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Selected Dietary Flavonoids Are Associated with Markers of Inflammation and Endothelial Dysfunction in U.S. Women[1,2]

Rikard Landberg,[3]* Qi Sun,[4] Eric B. Rimm,[4–6], Aedin Cassidy,[7] Augustin Scalbert,[8] Christos S. Mantzoros,[9] Frank B. Hu,[4–6] and Rob M. van Dam[4,10]

[3]Department of Food Science, Swedish University of Agricultural Sciences, SE-75007 Uppsala, Sweden; [4]Department of Nutrition, Harvard School of Public Health, Boston, MA 02115; [5]Channing Laboratory, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA 02115; [6]Department of Epidemiology, Harvard School of Public Health, Boston, MA 02115; [7]School of Medicine, University of East Anglia, Norwich NR4 7TJ, UK; [8]INRA, UMR 1019, Unité de Nutrition Humaine, Centre de Recherche de Clermont-Ferrand/Theix, 63122 Saint-Genes-Champanelle, France; [9]Division of Endocrinology, Diabetes and Metabolism, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, MA 02215; and [10]Departments of Epidemiology and Public Health, and Medicine, Yong Loo Lin School of Medicine, National University of Singapore, Singapore 117597

Abstract

Flavonoids show antiinflammatory effects in vitro and human intervention studies have suggested beneficial effects of flavonoid-rich foods on biomarkers of inflammation and endothelial function. In the present study, we assessed the relationship between flavonoid intake and biomarkers of inflammation and endothelial dysfunction in a cross-sectional study of participants from the Nurses' Health Study cohort. Intake of 6 flavonoid subclasses (flavonols, flavones, flavanones, flavan-3-ols, anthocyanidins, and polymeric flavonoids) was assessed using a FFQ administered in 1990. Also, the main food sources of these flavonoids were examined. Blood samples were collected in 1989–1990 and plasma C-reactive protein (CRP), IL-6, IL-18, soluble tumor necrosis factor receptor-2 (sTNF-R2), soluble intercellular adhesion molecule-1, soluble vascular adhesion molecule-1 (sVCAM-1), and E-selectin were measured in 1194–1598 women. The multivariate-adjusted geometric mean of plasma IL-8 were lower for women in the highest intake quintile of flavones, flavanones, and total flavonoids compared with those in the lowest quintiles by 9% (Q1: 264 ng/L, Q5: 241 ng/L; *P*-trend = 0.019), 11% (Q1: 273 ng/L, Q5: 244 ng/L; *P*-trend = 0.011), and 8% (Q1: 276 ng/L, Q5: 55 ng/L; *P*-trend = 0.034), respectively. The multivariate-adjusted geometric mean for women in the highest intake quintile of flavonol compared with those in the lowest quintile was 4% lower for sVCAM-1 (Q1: 578 μg/L, Q5: 557 μg/L; *P*-trend = 0.012). Among flavonoid-rich foods, higher intake of grapefruit was significantly associated with lower concentrations of CRP and sTNF-R2. In summary, higher intakes of selected flavonoid subclasses were associated with modestly lower concentrations of inflammatory biomarkers. In particular, flavonoids typically found in citrus fruits were modestly associated with lower plasma IL-18 concentrations. J. Nutr. 141: 618–625, 2011.

Introduction

Inflammation is a fundamental biological process that plays an important role in atherosclerosis and thus the development of cardiovascular diseases (CVD)[11] (1–3). Increasing evidence also links inflammation and endothelial dysfunction with the group of alterations constituting metabolic syndrome: hyperglycemia, insulin resistance, hyperinsulinemia, hypertension, obesity, and dyslipidemia (4). Proinflammatory cytokines such as TNFα, IL-6, IL-18, and C-reactive protein (CRP), a biomarker of low-grade inflammation, have been associated with an increased risk of type 2 diabetes (5,6). TNFα, IL-6, and CRP also stimulate endothelial production of adhesion molecules, such as E-selectin and inter-cellular adhesion molecule 1 (ICAM-1), mediators of endothelial dysfunction in capillary and arteriolar endothelium (7).

Flavonoids represent a large and diverse group of secondary plant metabolites commonly found in various fruits, vegetables, legumes, dark chocolate, and beverages such as tea and wine (8–10). According to structural features, flavonoids can be divided into subgroups: flavonols, flavones, flavanones, anthocyanidins, isoflavones, monomeric flavan-3-ols, and polymeric flavan-3-ols

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[11] Abbreviations used: CRP, C-reactive protein; CVD, cardiovascular disease; MET, metabolic equivalent; NHS, Nurses' Health Study; sICAM-1, soluble intercellular adhesion molecule 1; sTNF-R2, soluble tumor necrosis factor receptor-2; sVCAM-1, soluble vascular cell adhesion molecule 1.

* To whom correspondence should be addressed. E-mail: rikard.landberg@lmv.slu.se.

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(i.e. proanthocyanidins, thearubigins, and theaflavins). Many of the flavonoids show antiinflammatory effects in vitro and in animal models (11–13). Due to the structural differences, bioactivity and bioavailability vary substantially between and also within the subclasses (14,15). Several short-term intervention studies have shown favorable effects on biomarkers of inflammation and endothelial function by intake of flavonoids or flavonoid-rich foods (16–19), whereas other studies have shown no effect (20–24). To date, only a limited number of observational studies have investigated the association between the extensive range of flavonoids or flavonoid-rich foods and biomarkers of inflammation and endothelial dysfunction (25–28). However, none of the previous studies used the latest flavonoid database from 2007 (9) and typically only 1 or 2 biomarkers of inflammation were investigated. Assessment of several different biomarkers of inflammation and endothelial function that partly reflect different mechanistic pathways may facilitate a more comprehensive understanding of the putative effects of flavonoids in relation to chronic diseases.

In the present study, using a recently updated database, we examined the association between intake of total flavonoids and 6 flavonoid subclasses as well as flavonoid-rich food sources in relation to plasma biomarkers of inflammation and endothelial dysfunction in a large number of apparently healthy women in the U.S. Nurses' Health Study (NHS).

Participants and Methods

Participants. The NHS was established in 1976 and included 121,700 female nurses residing in the United States (29). Every 2 y, participants have been mailed a follow-up questionnaire regarding potential risk factors to identify newly diagnosed cases of chronic diseases. FFQ have been mailed to participants every 2–4 y. The present study included a total number of 2115 women aged 43–70 y who were selected as controls for 2 nested case-control studies on type 2 diabetes and myocardial infarction (5,30). Participants had not been diagnosed with CVD, cancer, or type 2 diabetes mellitus at the time of the blood collection in 1989–1990. The Harvard School of Public Health and Brigham and Women's Hospital Human Subjects Committee Review Board approved the study protocol.

Blood collection and assessment of markers. Blood was collected in1989–1990 from 32826 women. Women willing to provide blood specimens were sent instructions and a phlebotomy kit by mail and blood specimens were returned on ice by overnight mail. When arriving at the laboratory, samples were centrifuged at 1200 × *g* for 15 min to separate plasma, buffy coat, and erythrocytes, and all parts were immediately frozen in liquid nitrogen at a temperature no higher than −130°C and kept until analysis. Ninety-seven percent of samples arrived within 26 h of phlebotomy. Quality control samples were routinely frozen along with study samples to monitor changes due to long-term storage and assay variability.

Biomarkers of inflammation and endothelial dysfunction were analyzed in the Clinical Chemistry Laboratory at Children's Hospital in Boston, except for IL-18, which was analyzed at the Division of Endocrinology, Diabetes and Metabolism, Department of Medicine, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston. The numbers for the different biomarkers vary somewhat, because measurements were conducted in different years with the size of the nested case-control studies increasing over time [CRP, $n = 1598$; IL-6, $n = 1158$; IL-18, $n = 1087$; soluble tumor necrosis factor receptor 2 (sTNF-R2), $n = 1194$; soluble intercellular adhesion molecule-1 (sICAM), $n = 1199$; soluble vascular adhesion molecule-1 (sVCAM), $n = 1199$; E-selectin, $n = 1199$]. The concentrations of plasma sICAM-1, sVCAM-1, E-selectin, IL-6, and sTNF-R2 were measured by commercial ELISA (R&D Systems). Plasma high-sensitive CRP concentrations were measured by a latex-enhanced turbidimetric assay on a Hitachi 911 (Denka Seiken). The plasma IL-18 concentration was measured by an ELISA (MBL). The inter-assay CV were <10% for all markers. It has been demonstrated that processing time did not substantially affect concentration of investigated markers (31).

Assessment of flavonoid intakes. In 1990, a semiquantitative FFQ containing 131 food items was mailed to the participants. A standard portion size was given for each food item and cohort members were asked to choose 1 from 9 possible frequency responses ranging from "never" to "6 times/d" for each food. The relative validity and reproducibility of the FFQ have been previously reported and the correlation between flavonoid-rich foods such as grapefruits, oranges, apple, and tea intakes measured by diet records and FFQ were 0.76, 0.84, 0.70, and 0.77, respectively (32).

A database for assessment of intake of total flavonoids and 6 subclasses commonly consumed in the U.S. diet was constructed and included in the Harvard nutrient database as described by Cassidy et al. (33). Briefly, data from the latest USDA databases on flavonoids and proanthocyanidins (9,34) were extended with information from a European database (35) and peer-reviewed literature. Intakes of individual flavonoids 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 6 main subclasses commonly consumed in the U.S. diet. These were flavonols (quercetin, kampferol, myricetin, isohamnetin), flavones (luteolin, apigenin), flavanones (eriodicytol, hesperetin, naringenin), anthocyanidins (cyanidin, delphinidin, malvidin, pelargonidin, petunidin, peonidin), monomeric flavan-3-ols (catechins, epicatechins), and polymeric flavan-3-ols (including oligomeric and polymeric proanthocyanidins, theaflavins, and thearubigins). Total flavonoid intake was calculated as the sum of the 6 subclasses and expressed in mg/d. Flavonoid intakes were adjusted for total energy intake by the residual method (36). We also examined the association of flavonoid-rich foods, including tea, onions, apples, berries (strawberry + blueberry), oranges (including juice), grapefruit (including juice), and total citrus (oranges + grapefruit including juices) with the biomarkers. Fresh and frozen strawberries and blueberries represented 95% of the per capita availability of berries in the US in 1990 (37). Intakes of foods were expressed as servings, equivalent to 237 g (coffee and tea), 186 g (juice), 110 g (onions), 138 g (apples), 37 g (berries), 131 g (oranges), and 65.5 g (grapefruit).

Assessment of other variables. Body weight was self-reported in 1990. BMI was calculated as weight (kg) divided by squared height (m^2). Physical activity was assessed in hours per week spent on common leisure-time physical activities expressed as metabolic equivalent (MET) h/wk in 1988 (38). Mean daily alcohol intake and total coffee intake (sum of caffeinated and decaffeinated coffee) were assessed in the 1990 FFQ. Aspirin intake was assessed from 1988 and hormone therapy and menopausal status were assessed in 1990.

Statistical analysis. We calculated Spearman's rank correlation coefficients for flavonoid intakes and biomarkers. Plasma biomarker variables were log-transformed to better approximate a normal distribution. ANCOVA was used to calculate age-adjusted (5-y categories) and multivariable-adjusted geometric least square means and their 95% CI of biomarkers for quintiles of flavonoid intakes. Foods were categorized a priori into 4–5 groups with cutoff values chosen to obtain wide intake ranges including at least 4% of the intake distribution in each category (total citrus fruits including juices: <1/mo, 1/mo to 1/wk, 1/wk to 1/d, 1/d to 2/d, ≥2/d; grapefruit including juice: <1/mo, 1/mo to 1/wk, 1/wk to 1/d, ≥1/d; apples: same categories as for grapefruit; onions: same categories as for grapefruit; tea: <1/mo, 1/mo to 1/wk, 1/wk to 2/d, ≥2/d). Because biomarkers were analyzed at different occasions, all models were adjusted for batch. In multivariable models, means were adjusted for age (5-y categories), BMI (entered as a continuous variable), smoking status (never smoked, past smoker, and current smoker of 1–14 and ≥15 cigarettes/d), alcohol consumption (nondrinker and 0–4.9, 5.0–9.9, 10–14.9, and ≥15 g/d), energy intake, current aspirin use (yes/no), and menopausal hormone therapy (premenopausal and never, past, and current user), physical activity

(quintiles of MET h/wk). We also conducted sensitivity analysis by further adjusting models for nutrients and foods that had previously been associated with inflammation and endothelial dysfunction, including vitamin C, *trans*-fatty acids, total (n-3) fatty acids, and cereal fiber. Age-adjusted and multivariable models where flavonoids were entered as continuous variables were also tested. *P*-values for trend were calculated by entering the median value of each category of the dietary exposure variable into the model as a continuous variable. All analyses were conducted using SAS version 9 (SAS Institute).

Results

We observed a ~5-fold difference in total flavonoid intake between the highest and lowest quintiles of the study population (median: 801 mg/d in the highest quintile vs. 107 mg/d in the lowest) (**Table 1**). Women in the higher intake categories were less likely to smoke and to use aspirin. They also drank less alcohol and coffee and more tea compared with those in the lower quintiles. The median intake for flavonoid subclasses ranged from 2 to 141 mg/d and the main contributing foods were tea, apples, and citrus fruits (including juices) (**Table 2**). Significant Spearman correlation coefficients in the range of 0.15–0.95 were observed between flavonoid subclasses and flavonoid-rich foods such as tea, onion, apples, berries (strawberry + blueberry), oranges (including juice), grapefruit (including juice), and total citrus (oranges + grapefruit including juices).

We calculated partial Spearman correlation coefficients between flavonoid subclasses and biomarkers of inflammation and endothelial dysfunction and found that several flavonoid classes were inversely correlated with plasma IL-18, CRP, sVCAM-1, sICAM-1, and sTNF-R2 concentrations in age-adjusted models (data not shown). After multivariable adjustment, the correlation between Il-18 and flavones (−0.069; P = 0.033), anthocyanidins (−0.079; P = 0.014) ,and total flavonoids (−0.075; P = 0.020) remained significant as did the correlation between flavonols and sVCAM-1 (−0.078; P = 0.012) (**Table 3**). A borderline significant correlation was observed between intake of flavanones and sTNF-R2 (−0.061; P = 0.05) (Table 3).

To avoid the potential impact of extreme intakes and to evaluate whether associations were monotonous, we further investigated significant correlations in an analysis of biomarker concentrations by quintiles of flavonoid intakes. After multivariable adjustment, higher intake of total flavonoids was associated with lower concentrations of IL-18 (Q1: 276 ng/L, Q5: 255 ng/L; *P*-trend = 0.034). The flavonoid classes flavones (Q1: 264 ng/L, Q5: 241 ng/L; *P*-trend = 0.019) and flavanones (Q1: 273 ng/L, Q5: 244 ng/L; *P*-trend = 0.011) were also associated with lower IL-18 concentrations (**Table 4**). In addition, higher intakes of flavonols were associated with lower sVCAM-1 concentrations (Q1: 578, Q5: 557 μg/L; *P*-trend = 0.008)

TABLE 1 Characteristics in 1990 according to quintiles of total flavonoid intake in the NHS[1]

	Total flavonoids[2]				
	Q1, *n* = 403	Q2, *n* = 402	Q3, *n* = 402	Q4, *n* = 403	Q5, *n* = 402
Median [IQR],[3] *mg/d*	107 [47]	174 [31.7]	243 [44.8]	375 [88.9]	801 [455.3]
Age, *y*	57 ± 7	58 ± 7	58 ± 7	58 ± 7	57 ± 7
BMI, *kg/m²*	25.9 ± 5.5	25.1 ± 4.4	25.2 ± 4.5	25.4 ± 4.6	25.2 ± 4.4
Current smoker, *%*	28	18	14	10	15
Physical activity, *MET h/wk*	15.2 ± 15.9	18.6 ± 18.8	19.1 ± 17.2	18.8 ± 21.6	16.3 ± 16.0
Postmenopasual hormone use, *%*	31	39	36	32	33
Hypertension, *%*	15	18	19	18	17
Hypercholesterolemia, *%*	29	31	34	32	26
Total energy intake, *MJ/d*	7.2 ± 2.3	7.7 ± 2.2	7.5 ± 2.0	7.5 ± 1.9	7.1 ± 2.1
Alcohol consumption, *g/d*	7.0 ± 11.4	6.2 ± 9.8	4.9 ± 8.5	5.1 ± 9.3	5.5 ± 10.2
Aspirin use, *%*	61	66	66	64	58
Glycemic load	99 ± 21	104 ± 20	109 ± 19	108 ± 20	108 ± 19
Vitamin C intake, *mg/d*	252 ± 355	326 ± 366	344 ± 376	328 ± 359	318 ± 377
Total *trans* fat, *% of energy*	1.6 ± 0.6	1.4 ± 0.5	1.4 ± 0.6	1.4 ± 0.5	1.5 ± 0.6
Total (n-3) fat,[4] *% of energy*	0.7 ± 0.3	0.7 ± 0.2	0.7 ± 0.3	0.7 ± 0.2	0.7 ± 0.2
Cereal fiber intake, *g/d*	5.1 ± 3.8	5.3 ± 3.1	5.6 ± 3.0	5.2 ± 2.8	5.2 ± 3.3
Whole grain, *g/d*	18.8 ± 20.4	20.4 ± 15.5	23.3 ± 16.9	20.5 ± 15.1	19.8 ± 17.3
Coffee,[5] *servings/d*	2.8 ± 2.0	2.7 ± 1.8	2.4 ± 1.7	2.1 ± 1.6	1.6 ± 1.6
Tea, *servings/d*	0.1 ± 0.4	0.1 ± 0.1	0.2 ± 0.2	0.7 ± 0.4	2.4 ± 1.4
Onions, *servings/wk*	3.1 ± 6.1	3.1 ± 3.2	3.0 ± 3.0	3.3 ± 3.6	2.8 ± 3.2
Apples,[6] *servings/wk*	1.0 ± 6.1	2.0 ± 1.6	3.1 ± 2.3	2.8 ± 2.6	3.1 ± 4.1
Berries, *servings/wk*	0.6 ± 1.1	1.0 ± 1.0	1.3 ± 1.3	1.3 ± 1.4	1.5 ± 2.7
Oranges,[7] *servings/wk*	2.5 ± 4.5	4.4 ± 3.6	5.2 ± 4.2	5.1 ± 5.3	4.4 ± 4.7
Grapefruit,[7] *servings/wk*	0.9 ± 1.9	1.3 ± 2.0	1.5 ± 2.4	1.6 ± 2.5	1.3 ± 2.5

[1] Values are means ± SD. One serving corresponds to 237 g (coffee and tea), 110 g (onions), 138 g (apples), 37 g (berries), 131 g (oranges), 65.5 g (grapefruit), and 186 g (orange/grapefruit juice).
[2] Total U.S. flavonoids.
[3] [75th–25th percentile].
[4] 18:3(n-3) + 20:5(n-3) + 22:6(n-3).
[5] 1 serving = 237 g (coffee and tea), 110 g (9 g/slice, onions), 138 g (apples), 37 g (berries), 131 g (oranges), and 65.5 g (grapefruit).
[6] Apples and pears were assessed by the same question in the FFQ. According to per capita consumption, apples accounted for 88% of apples and pears availability in the US in 1990 (37). Reported values were therefore multiplied by 0.88.
[7] Sum of juice and fruit.

TABLE 2 Estimated intake of total flavonoids and subclasses and their main contributing foods in the NHS[1]

Flavonoid subclass	Median intake, *mg/d*	Main contributing foods[2]
Total flavonoids	243	Tea (53%), apples (14%), citrus fruits[3] (12%)
Polymeric flavan-3-ols[4]	141	Tea (61%) and apples (18%)
Flavanones	31	Oranges[3] (81%), grapefruit[3] (18%)
Monomeric flavan-3-ols	24	Tea (78%) and apples (6%)
Flavonols	15	Tea (33%) and apples (10%)
Anthocyanidins	10	Berries (62%)
Flavones	2	Oranges[3] (68%)

[1] Intakes are derived from 1990 (n = 2012).
[2] Based on the entire cohort (n = 121,700).
[3] Including juice.
[4] Include oligomeric and polymeric proanthocyanidins, theaflavins, and thearubigins.

(**Table 5**). After further adjustment for quintiles of (n-3) fatty acids, *trans*-fatty acids, vitamin C, and cereal fiber, the associations remained virtually the same (data not shown).

We also investigated the associations with biomarkers of the main food sources for the flavonoid subclasses for which we observed significant inverse associations. The examined foods were tea, onions, apples, berries (strawberries and blueberries), grapefruit (including juice), oranges (including juice), and total citrus (sum of grapefruit, oranges, and juices). Plasma concentrations of IL-18 tended to be lower with higher total citrus intakes (284 ng/L for <1/mo vs. 231 ng/L for ≥2/d; P-trend = 0.007) (**Fig. 1***A*). This trend was mainly attributed to orange juice and not to orange or grapefruit intake (Fig. 1*B*), for which the range of intake was smaller (data not shown). However, grapefruit (including juice) intake (Fig. 1*C,D*), but not total citrus intake (Fig. 1*E,F*), was significantly associated with lower CRP and TNF-R2 concentrations. None of the other tested foods were substantially associated with differences in biomarkers (data not shown). The results did not substantially change after adjustment for quintiles of (n-3) fatty acids, *trans*-fatty acids, vitamin C, and cereal fiber (data not shown).

Discussion

Over the range of dietary intakes in this population, we found that total flavonoids, flavanones, and flavones were inversely associated with IL-18 concentrations and that flavonols were inversely associated with sVCAM concentrations. The associated biomarker changes were in the range of 4–11% (Q5 vs. Q1) and were all independent of a range of other evaluated lifestyle and dietary determinants of inflammation and endothelial dysfunction. In addition, intake of citrus fruits, which are rich sources of flavones and flavanones, was associated with a 20% lower IL-18 concentration (≥2 servings/d vs. <1 serving/mo) and intake of grapefruit was associated with a 21% lower CRP and 8% lower TNF-R2 concentration (≥1 servings/d vs. <1 serving/mo).

Inflammation and endothelial dysfunction are recognized as important contributors to the development of atherosclerosis (1,39,40). CRP is a marker of low-grade systemic inflammation and was an independent predictor of CVD in several studies (41,42). IL-6 and TNFα are both proinflammatory cytokines, which induce hepatic production of CRP and other acute phase proteins (43). We examined a soluble TNFα receptor, which is activated by TNFα and other cytokines (44). TNFα receptor is a more stable marker of TNFα levels (30) and has been related to obesity and coronary heart disease (45).

Recently, IL-18, a proinflammatory cytokine, was shown to independently predict type 2 diabetes incidence (46). A 10% change in IL-18, similar to the associated change of citrus fruit flavonoids observed in the present study, was associated with an ~3% lower risk of type 2 diabetes in that study (46). The exact role of IL-18 in diabetes etiology is unclear, but it amplifies the inflammatory cascade by inducing expression of other proin-

TABLE 3 Partial Spearman rank correlation coefficients between intake of flavonoid classes and biomarkers of inflammation and endothelial dysfunction in the NHS[1–3]

Biomarker	Flavonols	Flavones	Flavanones	Anthocyanidins	Monomeric flavan-3-ols	Polymeric flavan-3-ols	Total flavonoids
IL-6	0.003	−0.009	−0.018	−0.011	−0.012	−0.015	−0.014
IL-18	−0.057	−0.069*	−0.061*	−0.079*	−0.057	−0.060	−0.075*
CRP	0.015	−0.030	−0.039	−0.025	0.044	0.043	0.030
sVCAM-1	−0.078*	−0.009	−0.005	0.035	−0.030	−0.013	−0.027
sICAM-1	−0.033	−0.024	−0.049	−0.020	−0.012	−0.0003	−0.018
sTNF-R2	−0.047	−0.031	−0.061	0.011	0.033	0.041	0.027
E-selectin	−0.016	0.017	−0.005	0.0003	0.009	0.008	0.006

[1] Intakes are derived from 1990.
[2] Model adjusted for age (<50 y; 50 to <55 y; 55 to <60 y; 60 to <65 y; ≥65 y), biomarker assay batch (1–5 batches), BMI (continuous), physical activity (MET h/wk), smoking status (never; past; current, <15 cigarettes/d; current ≥15 cigarettes/d), alcohol consumption (nondrinker; >0 to <5, 5 to <15, ≥15 g/d), current aspirin use (yes/no), postmenopausal hormone therapy (premenopausal and never, past, current user), energy intake (continuous), coffee intake (<1 cup/mo, 1 cup/mo to 4 cups/wk, 5–7 cups/wk, ≥2 cups/d).
[3] Where n = 1040 (IL-6), 981 (IL-18), 1419 (CRP), 1074 (sVCAM-1), 1074 (sICAM-1), 1069 (sTNF-R2), and 1079 (E-selectin). *$P < 0.05$.

TABLE 4 Adjusted geometric mean (and 95% CI) plasma concentrations of IL-18 (ng/L) by categories of flavonoid classes in the NHS[1]

	Energy adjusted intake (quintiles)					
	Q1	Q2	Q3	Q4	Q5	*P*-trend[2]
Flavonols, *mg/d*						
Median	7.4	11.0	14.8	20.7	35.4	
Age-adjusted[3]	282 (267, 299)	270 (255, 285)	260 (246, 275)	265 (250, 281)	264, (249, 279)	0.18
Multivariate-adjusted[4]	284 (267, 301)	270 (254, 286)	258 (244, 273)	267 (252, 283)	259, (244, 274)	0.09
Flavones, *mg/d*						
Median	0.53	1.0	1.5	2.2	3.2	
Age-adjusted	269 (255, 284)	288 (272, 306)	271 (256, 287)	272 (256, 288)	241, (227, 256)	0.002
Multivariate-adjusted	264 (250, 280)	284 (267, 301)	270 (255, 285)	278 (262, 295)	241, (226, 256)	0.019
Flavanones, *mg/d*						
Median	5.0	15.6	31.4	49.2	79.2	
Age-adjusted	279 (264, 294)	277 (262, 294)	268 (253, 284)	273 (257, 289)	243, (229, 258)	0.001
Multivariate-adjusted	273 (258, 289)	275 (259, 291)	270 (255, 286)	273 (257, 290)	244, (230, 260)	0.011
Anthocyanidin, *mg/d*						
Median	2.8	6.0	10.0	15.3	24.6	
Age-adjusted	275 (260, 292)	274 (259, 290)	272 (257, 288)	251 (237, 266)	268, (253, 285)	0.22
Multivariate-adjusted	273 (257, 290)	277 (261, 293)	273 (257, 289)	249 (235, 264)	265, (249, 281)	0.14
Monomeric flavan-3-ols, *mg/d*						
Median	7.0	14.3	23.6	55.9	153	
Age-adjusted	283 (267, 299)	264 (250, 280)	264 (248, 280)	266 (252, 282)	264, (249, 279)	0.34
Multivariate-adjusted	279 (262, 296)	266 (251, 281)	267 (251, 284)	263 (248, 278)	262, (247, 278)	0.29
Polymeric flavan-3-ols, *mg/d*						
Median	48.5	91.9	141	235	557	
Age-adjusted	272 (258, 288)	274 (258, 290)	269 (254, 285)	263 (248, 278)	263, (248, 278)	0.25
Multivariate-adjusted	271 (255, 288)	277 (260, 294)	266 (251, 282)	262 (247, 277)	261, (246, 276)	0.21
Total flavonoids, *mg/d*						
Median	106.5	174.1	242.7	374.9	801.0	
Age-adjusted	281 (266, 297)	274 (258, 291)	262 (247, 277)	266 (251, 282)	258, (244, 273)	0.042
Multivariate-adjusted	276 (260, 293)	279 (263, 296)	262 (247, 278)	264 (249, 280)	255, (241, 270)	0.034

[1] Intakes are derived from 1990.
[2] *P*-value from multiple linear regression models where log IL-18 concentration was modeled on medians of intake quintiles entered as a continuous variable.
[3] Model adjusted for age (<50 y; 50 to <55 y; 55 to <60 y; 60 to <65 y; ≥65 y), energy intake.
[4] Model adjusted for age (<50 y; 50 to <55 y; 55 to <60 y; 60 to <65 y; ≥65 y), biomarker assay batch (1–5 batches), BMI (continuous), physical activity (MET h/wk), smoking status (never; past; current, <15 cigarettes/d; current, ≥15 cigarettes/d), alcohol consumption (nondrinker; >0 to <5, 5 to <15, ≥15 g/d), current aspirin use (yes/no), postmenopausal hormone therapy (premenopausal and never, past, current user), energy intake (continuous), coffee intake (<1 cup/mo, 1 cup/mo to 4 cups/wk, 5–7 cups/wk, ≥2 cups/d).

flammatory mediators and is induced by redox-balance and TNFα through the NF-κB activation (47). To our knowledge, no previous study has investigated associated effects of flavonoid intake in relation to IL-18.

Cell adhesion molecules, including E-selectin, sICAM-1, and sVCAM-1, are markers of endothelial dysfunction, which may be a common pathogenic precursor of CVD and type 2 diabetes (5,48). E-selectin is a surface leukocyte adhesion molecule,

TABLE 5 Adjusted geometric mean (and 95% CI) plasma concentrations of sVCAM-1 (μg/L) by categories of intakes of flavonols in the NHS[1]

	Energy adjusted intake (quintiles)					
	Q1	Q2	Q3	Q4	Q5	*P*-trend[2]
Flavonols, *mg/d*						
Median	7.4	11.0	14.8	20.7	35.4	
Age-adjusted[3]	578 (561, 595)	591 (572, 609)	573 (555, 592)	561 (545–578)	560 (543–578)	0.027
Multivariate-adjusted[4]	578 (560, 596)	594 (575, 613)	577 (558, 596)	562 (545–579)	557 (539–575)	0.012

[1] Intakes are derived from 1990.
[2] *P*-value from multiple linear regression models where log sVCAM-1 concentration was modeled on medians of flavonol quintiles entered as a continuous variable.
[3] Model adjusted for age (<50 y; 50 to <55 y; 55 to <60 y; 60 to <65 y; ≥65 y), energy intake, and biomarker assay batch (3 batches).
[4] Model adjusted for age (<50 y; 50 to <55 y; 55 to <60 y; 60 to <65 y; ≥65 y), biomarker assay batch (1–5 batches), BMI (continuous), physical activity (MET h/wk), smoking status (never; past; current, <15 cigarettes/d; current, ≥15 cigarettes/d), alcohol consumption (nondrinker; >0 to <5, 5 to <15, ≥15 g/d), current aspirin use (yes/no), postmenopausal hormone therapy (premenopausal and never, past, current user), energy intake (continuous), coffee intake (<1 cup/mo, 1 cup/mo to 4 cups/wk, 5–7 cups/wk, ≥2 cups/d).

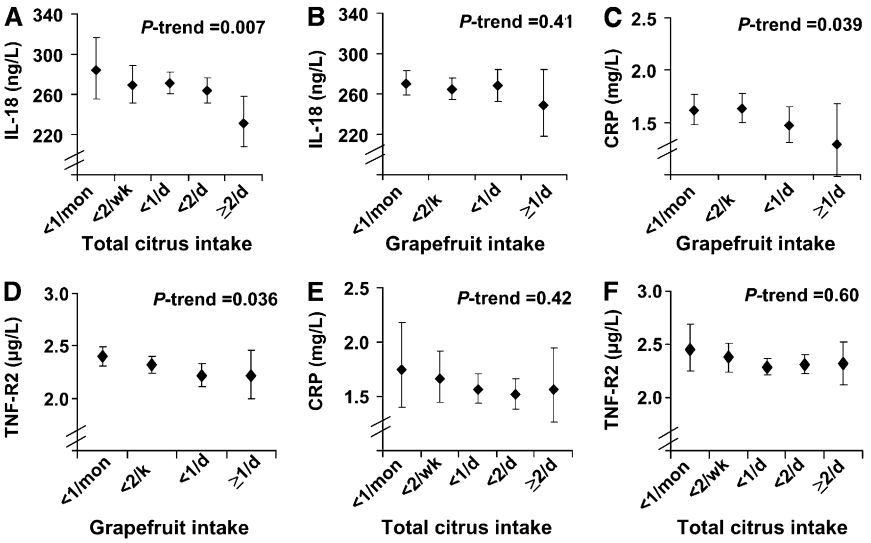

FIGURE 1 Multivariable-adjusted geometric means (95% CI) of plasma concentrations of the inflammation biomarkers IL-18 (*A,B*), CRP (*C,E*), and TNF-R2 (*D,F*) by serving categories of total citrus (oranges + grapefruit + juices) (*A,E,F*) and grapefruit (fruit + juice) (*B,C,D*) in the NHS (n = 1087–1598). Models were adjusted for the same covariates as described in Table 3. One serving corresponds to 131 g (oranges), 65.5 g (grapefruit), and 186 g (juices).

whereas sICAM-1 is a soluble leukocyte adhesion molecule. They are both expressed as a result of inflammatory stimuli of the endothelium (3). sVCAM-1 is mainly expressed in atherosclerotic plaques and has been suggested to be a marker of advanced atherosclerosis (49). Our finding of lower concentrations of IL-18 and sVCAM-1 with higher intakes of specific flavonoids and lower CRP and TNF-R2 with intake of citrus fruits may therefore have implications for the development of CVD and type 2 diabetes.

Although several epidemiological studies and clinical intervention studies have suggested beneficial effects of flavonoid-rich foods for inflammation and endothelial dysfunction biomarkers (18,26,50–53), few studies have directly investigated flavonoid intakes and none, to our knowledge, using the updated USDA database from 2007 (9). In the studies conducted to date, typically only a limited number of flavonoid subclasses or individual compounds have been examined. In a small cross-sectional analysis in 344 apparently healthy U.S. women, no association between intakes of total flavonols and flavones and plasma CRP and IL-6 concentrations was observed. In contrast, a large cross-sectional study including 8335 U.S. adults (25) found significant inverse associations between intakes of flavonols, anthocyanidins, isoflavones, and total flavonoids and lower CRP concentrations. The inconsistency of the results may be attributed to the small sample size in the first study, giving low statistical power to detect any associations, or it may be due to differences in calculated flavonoid intakes.

Our observation that intakes of the citrus fruit flavonoids were inversely associated with plasma IL-18 is supported by results from several laboratory and animal studies showing effects on biomarkers of inflammation and endothelial dysfunction (54–57). Moreover, a human intervention study showed significantly lower CRP concentrations after consumption of juice containing oranges and black currents for 28 d compared with a control beverage (16). In a very recent study, inflammation induced by a high-fat, high-carbohydrate meal was significantly reduced in participants consuming orange juice compared with a glucose control drink (58). In the present study, we also observed that higher total citrus fruit consumption was associated with lower IL-18 concentrations and that grapefruit consumption was associated with lower CRP and TNF-R2 concentrations. A cross-sectional study in children reported a borderline significant inverse association between intakes of a combination of citrus fruits and melons and CRP concentration (27), supporting our observations. However, Chun et al. (25) observed no relationship between citrus fruits, citrus fruit juice intakes, intake of flavones, or flavanones and CRP concentrations in adults. The difference in results may be explained by the fact that participants in the study by Chun et al. (59) were younger, that different dietary assessment methods were used, or the intakes of flavanones and flavones were higher in the present study. The median daily intake of flavanones in the present study was, e.g., twice as high as the mean intake in the study by Chun et al. (59).

We observed an inverse association between flavonol intake and plasma sVCAM-1, but no associations were found between intakes of tea, onions, or apples, 3 major food sources of flavonols, and sVCAM-1. This may be due to the rather small variation in intake of these foods and it may also be due to some misclassification of apple intake, because apples and pears were assessed by the same question in the FFQ, although the per capita availability of apples accounted for 88% of the total availability of apples and pears in the US in 1990 (37). To our knowledge, no previous observational study has investigated the association between flavonol and cellular adhesion molecules.

Flavonoid compounds are structurally diverse and therefore specific structural characteristics may be responsible for different bioactivities. Mounting evidence supports that mechanisms other than the classical hydrogen-donating antioxidative activity per se may be responsible for putative antiinflammatory effects of specific flavonoids (60). Several flavonoids have, e.g., been shown to inhibit the expression of NF-κB, an important regulator of a number of cytokines and cell adhesion molecules. NF-κB regulates the activity of the enzyme transforming inactive pro-IL-18 to active IL-18, and hence citrus flavonoids may in that way indirectly inhibit the formation of active IL-18, as was recently suggested for (n-3) fatty acids (61).

Our study has several limitations. First, because of the cross-sectional design, we cannot infer the causality from our results. Second, despite extensive adjustments for potential confounding factors, we cannot rule out that part of the observed associations might be due to a healthy lifestyle or due to other bioactive compounds present in flavonoid-rich foods. Third, some measurement errors are likely due to imperfect measurement of food intake by FFQ, food composition data, and biomarker measurements. For example, it was not possible to differentiate dark

chocolate, which is a rich source of monomeric and polymeric flavan-3-ols, from lighter chocolate. Fourth, we evaluated many associations, which increases the likelihood of chance findings and our findings require confirmation in independent studies. The present study also has several strengths, including the use of a validated FFQ, which reflect long-term food intake. We also used an updated flavonoid database containing a large number of foods that are representative for an American population for the assessment of 6 flavonoid subclasses. Moreover, 7 different biomarkers were measured in a large number of individuals, which allowed us to comprehensively assess the role of flavonoids associated with potentially different aspects of inflammation and endothelial function. The analyzed biomarkers have been proven stable throughout collection and laboratory processing (31). Because all biomarkers except IL-18 were analyzed at different occasions over the period of 1994–2004, we adjusted for batch to take into account potential differences in losses over time and laboratory drift. Furthermore, biomarkers used in this study have been shown to be stable within individuals over long time periods (1–12 y depending on biomarker) showing that a single sample will represent individuals' long-term concentrations with acceptable precision (62–64).

In summary, this study suggests that higher intakes of selected subclasses of flavonoids are associated with modestly lower plasma concentrations of inflammatory biomarkers. Particularly, flavonoids from citrus fruits appear to be consistently associated with modestly lower concentrations of IL-18.

Acknowledgments

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