Arteriosclerosis, Thrombosis, and Vascular Biology

CLINICAL AND POPULATION STUDIES

Higher Habitual Dietary Flavonoid Intake Associates With Less Extensive Abdominal Aortic Calcification in a Cohort of Older Women

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BACKGROUND: The extent of abdominal aortic calcification (AAC) is a major predictor of vascular disease events. We have previously found regular apple intake, a major source of dietary flavonoids, associates with lower AAC. Whether total dietary flavonoid intake impacts AAC remains unknown. Here, we extend our observations to habitual intakes of total flavonoids, flavonoid subclasses, and specific flavonoid-containing foods, with the odds of extensive AAC.

METHODS: We conducted cross-sectional analyses on 881 females (median [interquartile range] age, 80 [78–82] years; body mass index, 27 [24–30] kg/m^2) from the PLSAW (Perth Longitudinal Study of Ageing Women). Flavonoid intake was calculated from food-frequency questionnaires. Calcifications of the abdominal aorta were assessed on lateral lumbar spine images and categorized as less extensive or extensive. Logistic regression was used to investigate associations.

RESULTS: After adjusting for demographic, lifestyle and dietary confounders, participants with higher (Q4), compared with lower (Q1) intakes, of total flavonoids, flavan-3-ols, and flavonols had 36% (odds ratio [95% CI], 0.64 [0.43–0.95]), 39% (0.61 [0.40–0.93]) and 38% (0.62 [0.42–0.92]) lower odds of extensive AAC, respectively. In food-based analyses, higher black tea intake, the main source of total flavonoids (75.9%), associated with significantly lower odds of extensive AAC (2–6 cups/d had 16%–42% lower odds compared with 0 daily intake). In a subset of nonconsumers of black tea, the association of total flavonoid intake with AAC remained (Q4 versus Q1 odds ratio [95% CI], 0.11 [0.02–0.54]).

CONCLUSIONS: In older women, greater habitual dietary flavonoid intake associates with less extensive AAC.

GRAPHIC ABSTRACT: A graphic abstract is available for this article.

Key Words: aorta, abdominal ■ flavonoids ■ observational study ■ tea ■ vascular calcification ■ vascular diseases

Flavonoids, found in foods and beverages such as black tea, cocoa, red wine, and fruits, have been identified as associating with lower risk of cardiovascular disease (CVD) mortality in a meta-analysis of cohort studies including our own.[1,2] In addition, we have previously reported that tea, a major source of flavonoids, associates with lower CVD mortality.[3] About mechanisms, flavonoids have demonstrated anti-inflammatory and anti-oxidative activities[4] that have been hypothesized to play a role in the prevention of vascular calcification.[5,6]

Vascular calcification occurs as part of the pathological progression of atherosclerosis and arteriosclerosis.[7] Oxidative stress and inflammatory processes play a central role in the development of vascular calcification, cueing vascular smooth muscle cells to adopt an osteoblastic phenotype and deposit calcium within the arterial wall.[5] Calcifications found within the intimal arterial wall layer occur concomitant with atherosclerosis and correlate with the extent of overall plaque burden.[8,9] Calcifications of the medial arterial wall layer are found in arteriosclerosis at sites of elastic fiber degradation and contribute

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Nonstandard Abbreviations and Acronyms

AAC	abdominal aortic calcification
AAC24	AAC 24-point scoring method
CAIFOS	Calcium Intake Fracture Outcome Study
CKD	chronic kidney disease
eGFR	estimated glomerular filtration rate
Hmox-1	heme oxygenase-1
hs-CRP	high-sensitivity C-reactive protein
hs-cTnI	high-sensitivity cardiac troponin I
Nrf2	NF-E2-related factor 2
OR	odds ratio
PLSAW	Perth Longitudinal Study of Ageing Women

Highlights

- Higher habitual dietary flavonoid intake was observed to associate with a lower risk of extensive abdominal aortic calcification in older women.
- Greater intakes of black tea, the main source of dietary flavonoids (≈75%), also associated with a lower risk of extensive abdominal aortic calcification.
- In a subgroup of those women who do not drink black tea, higher total nontea flavonoid intake beneficially associated with a lower risk of extensive abdominal aortic calcification.

to vascular stiffening.[8,9] Intimal and medial calcifications often co-exist within the abdominal aorta,[8] which is one of the first vascular beds to calcify.[10] Extensive abdominal aortic calcification (AAC) identifies people with marked structural vascular disease and indicates a greater risk for cardiovascular events.[11,12]

In this population, we have previously reported that a higher intake of apples, a major flavonoid-containing food, associates with lower odds of extensive AAC.[13] We have also seen cruciferous vegetables, another contributor to flavonoid intake, associates with lower odds of extensive AAC in this cohort.[14] Other groups have observed habitually drinking black tea[15–17] and regularly consuming chocolate,[18] associates with lower calcifications of various vascular beds. Therefore, in this study, we investigate associations between habitual intakes of total flavonoids, flavonoid subclasses, and specific flavonoid-containing foods with AAC, to expand the understanding of population-based dietary determinants that may impact this marker for clinical CVD.

METHODS

Study Design

The data that support the findings of this study are available from the corresponding author upon reasonable request in-line with governing ethical considerations (Supplemental Major Resources Table). This observational investigation was conducted as part of the PLSAW (Perth Longitudinal Study of Ageing Women), a large cohort study of older, White, female adults residing in Western Australia. As described in detail previously,[19] the PLSAW cohort was initially recruited in 1998, as part of a large-scale, randomized, controlled trial, examining the effects of supplemental calcium on fracture risk in the CAIFOS (Calcium Intake Fracture Outcome Study). Of the 5586 women approached, 1500 women were recruited to trial and received 1.2 g of calcium carbonate daily or a matching placebo for 5 years. Eligible women were postmenopausal, ambulant and not using bone-active agents, including hormone-replacement therapy. Following completion of CAIFOS, from 2003 onwards, consenting participants were followed observationally, as part of the PLSAW ongoing prospective investigation into the role of environmental, physiological, and genetic factors in the development of chronic diseases of aging. For the current analysis, we report on data collected in 2003. At this time, participants attended study centers for medical assessment and completed questionnaires on food and beverage consumption, physical activity, and socioeconomic status; professional staff obtained measures of anthropometry, conducted radiographic imaging and collected bloods. Of the original 1500 participants in 1998, 1171 completed a food-frequency questionnaire with plausible energy intakes (2100–14 700 kJ/d) in 2003. After excluding participants with missing outcome (n=86), or covariate information (n=29), data were available for 1057 females. The further exclusion of those with a history of atherosclerotic cardiovascular disease (n=175) gave a population of 881. This study was conducted according to the guidelines laid down in the Declaration of Helsinki, and ethical approval was granted by the Human Ethics Committee of the University of Western Australia (approval number 05/06/004/H50). All participants provided written informed consent. The PLSAW cohort registration ID is ACTRN12617000640303.

Exposures

A semiquantitative self-administered food-frequency questionnaire developed by the Cancer Council of Victoria was used to assess dietary intake,[20] and an additional questionnaire captured information pertinent to tea and coffee consumption.[21] For both questionnaires, respondents were asked to indicate their usual frequency of consumption during the past year. Intakes of foods were evaluated using a 9-category frequency scale that ranged from never to 6 or more eating occasions per day. Intakes of black tea and coffee were evaluated using open-ended questions. Flavonoid consumption was estimated, as the product of serving size by intake frequency and flavonoid content, using the United States Department of Agriculture flavonoid-food composition databases, which we have previously assessed against Phenol-Explorer, for consistency in flavonoid intake estimation (United States Department of Agriculture Database for the Flavonoid Content of Selected Foods, Release 2.1; United States Department of Agriculture Database for the Isoflavone Content of Selected

Foods, Release 2.0; United States Department of Agriculture Database for the Proanthocyanidin Content of Selected Foods, Release 1).[22] We derived intakes of 7 subclasses as follows: flavanones (eriodictyol, hesperetin, and naringenin), anthocyanins (cyanidin, delphinidin, malvidin, pelargonidin, petunidin, and peonidin), flavan-3-ols (catechins and epicatechins, theaflavins, and thearubigins), proanthocyanidins (dimers, trimers, 4–6 mers, 7–10 mers, and polymers), flavonols (quercetin, kaempferol, myricetin, and isohamnetin), flavones (luteolin and apigenin), and isoflavones (daidzein, genistein, and glycitein). Total flavonoid intake was estimated as the sum of all subclasses. Exposures of interest were intakes of total flavonoids and flavonoid subclasses with mean intakes of ≥5 mg/d. For the calculation of intake for total flavonoids and flavonoid subclasses, all flavonoid-containing foods and beverages reported in the questionnaires were included. In food-based analysis, flavonoid-containing foods of interest were those which contributed the most to flavonoid intake. As we have previously reported on associations of apples, oranges, and total fruit, with the odds of AAC in this cohort,[13] to prevent duplication of results, our food and beverage exposures for this study included: black tea, fruit juice, chocolate, and red wine.

Outcome

Calcifications of the abdominal aorta were assessed using digitally enhanced lateral single-energy images of the thoracolumbar spine obtained by dual-energy X-ray absorptiometry machine (Hologic 4500-A Hologic Inc, Bedford, MA). An experienced investigator (J.T. Schousboe) scored each image for AAC using a semiquantitative 24-point scoring method (AAC24) while blinded to clinical data.[23] Scores for AAC were classified as not extensive (AAC24 scores 0–5) or extensive (AAC24 scores 6–24), as scores ≥6 mark generalized atherosclerosis,[19] predict future CVD,[12] and have been previously used to investigate the cause of AAC.[13,14] In an exploratory analysis, we examined further thresholds for classifying the extent of calcification, investigating the moderate to severe AAC cut point (AAC24 ≥2) and the no versus any AAC cut point (AAC24 ≥1).

Covariates

Data on age, education, smoking habits, physical activity, anthropometry, medication use, blood biochemistry, and diet were obtained from the medical assessment and accompanying questionnaires. Energy, macronutrient, and micronutrient intakes from foods and beverages were estimated with FOODWORKS PROFESSIONAL (Xyris, Brisbane, Australia) using the Australian Food Composition Database (NUTTAB 95; Australian Government Nutrient Database, Canberra, Australia). Prevalent diabetes was determined by medication use and coded (T89001–T90009) using the International Classification of Primary Care-Plus method. Previous atherosclerotic cardiovascular disease was determined from discharge diagnoses from hospital records (lookback period from 1980 to 2003).

Biochemistry

Venous blood samples were acquired following an overnight fast (from 2200 hours) by a trained phlebotomist, processed within an hour of blood collection and stored at −80 °C. Routine analysis was performed in a commercial laboratory for total cholesterol using a Hitachi 917 auto analyzer (Roche diagnostics). hs-CRP (High-sensitivity C-reactive protein) was measured using a highly sensitive latex immunoassay (CRP Vario, Sentinel Diagnostics, Abbott Diagnostics Europe, Milan, Italy). hs-cTnI (High-sensitivity cardiac troponin I) was measured using an Abbott ARCHITECT i2000SR STAT hsTnI assay. Creatinine and cystatin C were measured in serum. Creatinine was measured using an isotope dilution MS-traceable Jaffe kinetic assay on a Hitachi 917 analyzer (Roche Diagnostics GmbH). Cystatin C was measured using a fully automated particle-enhanced immunoturbidimetric assay with Sentinel Diagnostics reagents (Sentinel CH) on the Architect ci 16200 System (Abbott Laboratories). The estimated glomerular filtration rate (eGFR) was calculated using the Chronic Kidney Disease Epidemiology Collaboration creatinine and cystatin C equation.[24] Stages of chronic kidney disease (CKD) were determined on the basis of eGFR by standard criteria.[25]

Statistical Analysis

Logistic regression was used to investigate associations of our exposures with the odds of extensive AAC. We explored nonlinearity of the associations using cubic splines; the test of nonlinearity used a χ^2 likelihood ratio test to compare the model with only the linear term to the model that included the cubic spline terms. As we found no indications for nonlinear associations for the main models, all analyses were performed assuming linearity (except for red wine, which showed a significant departure from linearity and was therefore modeled using a cubic spline). Collinearity diagnostics for the exposure variables were tested using variance inflation factors, with no violations found. All exposures were entered as continuous variables and we derived the reported odds ratios (OR) and 95% CIs from these model coefficients. For flavonoids, ORs are reported for the median intake in each quartile with the first quartile median as the reference point. For foods and beverages, ORs are reported across common serving sizes (eg, 1, 2, and 3 cups/d), with zero consumption as the reference group in analyses of black tea, chocolate, and fruit juice. In the analysis of red wine, modest red wine consumers were set as the reference group, to account for abstainer bias.[26] Three main models of adjustment were utilized: (1) minimally adjusted (Model 1): age (years) and the Calcium Intake Fracture Outcome Study treatment code (placebo/treatment); (2) multivariable-adjusted (Model 2): age (years), body mass index (BMI; kg/m^2), smoking (never or former/current), energy expended in physical activity (kJ/d), alcohol intake (g/d), dietary energy intake (kJ/d), the Calcium Intake Fracture Outcome Study treatment code (placebo/treatment), antihypertensive medication use (yes/no), and statin use (yes/no); (3) multivariable-adjusted including potential dietary confounders: (g/d) saturated fat, polyunsaturated fat, monounsaturated fat, sodium, and fiber. As diabetes (present/absent), eGFR, and CKD (stages 1–5) may be potential mediators on the causal pathway linking flavonoid intake with AAC, we report the addition of these terms to Model 3 separately. In additional analysis, smoking years and cigarettes smoked per day were added to models 2 and 3 (in place of smoking [never or former/current]) to further examine the impact of smoking. We then conducted sensitivity analyses to

investigate possible confounding by other nutrients that may influence calcific processes.[6,27] In this analysis, (mg/d) calcium, zinc, magnesium, phosphorus, and (µg/d) folate were added to Model 3. In the food-based investigation, we considered coffee intake a potential confounder of tea intake, and thus, during sensitivity analysis, coffee intake (mL/d) was added to Model 3. In secondary analyses, we investigated whether associations differed among subgroups, stratifying at thresholds relevant for CVD risk, including age (≥80 years), BMI (≥30 kg/m^2), smoking status (ever versus never), total cholesterol (≥5.5 mmol/L), hs-CRP (≥2.0 mg/dL), renal function (CKD grades 1 and 2 versus 3–5), and hs-cTnI, which we dichotomized at the cohort median value (≥4.1 ng/L). In exploratory analysis, we stratified by those who drank and did not drink black tea and investigated the association of total nontea flavonoid intake with the odds of extensive AAC, adjusting for tea-derived flavonoid intake, among the black tea consuming group. Associations were considered statistically significant at $P \leq 0.05$ (2-tailed). Analyses were performed using R-4.2.1 Statistical Software.[28] Figures were generated using the rms R package[29] with the *x*-axis truncated in all graphs, at 2 SDs above the mean of the exposure, for visualization purposes.

RESULTS

Study Population

In this cohort of postmenopausal White females, all were ≥75 years old, most had never smoked, none used hormone-replacement therapy and ≈58% had a BMI within the normal range for older adults (≥23.0–≤29.9 kg/m^2; Table 1). In total, <7% were diabetic, ≈50% were hypertensive and 30% used lipid-lowering medication. Extensive AAC was present in ≈25% of participants. Characteristics of higher flavonoid consumers were similar to lower consumers in terms of age, BMI, smoking status, physical activity, medication use, and blood cholesterol, although participants with the highest flavonoid intakes had greater consumption of all nutrients, slightly better eGFR and slightly less hs-CRP (Table 1). Higher flavonoid consumers appeared to consume more flavonoids from several flavonoid sources (eg, black tea, pome fruits, fruit juice, chocolate, oranges, and cruciferous vegetables) rather than just 1 or 2 unique food items. The range of total daily flavonoid intake was wide (54–4250 mg) with participants consuming a median (IQR) of 1188 (719–1611) mg/d. Subclasses that contributed the most to total flavonoid intake were flavan-3-ols (72.4%) and proanthocyanidins (17.1%), with flavanones (3.8%), flavonols (3.1%), anthocyanins (3.1%), isoflavones (0.4%), and flavones (0.2%) contributing less. The major source of dietary flavonoids was black tea (75.9%), with pome fruits (5.7%), fruit juice (3.4%), chocolate (3.4%), oranges (1.7%), red wine (1.4%), and green beans (1.4%) contributing lesser amounts. Strawberries (0.9%), banana (0.8%), peaches (0.8%), nuts (0.8%), tinned fruit (0.7), and leguminous beans (0.6%) were minor contributors to total flavonoid intake. Other flavonoid-containing foods, such as tofu, beer, and onions contributed <0.5% to total flavonoid intake. Black tea was also the major dietary source of several flavonoid subclasses including flavan-3-ols (97.9%) and flavonols (69.6%) but not proanthocyanidins (13.7%).

Associations Between Habitual Flavonoid Intake and AAC

After adjusting for demographic and lifestyle confounders, participants with higher (Q4), compared with lower (Q1), intakes of total flavonoids, flavan-3-ols, flavonols, and flavanones, had 37% (OR [95% CI], 0.63 [0.43–0.92]), 38% (0.62 [0.41–0.93]), 40% (0.60 [0.41–0.87]), and 31% (0.69 [0.49–0.97]) lower odds of extensive AAC, respectively (Model 2, Table 2). With additional adjustment for dietary confounders of CVD, including saturated fat, polyunsaturated fat, monounsaturated fat, sodium, and fiber, all associations remained statistically significant except for that of the flavanone subclass (Model 3, Table 2, Figure 1). Intakes of other subclasses (ie, anthocyanins and proanthocyanidins) were not associated with AAC. Sensitivity analysis showed the results were unchanged using an alternative model of smoking adjustment incorporating pack-years of smoking and cigarettes per day (data not shown). Given the potential for other nutrients to influence calcific processes, we performed additional sensitivity analyses, on those flavonoid exposures that showed significant associations in Model 3. Here, intakes of calcium, zinc, magnesium, phosphorus, and folate were adjusted for. The addition of these factors, either individually or collectively to Model 3, did not materially affect the findings (data not shown). In a further sensitivity analysis, the individual inclusion of potential mediators, including prevalent diabetes, eGFR and categorical grades of CKD, to Model 3, did not alter any of the results (data not shown).

Associations Between Habitual Intake of Specific Flavonoid-Containing Foods and AAC

Higher habitual black tea intake was associated with lower odds of extensive AAC after multivariable adjustment for demographic and lifestyle factors. In comparison to those consuming zero cups of black tea per day, those consuming 2, 4, and 6 cups per day showed 16% (OR [95% CI], 0.84 [0.71–0.99]), 30% (0.70 [0.50–0.97]), and 42% (0.58 [0.35–0.96]) lower odds respectively of extensive AAC (Model 2; Table 3). The inverse association of black tea intake with AAC remained statistically significant following further adjustment for dietary confounders (Model 3; Table 3 and Figure 2) as well as after the individual addition of prevalent diabetes, eGFR, CKD grade, and coffee consumption to the model (data not shown). Sensitivity analysis showed the results were

Table 1. Characteristics of the Study Participants*

	Total population (n=881)	Total flavonoid intake quartile			
		Q1 (n=221)	Q2 (n=220)	Q3 (n=220)	Q4 (n=220)
Demographics					
Total flavonoid intake, mg/d	1188 [719–1611]	344 [228–549]	924 [842–1058]	1404 [1283–1514]	1955 [1760–2253]
Age, y	80 [78–82]	80 [77–82]	80 [78–82]	80 [78–82]	79 [78–82]
BMI, kg/m^2	27 [24–30]	26.3 [23.6–30.3]	27.1 [24.0–30.2]	26.4 [23.8–29.7]	26.5 [24.1–29.4]
Smoking, n (%)					
Never	579 (65.7)	138 (62.4)	142 (64.5)	152 (69.1)	147 (66.8)
Current/former	302 (34.3)	83 (37.6)	78 (35.5)	68 (30.9)	73 (33.2)
Duration of smoking, y	22 [5–41]	26 [6–42]	22 [6–42]	21 [6–37]	22 [4–36]
Cigarettes per day	4 [1–6]	4 [2–6]	2 [1–5]	4 [2–6]	4 [1–6]
Physical activity, kJ/d	102 [29–185]	89 [0–185]	119 [51–187]	115 [46–195]	90 [0–174]
Medication use					
Antihypertensive [yes], n (%)	499 (56.6)	123 (55.7)	125 (56.8)	128 (58.2)	123 (55.9)
Statins [yes], n (%)	264 (30.0)	69 (31.2)	64 (29.1)	66 (30.0)	65 (29.5)
Comorbidities					
Prevalent diabetes [yes], n (%)	57 (6.5)	25 (11.3)	12 (5.5)	9 (4.1)	11 (5.0)
Clinical markers†					
Total cholesterol, mmol/L	5.3 [4.7–6.0]	5.3 [4.8–6.0]	5.0 [4.6–5.9]	5.3 [4.6–6.2]	5.4 [4.7–6.0]
hs-CRP, mg/dL	2.1 [1.2–3.7]	2.3 [1.3–4.8]	2.2 [1.3–4.3]	1.9 [1.1–4.2]	2.15 [1.1–4.3]
hs-cTnI, ng/L	4.1 [3.1–5.8]	4.0 [3.1–5.6]	4.1 [3.2–6.1]	4.1 [2.8–5.7]	4.1 [3.0–5.7]
eGRF, mL/min per 1.73 m^2	64.0 [53.0–71.5]	63.6 [53.8–71.2]	62.5 [53.1–71.0	64.6 [55.3–71.5]	65.1 [54.4–72.0]
Dietary intake					
Energy, kj	6572 [5406–7983]	6182 [4980–7308]	6069 [5081–7663]	6747 [5586–7941]	7265 [6078–8711]
Saturated fat, g/d	23 [17–30]	19 [15–29]	22 [17–28]	24 [19–31]	26 [20–35]
Polyunsaturated fat, g/d	10 [7–13]	9 [7–12]	9 [7–13]	10 [7–13]	11 [8–15]
Monounsaturated fat, g/d	20 [16–26]	19 [14–24]	19 [15–26]	21 [16–26]	23 [18–30]
Simple carbohydrates, g/d	86 [69–108]	83 [61–101]	81 [61–99]	86 [72–110]	102 [78–120]
Alcohol, g/d	2 [0–9]	1 [0–10]	2 [0–9]	3 [0–11]	1 [0–8]
Sodium, mg/d	1895 [1533–2397]	1804 [1450–2188]	1764 [1487–2363]	1894 [1584–2322]	2146 [1758–2692]
Magnesium, mg/d	275 [225–339]	259 [214–316]	252 [203–321]	272 [228–338]	307 [253–371]
Zinc, mg/d	9 [7–12]	9 [7–11]	9 [7–11]	9 [8–12]	11 [8–13]
Folate, µg/d	250 [199–320]	233 [184–294]	233 [184–312]	246 [207–308]	285 [229–355]
Fiber, g/d	20 [16–26]	19 [16–24]	19 [15–24]	20 [16–25]	23 [18–29]
Selected flavonoid sources					
Black tea	750 [250–1000]	0 [0–250]	500 [500–500]	750 [750–1000]	1250 [1000–1500]
Pome fruits	59 [23–107]	54 [20–105]	55 [21–98]	51 [22–105]	79 [35–121]
Fruit juice	13 [0–89]	6 [0–89]	12 [0–89]	11 [0–79]	34 [2–122]
Chocolate	2 [1–8]	2 [0–8]	2 [0–5]	3 [1–7]	3 [1–11]
Oranges	36 [6–87]	30 [3–83]	32 [9–80]	38 [9–92]	41 [5–92]
Red wine	0 [0–10]	0 [0–10]	0 [0–9]	0 [0–19]	0 [0–9]
Cruciferous	28 [15–44]	26 [14–42]	28 [15–44]	26 [14–41]	31 [17–46]

BMI indicates body mass index; hs-CRP, high-sensitivity C-reactive protein; hs-cTnI, high-sensitivity cardiac troponin I; and IQR, interquartile range.
*Data expressed as median [IQR] or n (%) unless otherwise stated.
†Clinical markers measured in participant subset (n=824–857).

unchanged using an alternate model of smoking adjustment incorporating pack-years of smoking and cigarettes per day (data not shown). Higher intakes of dark chocolate, fruit juice, or red wine did not associate with lower AAC (Table 3).

Associations Between Habitual Intake of Nontea Flavonoids and AAC

Given the major source of total dietary flavonoid intake was black tea (75.9%), to disentangle the impact of tea

Table 2. Odds of Extensive AAC24 ≥6 by Quartiles of Habitual Dietary Flavonoid Intake Among Older Postmenopausal Women (n=881)*

	Dietary flavonoid intake			
	Q1 (n=221)	Q2 (n=220)	Q3 (n=220)	Q4 (n=220)
Total flavonoids				
AAC24 ≥6, n	68	57	57	44
Intake, mg/d†	344 [54–719]	924 [721–1190]	1400 [1190–1610]	1950 [1610–4250]
Odds ratio (95% CI)				
Model 1	Reference	0.85 (0.74–0.98)	0.75 (0.58–0.96)	0.64 (0.44–0.94)
Model 2	Reference	0.84 (0.74–0.97)	0.73 (0.57–0.94)	0.63 (0.43–0.92)
Model 3	Reference	0.85 (0.74–0.98)	0.74 (0.57–0.97)	0.64 (0.43–0.95)
Anthocyanins				
AAC24 ≥6, n	54	67	50	55
Intake, mg/d†	14 [1–20]	24 [20–30]	37 [30–47]	63 [47–192]
Odds ratio (95% CI)				
Model 1	Reference	0.97 (0.91–1.03)	0.93 (0.80–1.08)	0.86 (0.62–1.18)
Model 2	Reference	0.95 (0.89–1.02)	0.89 (0.77–1.05)	0.79 (0.57–1.10)
Model 3	Reference	0.99 (0.91–1.08)	0.99 (0.82–1.19)	0.97 (0.65–1.45)
Flavan-3-ols				
AAC24 ≥6, n	65	63	53	45
Intake, mg/d†	25 [4–334]	638 [334–943]	957 [943–1260]	1570 [1260–3750]
Odds ratio (95% CI)				
Model 1	Reference	0.83 (0.70–0.97)	0.75 (0.59–0.96)	0.62 (0.41–0.93)
Model 2	Reference	0.83 (0.70–0.97)	0.75 (0.58–0.96)	0.62 (0.41–0.93)
Model 3	Reference	0.82 (0.69–0.97)	0.74 (0.57–0.96)	0.61 (0.40–0.93)
Proanthocyanidins				
AAC24 ≥6, n	59	53	56	58
Intake, mg/d†	92 [24–124]	150 [125–178]	211 [178–255]	318 [256–1060]
Odds ratio (95% CI)				
Model 1	Reference	1.02 (0.94–1.10)	1.04 (0.88–1.22)	1.07 (0.79–1.46)
Model 2	Reference	0.99 (0.92–1.08)	0.99 (0.84–1.17)	0.98 (0.71–1.34)
Model 3	Reference	1.06 (0.95–1.19)	1.13 (0.91–1.42)	1.27 (0.83–1.94)
Flavanones				
AAC24 ≥6, n	63	61	54	48
Intake, mg/d†	8 [0–16]	26 [16–36]	48 [36–63]	87 [63–237]
Odds ratio (95% CI)				
Model 1	Reference	0.92 (0.85–1.00)	0.84 (0.70–0.99)	0.70 (0.50–0.99)
Model 2	Reference	0.92 (0.85–0.99)	0.83 (0.70–0.99)	0.69 (0.49–0.97)
Model 3	Reference	0.94 (0.86–1.03)	0.87 (0.72–1.06)	0.76 (0.52–1.13)
Flavonols				
AAC24 ≥6, n	67	65	47	47
Intake, mg/d†	14 [1–23]	29 [23–35]	42 [35–47]	57 [47–121]
Odds ratio (95% CI)				
Model 1	Reference	0.84 (0.74–0.96)	0.73 (0.57–0.93)	0.62 (0.43–0.90)
Model 2	Reference	0.83 (0.73–0.95)	0.72 (0.56–0.91)	0.60 (0.41–0.87)
Model 3	Reference	0.84 (0.73–0.97)	0.73 (0.57–0.95)	0.62 (0.42–0.92)

Model 1 adjusted for: age and the Calcium Intake Fracture Outcome Study treatment code; Model 2 adjusted for age, BMI, smoking, energy expended in physical activity, alcohol intake, antihypertensive medication use, statin use, the Calcium Intake Fracture Outcome Study treatment code and dietary energy intake; and Model 3 adjusted for all covariates in Model 2 plus intakes of saturated fat, polyunsaturated fat, monounsaturated fat, sodium, and fiber. AAC24, abdominal aortic calcification 24-point scoring method; and BMI, body mass index.

*Odds ratios (95% CI) for extensive abdominal aortic calcification, obtained from logistic regression models. All exposures were entered as linear continuous terms (as there was no evidence of nonlinearity) and we derived the reported odds ratios and 95% CIs from these model coefficients at specific levels of flavonoid intake (ie, median of Q2, Q3, and Q4) with the first quartile median as the reference point.

†Median; range in brackets [all such values]. Intake quartiles are mutually exclusive.

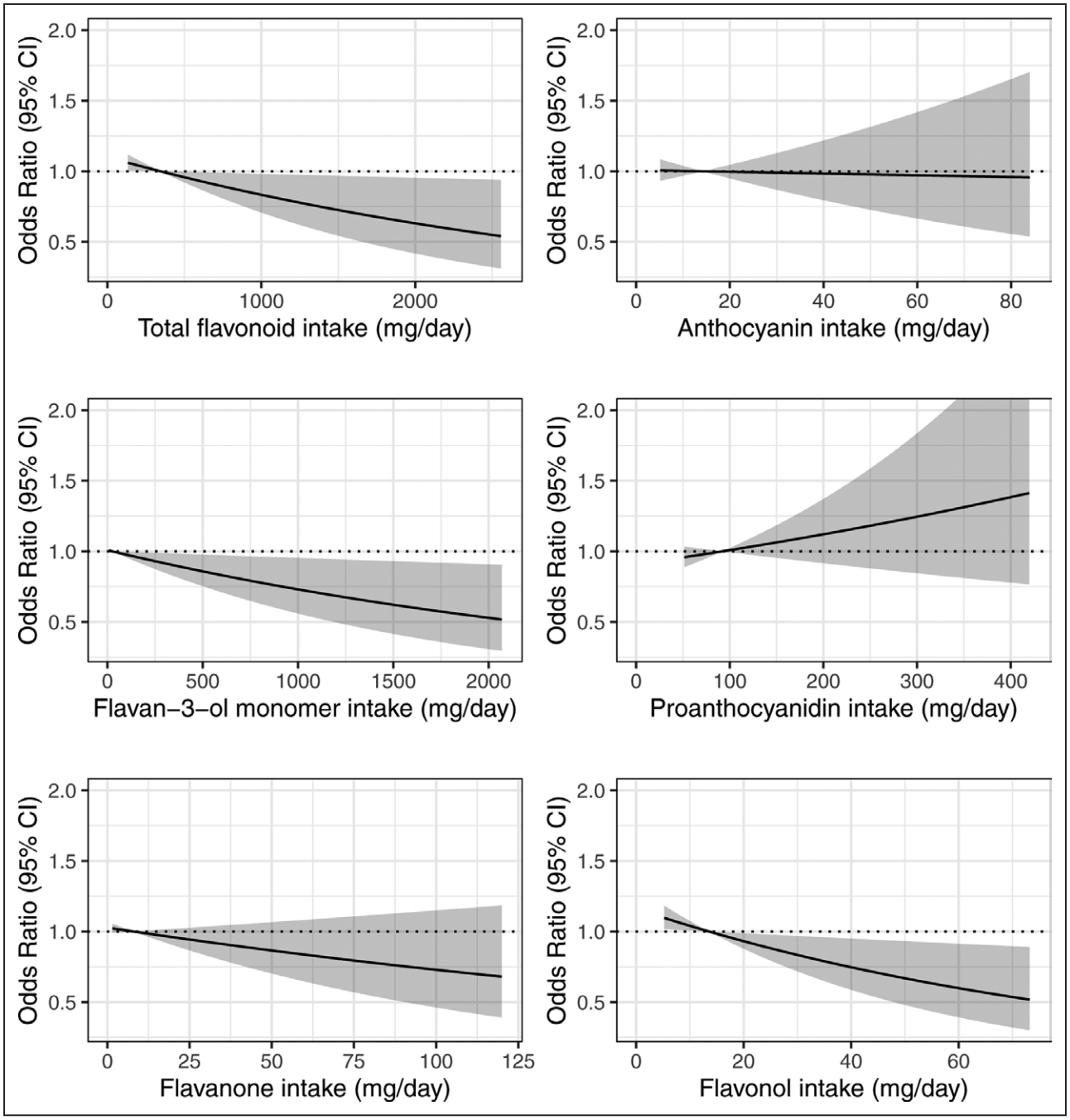

Figure 1. Logistic regression results describing the association between habitual dietary flavonoid intake and the odds of extensive abdominal aortic calcification (AAC; AAC 24-point scoring method [AAC24] ≥6; n=881).
Odds ratios are comparing the specific level of flavonoid intake (horizontal axis) to the median intake for participants in the lowest intake quartile. Horizontal axes are truncated at 2 SDs above the exposure means for visualization purposes. Adjusted for age, body mass index (BMI), smoking, energy expended in physical activity, alcohol intake, antihypertensive medication use, statin use, the Calcium Intake Fracture Outcome Study treatment code and intakes of dietary energy, saturated fat, polyunsaturated fat, monounsaturated fat, sodium, and fiber.

and nontea flavonoids on AAC, we conducted exploratory analysis on a subset of participants who did not consume black tea. In this subset, following adjustment for demographic and lifestyle confounders, intakes of nontea total flavonoids, proanthocyanidins and flavanones were inversely associated with the extent of AAC, with the lowest odds observed for those in Q4 (total flavonoids Q4 versus Q1 OR [95% CI], 0.13 [0.03–0.49]; proanthocyanidins Q4 versus Q1 OR [95% CI], 0.34 [0.17–0.69]; flavanones Q4 versus Q1 OR [95% CI], 0.43 [0.24–0.80]; Model 2; Table S1; data not shown for subclasses). These associations remained statistically significant following additional adjustment for dietary confounders (Model 3; Table S1; data not shown for subclasses). Among black tea consumers, there was no association of total nontea flavonoid intake with AAC (Model 2; Table S1).

Table 3. Odds of Extensive AAC24 ≥6 by Flavonoid-Rich Food and Beverage Intake Among Older Postmenopausal Women (n=881)*

	Dietary intake			
Black tea				
Intake (cups/d, mL)	0	2 [500]	4 [1000]	6 [1500]
Odds (95% CI)				
Model 1	Reference	0.82 (0.70–0.97)	0.68 (0.49–0.94)	0.56 (0.34–0.91)
Model 2	Reference	0.84 (0.71–0.99)	0.70 (0.50–0.97)	0.58 (0.35–0.96)
Model 3	Reference	0.81 (0.69–0.97)	0.67 (0.48–0.94)	0.55 (0.33–0.91)
Chocolate				
Intake, g/d	0	10	20	30
Odds ratio (95% CI)				
Model 1	Reference	1.09 (0.95–1.27)	1.20 (0.90–1.60)	1.31 (0.85–2.03)
Model 2	Reference	1.14 (0.97–1.34)	1.30 (0.94–1.80)	1.48 (0.91–2.40)
Model 3	Reference	1.10 (0.93–1.30)	1.21 (0.87–1.70)	1.34 (0.81–2.23)
Red wine				
Intake (glass/d, mL)	¼ [32.5]	½ [75]	¾ [107.5]	1 [150]
Odds ratio (95% CI)				
Model 1	Reference	0.88 (0.74–1.05)	0.98 (0.79–1.20)	1.26 (0.92–1.73)
Model 2	Reference	0.86 (0.70–1.06)	0.92 (0.69–1.22)	1.13 (0.74–1.74)
Model 3	Reference	0.87 (0.70–1.08)	0.94 (0.71–1.26)	1.18 (0.77–1.81)
Fruit juice				
Intake (cups/d, mL)	0	¼ [62.5]	½ [125]	1 [250]
Odds ratio (95% CI)				
Model 1	Reference	0.94 (0.85–1.05)	0.89 (0.72–1.10)	0.79 (0.52–1.21)
Model 2	Reference	0.95 (0.85–1.06)	0.89 (0.71–1.12)	0.80 (0.51–1.25)
Model 3	Reference	0.95 (0.85–1.07)	0.91 (0.71–1.15)	0.83 (0.52–1.33)

Model 1 adjusted for: age and the Calcium Intake Fracture Outcome Study treatment code; Model 2 adjusted for age, BMI, smoking, energy expended in physical activity, alcohol intake, antihypertensive medication use, statin use, the Calcium Intake Fracture Outcome Study treatment code and dietary energy intake; and Model 3 adjusted for all covariates in Model 2 plus intakes of saturated fat, polyunsaturated fat, monounsaturated fat, sodium, and fiber. AAC24 indicates abdominal aortic calcification 24-point scoring method; and BMI, body mass index.

*Odds ratios (95% CI) for extensive abdominal aortic calcification, obtained from logistic regression models. Exposures were entered as linear continuous terms (as there was no evidence of nonlinearity, except for red wine which was, therefore, modeled using a cubic spline). We then derived the reported odds ratios and 95% CIs from these model coefficients at specific levels of intake (eg, 1, 2, and 3 cups/d) with zero consumption set as the reference point for analyses of black tea, chocolate, and fruit juice, and in the analysis of red wine, modest consumers were set as the reference group, to account for abstainer bias.[26]

Associations Between Habitual Total Flavonoid Intake and AAC According to Risk Factor Status

To examine the possibility that the association of flavonoid intake with extensive AAC is modified in certain subgroups, we conducted analyses stratified by risk factors for CVD including age, BMI, smoking, hypercholesterolemia, hypertensive medication, lipid-lowering medication, hs-CRP, hs-cTnI, and renal function. Point estimates showed inverse associations of habitual total flavonoid intake with the odds of extensive AAC in all subgroups investigated (except for age ≥80 years), wherein statistical significance was reached in specific subpopulations including those of younger age (<80 years, total flavonoids Q4 versus Q1 OR [95% CI], 0.39 [0.21–0.73]; Model 3; Table 4), those with poorer eGFR (<60 mL/min per 1.73 m^2, total flavonoids Q4 versus Q1 OR [95% CI], 0.62 [0.40–0.96]; Model 3; Table 4) and those with elevated hs-cTnI (≥4.1 ng/L, total flavonoids Q4 versus Q1 OR [95% CI], 0.57 [0.32–0.99]; Model 3; Table 4).

Associations Between Habitual Total Flavonoid Intake and AAC Using Alternative Cut Points for Classifying the Extent of Calcification

In further sensitivity analysis, we examined the association of total flavonoid intake with AAC using alternative cut points for classifying the extent of calcification. Using the moderate to severe cut point (AAC24 ≥2), higher total flavonoid intake non-significantly associated with lower odds of AAC, following adjustment for demographic and lifestyle factors (Q4 versus Q1 OR [95% CI], 0.87 [0.61–1.24]; Model 2; Table S2). When no AAC,

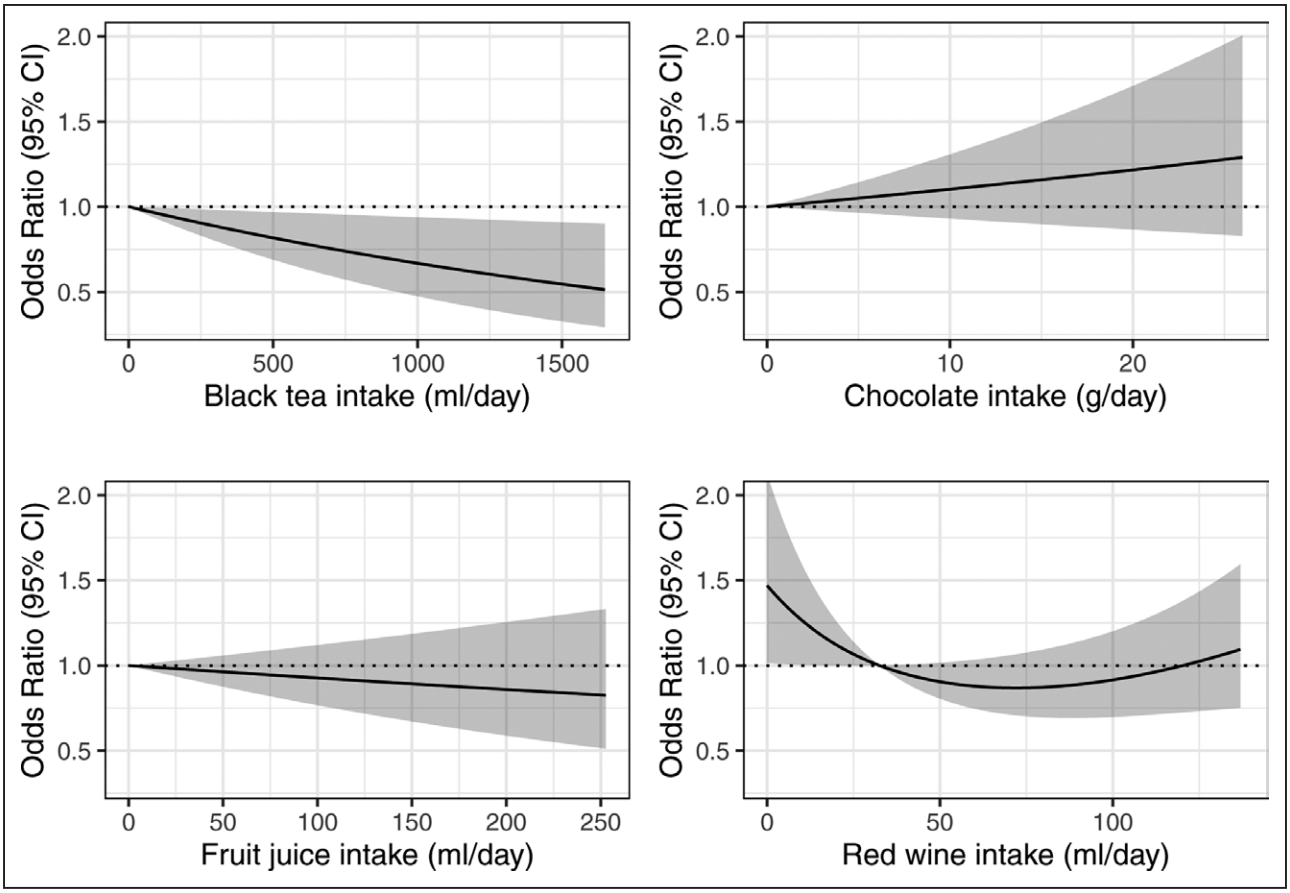

Figure 2. Logistic regression results describing the association between intakes of specific flavonoid-containing foods and beverages with the odds of extensive abdominal aortic calcification (AAC; AAC 24-point scoring method [AAC24] ≥6; n=881). Odds ratios are comparing the specific level of food or beverage intake (horizontal axis) to those with zero consumption set as the reference point for analyses of black tea, chocolate and fruit juice, and in the analysis of red wine, modest consumers were set as the reference group, to account for abstainer bias.[26] Horizontal axes are truncated at 2 SDs above the exposure means for visualization purposes. Adjusted for age, body mass index (BMI), smoking, energy expended in physical activity, alcohol intake, antihypertensive medication use, statin use, the Calcium Intake Fracture Outcome Study treatment code and intakes of dietary energy, saturated fat, polyunsaturated fat, monounsaturated fat, sodium, and fiber. Red wine showed departure from linearity and was thus modeled using a cubic spline term.

compared with any AAC was considered, a trend towards lower odds of any AAC was observed with higher total flavonoid consumption (Q4 versus Q1 OR [95% CI], 0.71 [0.47–1.07]; Model 2; Table S2).

DISCUSSION

In this cohort of postmenopausal women aged 70 years and older, we found higher habitual intakes of total flavonoids and certain subclasses (flavan-3-ols and flavonols) associate with significantly lower odds of extensive AAC following adjustment for demographics, lifestyle, and dietary factors. Of the main dietary sources of flavonoids, black tea intake, but not fruit juice, red wine, or chocolate, showed significant beneficial associations with AAC. Interestingly, when stratifying by those who drank and did not drink black tea, there was a beneficial association of total nontea flavonoid intake with AAC among nonconsumers of black tea but not among black tea consumers, implying flavonoids from sources other than black tea may play a role in our observed associations when tea-derived sources are low. Given the extent of AAC is a major predictor of vascular disease events, and that the intake of flavonoids associated with lower AAC in our cohort are easily achievable in the habitual diet, confirmation of our results, by clinical trials and in vivo models, may have important clinical and public health implications.

No prior observational studies have investigated the association of total dietary flavonoid intake with vascular calcification. Several cohort studies have, however, reported on intakes of major flavonoid-containing foods and vascular calcification.[13,15–17] In our earlier work on the PLSAW cohort, we observed higher apple intake, a major flavonoid-containing food, associates with lower AAC.[13] We have also seen cruciferous vegetables, another contributor to flavonoid intake, associates with lower odds of extensive AAC in this cohort.[14] In our current analysis of the PLSAW cohort, we build upon our prior research, finding higher black tea intake also associates with lower

Table 4. Odds of Extensive AAC24 ≥6 by Quartiles of Total Habitual Dietary Flavonoid Intake Among Older Postmenopausal Women Stratified by Risk Factors for Cardiovascular Disease*

(n=subgroup [case events])	Total dietary flavonoid intake			
	Q1	Q2	Q3	Q4
Age, y				
<80 (n=440 [94])	Reference	0.71 (0.57–0.89)	0.54 (0.36–0.82)	0.39 (0.21–0.73)
≥80 (n=441 [132])	Reference	0.99 (0.81–1.21)	0.98 (0.68–1.41)	0.97 (0.56–1.69)
BMI, kg/m^2				
<30 (n=669 [186])	Reference	0.85 (0.72–1.00)	0.75 (0.55–1.00)	0.64 (0.41–1.01)
≥30 (n=212 [40])	Reference	0.73 (0.50–1.05)	0.56 (0.28–1.08)	0.41 (0.15–1.13)
Smoking				
Never (n=579 [134])	Reference	0.84 (0.70–1.02)	0.73 (0.52–1.03)	0.62 (0.37–1.05)
Ever (n=302 [92])	Reference	0.84 (0.67–1.07)	0.73 (0.48–1.13)	0.63 (0.33–1.21)
Total cholesterol, mmol/L				
<5.5 (n=470 [130])	Reference	0.88 (0.72–1.06)	0.78 (0.55–1.11)	0.69 (0.41–1.18)
≥5.5 (n=386 [89])	Reference	0.79 (0.62–1.01)	0.65 (0.42–1.02)	0.53 (0.27–1.02)
Lipid-lowering therapy				
No (n=617 [132])	Reference	0.86 (0.71–1.03)	0.75 (0.54–1.06)	0.65 (0.39–1.09)
Yes (n=264 [94])	Reference	0.84 (0.65–1.07)	0.72 (0.46–1.13)	0.61 (0.31–1.21)
Hypertensive medication				
No (n=382 [81])	Reference	0.81(0.64,1.04)	0.69(0.44,1.07)	0.57(0.29,1.11)
Yes (n=499 [145])	Reference	0.87(0.73,1.05)	0.78(0.56,1.1)	0.69(0.41,1.15)
hs-CRP level, mg/dL				
<2.0 (n=400 [99])	Reference	0.83 (0.66–1.06)	0.72 (0.46–1.12)	0.60 (0.31–1.18)
≥2.0 (n=456 [120])	Reference	0.86 (0.71–1.06)	0.77 (0.53–1.11)	0.67 (0.38–1.17)
hs-cTnI				
<median (n=422 [98])	Reference	0.89 (0.71–1.11)	0.81 (0.54–1.22)	0.72 (0.39–1.35)
≥median (n=434 [121])	Reference	0.82 (0.67–1.00)	0.69 (0.48–0.99)	0.57 (0.32–0.99)
eGFR <60 mL/min per 1.73 m^2				
No (n=490 [112])	Reference	0.85 (0.69–1.05)	0.74 (0.50–1.09)	0.64 (0.35–1.15)
Yes (n=313 [99])	Reference	0.71 (0.48–1.05)	0.73 (0.49–1.10)	0.62 (0.40–0.96)

Models standardized for age, BMI, smoking, energy expended in physical activity, alcohol intake, antihypertensive medication use, statin use, the Calcium Intake Fracture Outcome Study treatment code and dietary energy intake plus intakes of saturated fat, polyunsaturated fat, monounsaturated fat, sodium, and fiber. Evers smokers were adjusted for pack-years of smoking and cigarettes per day. AAC24 indicates abdominal aortic calcification 24-point scoring method; BMI, body mass index; eGFR, estimated glomerular filtration rate; hs-CRP, high-sensitivity C-reactive protein; and hs-cTnI, high-sensitivity cardiac troponin I.

*Odds ratios (95% CI) for extensive abdominal aortic calcification, obtained from logistic regression models. All exposures were entered as linear continuous terms (as there was no evidence of nonlinearity, except for those with an eGFR of <60 mL/min per 1.73 m^2 which was, therefore, modeled using a cubic spline) and we derived the reported odds ratios and 95% CIs from these model coefficients at specific levels of flavonoid intake (ie, median of Q2, Q3, and Q4) with the first quartile median as the reference point.

AAC. This finding is in agreement with several cohort studies reporting higher black tea consumption associates with lower calcifications of the coronary artery,[16,17] and abdominal aorta.[15] Given black tea is widely consumed in Western countries, an impact of consumption on CVD risk could have important public health implications. We have also previously seen that higher chocolate intake among PLSAW participants, associates with lower carotid intima-media thickness/plaque and lower risk of CVD events in time to event analysis.[30] Djoussé et al[18] further report that higher chocolate intake cross-sectionally associates with lower risk of calcified plaques in the coronary arteries. Given these findings, we were surprised to not observe a beneficial association between chocolate intake and AAC. The reasons for these differential results are less certain, though may allude to differences in the relative contributions of cocoa's components on the underlying pathologies. Collectively, our findings show the strongest support for intake of flavan-3-ols and flavonols, both of which are present in black tea and apples.

Much of our previous research has identified beneficial associations between dietary flavonoid intake and CVD.[31–36] Given that more extensive AAC predicts higher risk of future ischemic stroke, myocardial infarction, and total CVD mortality,[11] the results of the present

study suggest that higher dietary flavonoid intake may impact clinical CVD risk at least, in part, through mechanisms relating to vascular calcification. Risk factors and specific drivers for intimal calcification include inflammation, oxidative stress, and hyperlipidemia, whereas medial calcification is often found with aging, cellular senescence, diabetes, and CKD.[5–7] The molecular mechanisms by which absorbed flavonoid metabolites potentially mitigate vascular calcification may involve the induction of protective signaling cascades, such as Nrf2 (NF-E2-related factor 2) and Hmox-1 (heme oxygenase-1) which reduce oxidant damage and inflammation,[37,38] or the downregulation of factors that produce reactive oxygen species, such as nicotinamide adenine dinucleotide phosphate oxidases.[5] It is also possible that flavonoids may protect against vascular calcification by supporting nitric oxide bioavailability, via endothelial nitric oxide synthase (eNOS) induction, as nitric oxide has been shown to prevent differentiation of vascular smooth muscle cells into osteoblastic cells.[39,40] It is relevant that the association of higher flavonoid intake was only clearly observed for lower odds of extensive AAC (AAC24 ≥6), but not moderate (AAC24 ≥2) or nil AAC (AAC24 ≥1). This could allude to differences in pathophysiology, whereby those with modest AAC may exhibit some localized dysfunction, compared with those with extensive AAC whom may display systemic dysregulation of calcification inhibitors/drivers. To our knowledge, no human clinical trials to date have considered vascular calcification as an endpoint following flavonoid or flavonoid-rich food intervention. In rodent models, quercetin, the most consumed dietary flavonol, was shown to significantly ameliorate adenine-induced vascular calcification in rats.[41,42] Overall, it is plausible that flavonoids potentially modulate vascular calcification via several mechanisms.

When investigating the associations between flavonoids with extensive AAC across strata of CVD risk factors and biomarkers, the association was statistically significant in those with elevated cardiac ischemia biomarkers (hs-cTnI) and poorer estimated renal function (eGFR). It is possible that the protective association of flavonoids with AAC is more prominent in these groups, as flavonoids may mitigate pathological processes which are amplified within these higher-risk populations. Although the small sample size prohibits us from ruling out a beneficial association of flavonoid intake with AAC among those with better hs-cTnI and eGFR status. Curiously, we found the protective association between total flavonoid intake and AAC was mitigated in the presence of increasing age. This could suggest the benefits of flavonoids on AAC diminish during later life, as physiological declines that occur with metabolic aging exceed the capacity for flavonoids to mitigate deterioration. Although overall, these analyses are hypothesis generating and should be considered with caution until replicated in future studies. However, when we stratified our analysis by consumers and nonconsumers of black tea, we found total nontea flavonoid intake associated with lower AAC among nonconsumers of black tea but not among black tea consumers. These results suggest the relative importance of nontea flavonoids may diminish as flavonoid intake from black tea increases. The implications are that populations with low black tea consumption may benefit by increasing intake of tea- or nontea-derived flavonoids.

This study has several limitations. The design is observational and as such, we are unable to confirm causality or exclude the possibility of confounding by unmeasured or residual factors. In particular, vitamin K, a nutrient for which we did not estimate intake values, has been proposed as preventive against vascular calcification.[43] However, given that black tea was the primary source of flavonoids in our population and that brews of black tea do not contain any significant quantities of vitamin K,[44] we think it highly unlikely that vitamin K could explain the associations observed in our study. Moreover, we conducted extensive sensitivity analysis showing the associations of total flavonoids, flavn-3-ols and flavonols with AAC are consistent, suggesting our findings are not likely spurious or easily attenuated. Indeed, the point estimates show strong beneficial associations, suggesting our results are most compatible with an important correlation, although considering multiplicity issues our results must be interpreted with caution. Another limitation of the present work is that our study relied on self-reports of dietary intake, which may have caused imprecision in estimates of dietary flavonoid consumption. We also measured AAC using dual-energy X-ray absorptiometry, which has an accuracy dependent on the imaging analysts, unlike other modalities such as computed tomography. However, measurement of AAC in our study was conducted by an imaging expert (J.T. Schousboe) who developed and validated the dual-energy X-ray absorptiometry method of scoring AAC[45] and in one of our recent meta-analyses, we found that imaging modalities (computed tomography, X-ray, or dual-energy X-ray absorptiometry) do not explain between-study heterogeneity between AAC and CVD risk or all-cause mortality.[11] Finally, our sample is also unlikely to be representative of all females, in terms of age, ethnicity, health status, and socioeconomic standing, and therefore, the generalizability of our results is limited and requires confirmation in other populations. Further observational studies would be beneficial to determine if the association of flavonoid intake with AAC is observed in populations with differing characteristics, such as males or other ethnic groups, as well as cohorts whose dietary patterns contribute differentially to the proportion and quantity of the flavonoid subclasses consumed.

We conclude that higher habitual intakes of total flavonoids, as well as the flavan-3-ol and flavonol subclasses,

associate with a lower propensity of the abdominal aorta to calcify. These associations appear largely driven by intakes of black tea, the primary contributor to total dietary flavonoid intake in Western populations. Although, we also observed a beneficial association between total flavonoid intake and AAC in nontea drinkers, suggesting flavonoids from sources other than black tea may also contribute to the protective association. As the extent of AAC is a major predictor of vascular disease events, a role for dietary flavonoids in the prevention of vascular calcification warrant further investigation.

ARTICLE INFORMATION

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Disclosures

None.

Supplemental Material

Figure S1
Tables S1–S2
Major Resources Table

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