and dietary guidelines, we conducted food-based analyses for the main dietary sources of anthocyanins. When we combined intakes of blueberries and strawberries and compared those who consumed >3 servings per week with those who rarely consumed these fruits, there was a trend toward

Table 2. Relationship Between Myocardial Infarction and Flavonoid Intake (Total and Subclasses in Quintiles) in Participants From the Nurses' Health Study II

	Quintiles of Flavonoid Intake Subclasses					
	1	2	3	4	5	P for Trend
Flavonols, mg/d	7.8	11.6	15.3	20.5	33.2	
Cases, n	99	76	83	66	81	
Person-years	329 022	330 993	331 848	330 730	328 659	
Age-adjusted model	1.0	0.73 (0.54–0.99)	0.78 (0.58–1.05)	0.60 (0.44–0.81)	0.71 (0.53–0.95)	0.009
Model 2*	1.0	0.84 (0.62–1.13)	0.93 (0.69–1.25)	0.71 (0.52–0.98)	0.81 (0.60–1.09)	0.10
Model 3†	1.0	0.85 (0.63–1.15)	0.95 (0.70–1.28)	0.71 (0.51–0.99)	0.79 (0.58–1.08)	0.08
Flavones, mg/d	0.6	1.0	1.4	1.9	2.9	
Cases, n	105	86	70	69	75	
Person-years	327 898	329 943	330 724	332 407	330 279	
Age-adjusted model	1.0	0.79 (0.60–1.05)	0.62 (0.46–0.84)	0.59 (0.43–0.79)	0.60 (0.45–0.81)	0.0001
Model 2*	1.0	0.95 (0.71–1.26)	0.81 (0.59–1.10)	0.82 (0.60–1.13)	0.92 (0.68–1.26)	0.36
Model 3†	1.0	0.98 (0.74–1.32)	0.86 (0.62–1.17)	0.89 (0.64–1.22)	1.00 (0.72–1.40)	0.75
Flavanones, mg/d	6.6	15.5	25.5	40.1	71.1	
Cases, n	109	75	59	89	73	
Person-years	328 444	330 702	331 126	331 375	329 605	
Age-adjusted model	1.0	0.69 (0.51–0.93)	0.52 (0.38–0.71)	0.77 (0.58–1.01)	0.60 (0.44–0.80)	0.004
Model 2*	1.0	0.80 (0.59–1.07)	0.64 (0.46–0.88)	1.01 (0.76–1.34)	0.85 (0.63–1.15)	0.66
Model 3†	1.0	0.82 (0.61–1.11)	0.67 (0.48–0.92)	1.07 (0.80–1.44)	0.91 (0.66–1.26)	0.96
Flavan-3-ols, mg/d	13.4	33.3	78.9	206.2	610.2	
Cases, n	99	64	81	77	84	
Person-years	328 919	330 959	331 080	330 939	329 355	
Age-adjusted model	1.0	0.66 (0.48–0.91)	0.84 (0.63–1.13)	0.79 (0.58–1.06)	0.82 (0.61–1.09)	0.40
Model 2*	1.0	0.76 (0.56–1.05)	0.94 (0.70–1.26)	0.88 (0.65–1.19)	0.86 (0.64–1.15)	0.53
Model 3†	1.0	0.77 (0.56–1.06)	0.94 (0.70–1.27)	0.87 (0.64–1.18)	0.82 (0.61–1.11)	0.37
Anthocyanins, mg/d	2.5	5.0	8.4	13.5	25.1	
Cases, n	126	81	66	73	59	
Person-years	324 793	330 336	331 831	332 148	332 143	
Age-adjusted model	1.0	0.63 (0.47–0.83)	0.50 (0.37–0.67)	0.54 (0.40–0.72)	0.42 (0.31–0.57)	<0.0001
Model 2*	1.0	0.76 (0.57–1.01)	0.66 (0.49–0.90)	0.77 (0.58–1.04)	0.62 (0.45–0.86)	0.006
Model 3†	1.0	0.80 (0.60–1.07)	0.71 (0.52–0.97)	0.85 (0.63–1.15)	0.68 (0.49–0.96)	0.047
Polymers, mg/d	65.4	110.1	160.9	256.7	578.6	
Cases, n	112	80	69	57	87	
Person-years	327 966	331 209	331 278	331 145	329 654	
Age-adjusted model	1.0	0.71 (0.53–0.95)	0.61 (0.45–0.82)	0.49 (0.36–0.68)	0.72 (0.54–0.95)	0.002
Model 2*	1.0	0.85 (0.64–1.14)	0.77 (0.57–1.04)	0.63 (0.45–0.86)	0.84 (0.63–1.12)	0.06
Model 3†	1.0	0.89 (0.66–1.19)	0.80 (0.59–1.08)	0.64 (0.46–0.89)	0.83 (0.62–1.11)	0.051
Total flavonoids, mg/d	117.4	187.1	260.7	389.9	804.7	
Cases, n	109	82	66	65	83	
Person-years	328 518	331 254	330 948	330 890	329 641	
Age-adjusted model	1.0	0.73 (0.55–0.98)	0.58 (0.43–0.79)	0.56 (0.41–0.76)	0.69 (0.52–0.92)	0.002
Model 2*	1.0	0.91 (0.68–1.22)	0.76 (0.56–1.04)	0.74 (0.54–1.01)	0.84 (0.62–1.12)	0.09
Model 3†	1.0	0.96 (0.72–1.28)	0.80 (0.58–1.09)	0.76 (0.55–1.05)	0.83 (0.61–1.12)	0.09

*Model 2 adjusted for age, physical activity, smoking, body mass index, alcohol, energy, menopausal status, postmenopausal hormone use, aspirin use, oral contraceptive use, and family history of myocardial infarction.

†Model 3 additionally adjusted for cereal fiber, saturated fatty acids, *trans* fatty acids, polyunsaturated fatty acids, monounsaturated fatty acids, and caffeine.

a decrease in the risk of MI (HR, 0.66; 95% CI, 0.40–1.08; *P*=0.09; the Figure). For the other main foods that contributed to flavonoid intake, we did not observe a significant reduction in risk with increased intake, except for onions, for which an intake of ≥5 times per week was significantly associated with reduced risk (HR, 0.27; 95% CI, 0.08–0.87; *P*=0.03).

Table 3. Associations Between Anthocyanin Intake and Risk of Myocardial Infarction Across Strata of Risk Factors for Participants From the Nurses' Health Study II

Risk factor	Cases n	Person-years	Quartile 5 vs 1	P for Trend	P for Interaction
Age, y					
<55	350	1 540 444	0.71 (0.50–1.03)	0.16	
≥55	55	110 807	0.49 (0.22–1.12)	0.07	0.42
BMI, kg/m^2					
<25	123	823 965	0.60 (0.33–1.09)	0.10	
≥25	282	827 287	0.73 (0.49–1.09)	0.20	0.60
Smoking					
Never	173	1 010 830	0.53 (0.31–0.92)	0.04	
Past	91	375 119	0.99 (0.51–1.91)	0.91	
Current	141	265 302	0.75 (0.42–1.34)	0.47	0.73
Physical activity					
Less than median	259	825 374	0.72 (0.47–1.11)	0.19	
More than median	146	825 877	0.63 (0.36–1.08)	0.12	0.68
Alcohol					
Nondrinker	204	668 944	0.75 (0.46–1.25)	0.61	
Drinker	201	982 307	0.60 (0.38–0.94)	0.03	0.26
Prevalent hypertension					
No	231	1 398 692	0.76 (0.49–1.18)	0.33	
Yes	174	252 560	0.59 (0.35–1.00)	0.05	0.53
History of diabetes mellitus					
No	336	1 609 342	0.65 (0.45–0.95)	0.04	
Yes	69	41 910	0.80 (0.34–1.86)	0.77	0.57

Multivariate model adjusted for age, physical activity, smoking, body mass index (BMI), alcohol, energy, menopausal status, postmenopausal hormone use, aspirin use, oral contraceptive use, family history of myocardial infarction, cereal fiber, saturated fatty acids, *trans* fatty acids, polyunsaturated fatty acids, monounsaturated fatty acids, and caffeine.

Discussion

In this prospective cohort study of well-characterized young and middle-aged women with 18 years of follow-up and repeated measures of dietary intake, we observed that a higher intake of anthocyanins was associated with a 32% reduction in risk of MI and that this inverse association was independent of established dietary and nondietary CVD risk factors. These compounds are present in red/blue fruits and vegetables and are readily incorporated into the habitual diet, and simple dietary change could have an impact on prevention efforts.

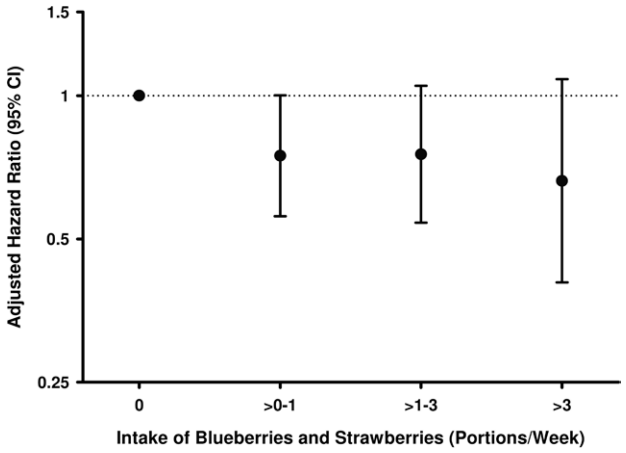

Figure. Multivariate-adjusted relative risk of myocardial infarction according to combined intake of strawberries and blueberries in the Nurses' Health Study II. Model adjusted for age, physical activity, smoking, body mass index, alcohol, energy, menopausal status, postmenopausal hormone use, aspirin use, oral contraceptive use, family history of myocardial infarction, cereal fiber, saturated fatty acids, trans fatty acids, polyunsaturated fatty acids, monounsaturated fatty acids, and caffeine.

Our current knowledge of modifiable risk factors to prevent MI in young and middle-aged women is limited. Over the last few decades, several small case-control studies have reported on the increased risk of MI associated with use of oral contraceptives, heavy smoking, history of diabetes mellitus, and hypertension in younger women.[2,3,23–25] From a dietary perspective, the only available case-control data suggest a reduction in risk with moderate alcohol consumption and a modest increase in risk with very high coffee intake (≥10 cups per day).[26,27] Our data suggest a potential role for flavonoids, specifically anthocyanins, in reducing risk.

To date, limited randomized, controlled trials have examined the impact of anthocyanins on blood pressure and endothelial function relative to other subclasses.[8] However, a recent 3-month randomized, controlled trial in dyslipidemic patients showed that anthocyanin intake improved the lipoprotein profile, resulting in an increase in high-density lipoprotein cholesterol levels and a decrease in low-density lipoprotein cholesterol levels, effects that were thought to be mediated via cholesteryl ester transfer protein inhibition.[28] A

growing body of evidence from animal models and in vitro experiments supports a cardioprotective role for anthocyanins and their degradation products or metabolites; however, the concentrations used in many cell culture experiments are frequently higher than are physiologically achievable through dietary intake. In addition, many in vitro studies focus on the parent anthocyanin glycosides, and their biological effects may not reflect the effects of in vivo degradation or the colonic metabolites that may be responsible for at least part of the cardiovascular bioactivity of anthocyanins.[7] In vitro, anthocyanins inhibit angiotensin-converting enzyme activity,[29] exert antiinflammatory effects,[30,31] and inhibit inducible nitric oxide synthase protein and mRNA expression and the activation of nuclear factor-κB and nitric oxide production in a dose-dependent manner.[32–34] Some of these biological effects were also observed after exposure to their metabolites, including protocatechuic acid.[30,31] In a rat heart model, anthocyanins increased cardiac glutathione concentrations and reduced infarct size after coronary occlusion and perfusion, which resulted in the myocardium being less susceptible to ischemia and reperfusion injury ex vivo.[35] In an apolipoprotein E–deficient mouse model, anthocyanins enhanced atherosclerotic plaque stabilization, suppressed the development of atherosclerotic lesions and the metabolite protocatechuic acid, and directly inhibited atherosclerosis development.[36–38] Anthocyanins have also been shown to act on a range of cells involved in atherosclerosis development, including suppressed tumor necrosis factor-α–induced monocyte chemoattractant protein-1 secretion in primary endothelial cells[39] and reduced expression of vascular endothelial growth factor–stimulated platelet-derived growth factor in vascular smooth muscle cells via deactivation of p38 mitogen-activated protein kinases and c-Jun N-terminal kinase.[40]

These mechanistic insights into anthocyanins are important because the pathogenetic mechanisms underlying MI in young and middle-aged women may differ from those in older women and men. For example, risk in younger women may particularly reflect atherosclerosis with plaque disruption and ulceration leading to MI with nonobstructive coronary artery disease.[4] Coronary artery spasm, a consequence of endothelial dysfunction, also may underlie CHD in younger women. Available data suggest that it is a frequent cause of acute coronary syndrome, with coronary spasm identified in 50% of patients.[5]

In an attempt to explore the biological pathways underlying the associations we observed, we added intermediate conditions, including history of hypertension, hypercholesterolemia, diabetes mellitus, and angina, to the multivariate models, but the risk estimates were not substantially attenuated. This suggests that other mechanisms beyond these may be involved. There was a suggestion of a stronger association in those with prevalent hypertension, although the interaction was not significant (Table 3), potentially because of a lack of statistical power. The addition of other plant-based food constituents, including potassium or folate, to our model also did not substantially attenuate the relationship, nor did adjusting for total intake of fruits and vegetables. These models suggest that the benefits are specific to a food constituent in anthocyanin-rich foods (including blueberries, strawberries, eggplants, blackberries, blackcurrants) and not necessarily to nonspecific benefits among participants who consume high intakes of fruits and vegetables.

We did not observe a substantial change in risk in the middle 3 quintiles of anthocyanin intake, but the difference in intake in these 3 quintiles was only 8 mg. With such small differences in intakes, measurement error or misclassification is likely to be greatest. However, when we examined the possible nonlinear relationship across the entire distribution of anthocyanin intake, we did not detect any significant deviations from linearity. To date, limited dose-response trials of anthocyanins and biomarkers of MI risk have been conducted, but given our knowledge of other subclasses, including the isoflavones and flavan-3-ols, there is likely a threshold of intake for a biological effect, and very low levels of intake are unlikely to be bioactive. When we compared extreme deciles of intake, those in the top decile had a 47% reduction in risk of MI, suggesting a continual dose-response at higher levels of habitual intake.

In food-based analyses, we similarly observed a trend toward a reduction in risk of MI with increasing intake of the 2 main sources of anthocyanins, strawberries and blueberries (which equated to almost 60% of total anthocyanin intake), with a 34% decrease in risk for those who consumed >3 portions per week compared with those who ate these fruits ≤1 time per month. These data are important from a public health perspective because these fruits can be readily incorporated into the habitual diet.

Previous prospective studies on flavonoids and CVD risk have been mixed,[41,42] in part because, until recently, databases did not contain the comprehensive range of flavonoids present in the diet. Two recent studies suggest inverse associations between increased flavanone and anthocyanin intake and fatal CVD risk in older women,[9,10] although 1 study was based on the earlier 2003 US Department of Agriculture database. Our data included both fatal CHD and nonfatal MI, and we had insufficient power to examine the association between anthocyanin intake and fatal CHD risk (n=36 cases). In relation to age, in these middle-aged women (median age, 48.9 years at diagnosis; age at baseline, 25–42 years), we observed a 32% reduction in risk in a comparison of extreme intake quintiles, a finding that supports our previous study in which we observed the greatest magnitude of effect on blood pressure in younger and middle-aged women.[11] This compares with the 2 previous studies of older women (mean age, 69 years; age range, 55–69 years at baseline, respectively) in which the magnitude of the association was lower: 18% and 9% reduction in risk, respectively.[9,10]

We hypothesized that increased flavan-3-ol intake would also be associated with a reduction in risk, given the wealth of mechanistic support for this subclass.[6,8,12] However, to the extent that we could assess intake, no relationship was apparent for habitual intake in this population. Our recent meta-analysis of flavan-3-ol randomized, controlled trials and CVD risk biomarkers suggested that >50 mg/d epicatechin, one of the main flavan-3-ol compounds, is required for beneficial

effects on systolic and diastolic blood pressures.[12] By their very nature, FFQs cannot capture all sources of flavonoids, and some sources of flavan-3-ols may not have been accurately captured. Specifically, dark chocolate is one of the main sources of flavan-3-ols, but its overall consumption in the 1990s was quite low, and most FFQs of that era did not assess different chocolate types.

There was a trend toward a reduction in risk of MI with increasing intake of flavonoid polymers and flavonols, although this did not reach statistical significance. The association with flavonoid polymer intake is intriguing because, to date, many of these compounds remain poorly defined, and we currently know little about their biological activity and bioavailability. This subclass includes proanthocyanidins, theaflavins, and thearubigins, found predominantly in the habitual diet in tea and apples, and the relative impact of these different constituents on biomarkers of CVD risk merits further investigation in future randomized, controlled trials.

The strengths of this study include the prospective design, focus on middle-aged and not older women, large sample size with long-term follow-up, repeat measures of dietary intake, detailed data on important risk factors and confounders for CVD risk, and comprehensive assessment of the range of flavonoid subclasses present in the habitual diet. The limitations of our study also warrant discussion. We adjudicated cases of acute MI using standard criteria, but we did not collect catheterization results. Therefore, although we speculated that coronary spasm may underlie CHD in younger women, we were unable to delineate which women had evidence of coronary spasm during their infarction. Although we adjusted for possible confounders that are strongly associated with MI risk (including body mass index, smoking, family history), there is still the possibility of residual or unmeasured confounding from additional unmeasured factors, which may be greatest when those consuming the highest and the lowest anthocyanin intakes are compared. However, given our detailed and updated adjustment for potential confounders, it is unlikely that these would account fully for the observed results, and in our stratified analyses, we showed that even in nonsmokers and in normal-weight, physically active participants, the point estimates remained similar (Table 3). Of all the flavonoid subclasses assessed, only anthocyanin intake was associated with a reduction in MI risk, suggesting something specific about this subclass. Our FFQ assesses intake of each food up to 6 times per day; therefore, even among the few participants who consume that many servings of blueberries or strawberries, we would be able to accurately assess their intakes. Our data may underestimate the true benefits of lowering blood pressure because we have only a dichotomous variable. It is likely that anthocyanin intake exerts benefits across the entire range of blood pressure, not just at an artificial threshold set for hypertension diagnosis. We used repeated measurements of diet to obtain a more accurate assessment of long-term flavonoid intake and to reduce measurement error. Mean cumulative dietary flavonoid intakes were calculated from a database developed from the most recent US Department of Agriculture databases[11] with additional input from other sources. These data sets allowed us to quantify a broad range of flavonoid subclass intakes more robustly than previous analyses. The flavonoid content of foods varies, depending on growing conditions and manufacturing processes, but despite this variation, these data allow us to rank order intakes and to compare high and low intakes in large population groups. Although correlations between the major dietary sources of flavonoids (fruits, vegetables, tea, wine) have been determined for our FFQ,[16,17] our FFQ has not been validated specifically for the intake of flavonoid subclasses. However, in a recent study, the sum of 7 flavonoid biomarkers measured in 24-hour urine samples was correlated with intakes of fruits and vegetables (0.43–0.66),[43] correlations similar to our validation studies. There are currently no specific biomarkers for anthocyanins because there is currently a limited understanding of their degradation and metabolism after ingestion. It is possible that our findings for anthocyanins might be due to other constituents found in the foods that contribute most to this subclass; however, the addition of other potentially beneficial constituents of fruits, including potassium, folate, and total fruit and vegetable intake, to our multivariate model did not substantially attenuate the relationship between anthocyanins and MI risk, suggesting that anthocyanins may be another important cardioprotective constituent. However, in a population-based study like ours, it is impossible to disentangle the relative influence of all the constituents of fruits and vegetables.

Our findings suggest that bioactive compounds present in red and blue fruits and vegetables commonly consumed in the habitual diet may be associated with a reduced risk of MI in young and middle-aged women. Further prospective studies, including studies with biomarkers of CHD risk to elucidate mechanisms, are needed to confirm these associations. Randomized trials focusing on commonly consumed anthocyanin-rich foods are also needed to examine dose-response effects and to be of long-enough duration to assess clinically relevant end points.

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Disclosures

None.

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CLINICAL PERSPECTIVE

To date, attention has focused on risk factors for coronary heart disease in older age groups, and risk factors may vary with age, particularly in women. Knowledge of modifiable risk factors to prevent myocardial infarction (MI) in young women is limited, particularly in relation to diet. Dietary flavonoids, bioactive compounds present in plant-based foods and drinks, exert potential beneficial effects on endothelial function and blood pressure in short-term trials, but the effects of habitual intakes on MI risk in younger women are unknown. The mechanisms underlying coronary heart disease in younger women may also differ, and coronary vasospasm, a consequence of endothelial dysfunction, may be important. We prospectively studied 93 600 young women from the Nurses' Health Study II for up to 18 years and examined the relationship between intakes of flavonoid subclasses and risk of MI. Individuals with a higher intake of 1 subclass, anthocyanins (responsible for the red/blue color of plants and present in strawberries, blueberries, and red wine), had a significantly lower risk of MI than women consuming low intakes. This 32% reduction in risk was independent of established dietary/lifestyle CVD risk factors, including smoking, body mass index, and fruit and vegetable intake. To relate these findings to public health, we showed that the combined intake of the main anthocyanin sources (strawberries and blueberries) was also associated with a reduction in MI risk. This study suggests that high anthocyanin intakes may reduce MI risk in young women. Intervention trials are needed to assess clinically relevant end points, and prevention efforts should focus on increasing intakes of commonly consumed anthocyanin-rich foods.

High Anthocyanin Intake Is Associated With a Reduced Risk of Myocardial Infarction in Young and Middle-Aged Women

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