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Research Article

Cancer Prevention Research

Interleukin-6 as a Potential Indicator for Prevention of High-Risk Adenoma Recurrence by Dietary Flavonols in the Polyp Prevention Trial

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Abstract

Serum interleukin-6 (IL-6), a proinflammatory cytokine, is considered an indicator of inflammation and may be an indicator of colorectal carcinogenesis given that inflammation can promote carcinogenesis. Flavonols, which can be found in fruits and vegetables, may inhibit colorectal carcinogenesis partly by inhibiting inflammation. We estimated odds ratios and 95% confidence intervals (95% CI) to determine whether serum IL-6 was associated with colorectal adenoma recurrence and flavonol intake and thus may serve as a risk indicator and as a response indicator to dietary flavonols. Serum IL-6 concentrations at baseline, year 1, and year 3 were measured in 872 participants from the intervention arm of the Polyp Prevention Trial, a 4-year trial that examined the effectiveness of a low-fat, high-fiber, high-fruit and vegetable diet on adenoma recurrence. Intake of flavonols, especially of isorhamnetin, kaempferol, and quercetin, was inversely associated with serum IL-6 concentrations (highest versus lowest flavonol intake quartile, 1.80 versus 2.20 pg/mL) and high-risk (OR, 0.51; 95% CI, 0.26-0.98) and advanced adenoma recurrence (OR, 0.17; 95% CI, 0.06-0.50). A decrease in IL-6 concentration during the trial was inversely associated with high-risk (OR, 0.44; 95% CI, 0.23-0.84) and advanced adenoma recurrence (OR, 0.47; 95% CI, 0.19-1.18). Individuals with above median flavonol intake and equal or below median IL-6 change after baseline had the lowest risk of recurrence of high-risk and advanced adenoma. Our results suggest that serum IL-6 may serve as a risk indicator and as a response indicator to dietary flavonols for colorectal cancer prevention. *Cancer Prev Res; 3(6); 764–75. ©2010 AACR.*

Introduction

Colorectal cancer is an important public health problem leading to $6.5 billion treatment costs and nearly 50,000 deaths in the United States annually (1, 2). Individuals with high-risk adenomas, defined as three or more adenomas or an advanced adenoma, are at high risk to progress to colorectal cancer (3). Dietary change, both feasible and safe, represents a viable strategy for preventing colorectal cancer; however, dietary intervention trials often showed no protection (4). There is scarcity of information about biomarkers that can predict a cancer-preventive response to dietary interventions.

Flavonols are a group of bioactive polyphenols that are present in fruits, vegetables, and beverages, such as apples, beans, onions, and tea, generally with a carbohydrate moiety attached (5, 6). The median daily flavonol consumption in the United States is approximately 5 to 6 mg/1,000 kcal with a range between 0 and 20 mg/1,000 kcal.[6] The major dietary flavonols, ordered by abundance, are quercetin (70% of total flavonols), kaempferol (16%), myricetin (12%), and isorhamnetin (2%). We previously showed that a flavonol-rich diet decreased the risk of advanced colorectal adenoma recurrence in the Polyp Prevention Trial (PPT; ref. 5). The protective associations were linked to higher flavonol intake (>17 mg/1,000 kcal) and were most pronounced in participants of the intervention arm of the PPT (5).

There are several molecular mechanisms by which flavonols may inhibit colorectal carcinogenesis, including the attenuation of inflammation (7–10). Inflammation is a complex process that involves many proinflammatory as

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well as anti-inflammatory cytokines. One of the key regulators of inflammation and carcinogenesis may be interleukin-6 (IL-6), a proinflammatory cytokine with pleiotropic function that is primarily involved in orchestrating and coordinating the innate and adaptive immune response and may act as a tumor promoter in colorectal neoplasms (11–13). The evidence in humans that high flavonol consumption decreases the concentrations of inflammatory markers in blood, including IL-6, is inconclusive (14, 15). Elevated blood IL-6 concentrations have been associated with colorectal adenoma presence (16) and potentially with progression, but no significant association has generally been observed with earlier stages of colorectal cancer (17, 18).

The aim of this study was to examine whether serum IL-6 concentrations were associated with both flavonol intake and colorectal adenoma recurrence and thus may serve as a risk indicator and as a response indicator to dietary flavonols.

Materials and Methods

Study design and population

The PPT was a 4-year randomized, multicenter, nutritional intervention trial to evaluate whether changing nutrition patterns toward a high-fiber, high-fruit and vegetable, and low-fat diet inhibits colorectal adenoma recurrence. Details of the study have been previously described (19–21). Study participants had at least one histologically confirmed colorectal adenoma identified by complete colonoscopy in the 6 months before study entry. Our study included 872 participants of the intervention arm (958 completed the study by having a final colonoscopy at the end of the 4-year trial) with available dietary data for any of the first 3 years of the trial and serum from baseline (T0) and either from year 1 (T1) or year 3 (T3). The institutional review boards of the National Cancer Institute and each participating center approved the study, and all participants provided written informed consent.

Exposure: lifestyle and dietary data

At baseline and at each of the four annual follow-up visits, participants completed an interviewer-administered questionnaire about demographics, family history, and use of medication or supplements and a self-administered, validated, modified Block-National Cancer Institute food frequency questionnaire (FFQ; ref. 22), which asked about the frequency of intake and portion size of 119 food and beverage items during the past year. Trained, certified nutritionists reviewed all FFQs with participants. Flavonol intake was estimated from questions on 55 food and beverage items using the 2007 U.S. Department of Agriculture (USDA) flavonoid database (23) and was calculated as the sum of isorhamnetin, kaempferol, myricetin, and quercetin. Because some participants had the final colonoscopy before filling out the final FFQ, the FFQ at the end of year 4 was excluded from this analysis. Compared with 24-hour dietary recall and 4-day food record data, the FFQ slightly overestimated fat (by approximately 2 g/d or 3% kcal/d) and underestimated fiber (by approximately 1.5 g/d) and fruit and vegetable intakes (by approximately 1.2 servings/d) and had acceptable correlations of macro- and micro-nutrients (21, 24).

Indicator: serum IL-6

At each annual visit, participants provided a venous blood sample after an overnight fast. All serum samples were stored at −70°C until analysis. Among the 872 participants, 23 and 69 had no available samples at T1 and T3, respectively. Serum IL-6 concentrations at T0, T1, and T3 were measured by the Clinical Support Laboratory of SAIC Frederick, Inc., using a commercially available multiplex 96-well ELISA kit (MS6000 Human ProInflammatory 9-Plex Ultra-Sensitive Kit K11007, Meso Scale Diagnostics) on a Sector Imager 6000 according to the manufacturer's recommendation (Meso Scale Diagnostics). Serum samples from T4 were not measured because some of these were taken after the final colonoscopy. All samples and standards were run in duplicate and the average of the duplicate was taken as value. Standard calibration curves were run on each plate using eight standards supplied by Meso Scale Diagnostics with a linear range of 0.2 to 10,000 pg/mL. None of the samples were below the detection limit. Five serum assay controls from Bioreclamation, Inc., and Gemini Bio-Products that cover a range of IL-6 concentrations were run on each plate with an interassay coefficient of variation between 11.4% and 20.9%.

Outcome: adenoma assessment

Participants had a full colonoscopy at T0, T1, and T4, the latter being used to determine adenoma recurrence. All lesions were examined for histologic features and degree of atypia by two independent pathologists. Adenoma recurrence was defined as advanced adenoma recurrence (≥1 adenoma of ≥1 cm in size, having ≥25% villous component, or exhibiting high-grade dysplasia; n = 49), high-risk adenoma recurrence (≥1 advanced adenoma or ≥3 pathologically confirmed adenomas; n = 100), or any adenoma recurrence (≥1 adenoma; n = 348).

Statistical analyses

Statistical analyses were done using Statistical Analysis Systems, version 9.1 (SAS, Inc.) software. The analysis focused on flavonol intake [baseline: T0; trial: mean(T1,2,3), in mg/d], serum IL-6 concentrations [baseline: T0; trial: geometric mean of T1 and T3 (T1,3); change: T(1,3-0), in pg/mL)], and colorectal adenoma recurrence at T4 (no adenoma versus advanced, high-risk, or any adenoma).

We examined baseline and follow-up measures of dietary exposure, serum indicators, and demographic variables by adenoma recurrence using Fisher's exact test for comparing two proportions, Wilcoxon rank-sum tests for comparing two continuous variables, and Kruskal-Wallis tests for comparing more than two continuous variables. Associations between dietary variables were also estimated using Pearson's correlation coefficients.

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Table 1. Characteristics of intervention group participants in the PPT by adenoma recurrence at the end of year 4 (n = 872)

Characteristics	Adenoma recurrence (T4)						
	No	Advanced		High-risk		Any	
	Median (IQR) or %*	Median (IQR) or %*	$P^{\dagger}$	Median (IQR) or %*	$P^{\dagger}$	Median (IQR) or %*	$P^{\dagger}$
Sample size	524	49		100		348	
Baseline (T0)							
Gender (% male)	64	69	0.53	76	0.02	70	0.05
Race (% Caucasian)	88	86	0.64	90	0.73	90	0.58
Education (% ≤high school)	22	31	0.21	31	0.07	27	0.11
Family history of colorectal cancer (% yes)	27	27	1.00	30	0.46	26	0.88
Smoker (% current)*	11	14	0.48	18	0.07	14	0.21
NSAID use (% yes)[‡]	35	31	0.54	33	0.65	37	0.67
Supplement use (% yes)[‡]	45	37	0.30	38	0.23	43	0.68
Hormone therapy (% yes)[‡]	13	8	0.38	6	0.04	9	0.05
Age (y)	60.0 (52.0-67.0)	66.0 (60.0-71.0)	0.0006	66.0 (58.0-71.0)	<0.0001	64.0 (57.0-70.0)	<0.0001
BMI (kg/m^2)	27.5 (24.8-30.3)	28.6 (26.1-32.4)	0.01	28.3 (25.4-31.1)	0.06	27.6 (25.1-30.6)	0.36
Physical activity (h/wk)[§]	8.50 (4.00-15.1)	7.16 (2.67-12.0)	0.19	8.27 (3.33-13.1)	0.21	8.46 (3.82-16.0)	0.93
Dietary intake							
Alcohol (g/d)	0.92 (0.00-9.78)	0.61 (0.00-2.10)	0.16	0.85 (0.00-8.61)	0.91	0.85 (0.00-10.3)	0.93
Energy (1,000 kcal/d)	1.84 (1.51-2.19)	1.83 (1.51-2.27)	0.91	1.85 (1.57-2.27)	0.40	1.89 (1.55-2.29)	0.10
Fat (% kcal/d)	35.6 (30.1-40.3)	35.8 (31.1-40.5)	0.57	35.4 (30.6-40.2)	0.98	35.6 (30.0-40.7)	0.89
Fat (g/d)	64.0 (49.1-79.8)	67.7 (46.8-81.1)	0.71	67.6 (49.1-81.9)	0.50	67.6 (49.4-83.5)	0.18
Fiber (g/d)	16.8 (12.9-22.9)	16.3 (13.2-23.8)	0.98	17.4 (13.4-23.1)	0.90	17.6 (13.5-22.6)	0.64
Fruits and vegetables (serv./d)	3.44 (2.56-4.67)	3.53 (2.54-4.59)	0.98	3.58 (2.55-4.74)	0.72	3.63 (2.61-4.91)	0.07
Flavonols (mg/d)	14.1 (9.40-20.8)	12.2 (7.83-18.1)	0.11	13.8 (8.65-19.9)	0.51	15.5 (10.1-22.8)	0.05
Isorhamnetin (mg/d)	0.24 (0.10-0.51)	0.19 (0.10-0.51)	0.49	0.22 (0.12-0.53)	0.98	0.25 (0.13-0.60)	0.06
Kaempferol (mg/d)	4.74 (2.54-8.04)	3.99 (1.98-5.19)	0.07	4.15 (1.98-7.26)	0.36	4.91 (2.70-9.38)	0.18
Myricetin (mg/d)	0.83 (0.51-1.44)	0.87 (0.53-1.34)	0.94	0.82 (0.45-1.36)	0.52	0.90 (0.51-1.52)	0.38
Quercetin (mg/d)	7.48 (5.01-11.3)	6.67 (4.46-10.9)	0.33	7.43 (4.49-11.5)	0.67	8.11 (5.40-11.6)	0.11
Dry beans (g/d)	7.54 (3.27-11.3)	4.90 (3.27-11.3)	0.25	7.00 (1.63-11.3)	0.44	7.54 (3.27-14.0)	0.29
Other vegetables (g/d)[‖]	16.8 (3.80-47.0)	12.8 (2.92-45.9)	0.66	16.3 (4.45-48.1)	0.73	21.7 (5.84-55.8)	0.04
Tea (g/d)	0.00 (0.00-72.0)	0.00 (0.00-38.6)	0.49	0.00 (0.00-32.1)	0.40	5.93 (0.00-72.0)	0.46
Apples (g/d)	18.8 (4.61-48.1)	19.7 (4.61-39.4)	0.88	18.4 (4.63-39.4)	0.81	16.6 (6.67-39.4)	0.71
Serologic measures (in pg/mL)							
IL-6	1.89 (1.36-2.81)	1.77 (1.40-2.62)	0.94	2.04 (1.45-2.75)	0.19	1.91 (1.36-2.92)	0.78
Intervention (T1,2,3[¶]):							
NSAID use (% yes)[‡]	22	20	1.00	22	0.90	23	0.62
Supplement use (% yes)[‡]	35	29	0.35	28	0.17	29	0.07
Hormone therapy (% yes)[‡]	11	8	0.64	6	0.15	7	0.08

(*Continued on the following page*)

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Table 1. Characteristics of intervention group participants in the PPT by adenoma recurrence at the end of year 4 (n = 872) (Cont'd)

Characteristics	Adenoma recurrence (T4)						
	No	Advanced		High-risk		Any	
	Median (IQR) or %*	Median (IQR) or %*	$P^{\dagger}$	Median (IQR) or %*	$P^{\dagger}$	Median (IQR) or %*	$P^{\dagger}$
BMI (kg/m^2)	26.8 (24.3-29.6)	28.8 (25.6-32.2)	0.004	27.9 (25.2-30.6)	0.01	27.3 (24.6-30.0)	0.18
Physical activity (h/wk)[§]	7.84 (4.19-13.3)	5.13 (2.89-8.25)	0.002	6.92 (4.02-11.5)	0.11	7.51 (4.14-13.5)	0.42
Dietary intake							
Alcohol (g/d)	0.90 (0.00-8.76)	0.98 (0.00-4.93)	0.24	0.98 (0.00-5.63)	0.42	0.96 (0.00-8.26)	0.51
Energy (1,000 kcal/d)	1.78 (1.52-2.09)	1.82 (1.60-1.99)	0.95	1.80 (1.56-1.99)	0.92	1.79 (1.54-2.07)	1.00
Fat (% kcal/d)	22.4 (18.5-26.6)	27.4 (22.2-30.6)	0.0002	25.1 (20.9-30.0)	0.0003	22.9 (19.7-28.1)	0.03
Fat (g/d)	38.9 (31.2-50.0)	47.1 (38.1-55.2)	0.004	44.2 (36.8-52.8)	0.004	41.3 (32.8-51.4)	0.10
Fiber (g/d)	32.1 (24.0-40.8)	29.4 (21.5-37.7)	0.08	29.4 (21.6-36.2)	0.01	30.9 (22.8-39.0)	0.13
Fruits and vegetables (serv./d)	5.72 (4.43-7.15)	4.98 (4.06-6.03)	0.01	5.24 (4.36-6.56)	0.03	5.65 (4.48-6.99)	0.54
Flavonols (mg/d)	29.7 (21.4-40.8)	21.0 (15.0-30.1)	0.0002	25.4 (16.2-36.1)	0.005	29.7 (21.0-38.9)	0.59
Isorhamnetin (mg/d)	0.73 (0.42-1.10)	0.59 (0.30-0.89)	0.02	0.61 (0.29-0.98)	0.01	0.73 (0.44-1.12)	0.89
Kaempferol (mg/d)	13.9 (7.83-21.5)	8.27 (5.19-14.4)	0.0002	10.9 (5.40-18.5)	0.01	13.6 (7.89-20.9)	0.40
Myricetin (mg/d)	0.99 (0.68-1.63)	0.91 (0.62-1.49)	0.18	0.89 (0.56-1.57)	0.06	1.06 (0.65-1.68)	1.00
Quercetin (mg/d)	13.3 (9.70-16.8)	11.1 (7.55-14.7)	0.007	12.1 (8.01-15.0)	0.01	13.3 (9.58-16.6)	0.98
Dry beans (g/d)	31.2 (15.3-54.6)	14.0 (7.37-35.2)	0.0001	23.0 (8.79-42.1)	0.005	30.3 (14.4-49.5)	0.26
Other vegetables (g/d)[‖]	72.3 (37.7-110)	51.5 (23.5-89.5)	0.01	57.5 (22.3-97.7)	0.01	71.4 (42.6-109)	0.88
Tea (g/d)	11.9 (0.00-72.0)	3.96 (0.00-48.9)	0.19	3.96 (0.00-55.2)	0.16	9.23 (0.00-91.3)	0.49
Apples (g/d)	57.6 (29.3-89.7)	45.3 (19.7-61.4)	0.03	53.0 (23.9-82.3)	0.33	53.5 (29.2-88.5)	0.79
Serologic measures (in pg/mL):							
IL-6 (T1)	1.89 (1.29-2.72)	2.03 (1.64-2.86)	0.12	2.27 (1.63-3.19)	0.002	1.92 (1.38-2.90)	0.17
IL-6 (T3)	2.03 (1.40-3.07)	2.21 (1.65-3.20)	0.31	2.44 (1.71-3.50)	0.01	2.05 (1.55-2.94)	0.37
IL-6 (T1,3[¶])	1.96 (1.43-2.77)	2.11 (1.66-3.07)	0.12	2.33 (1.68-3.26)	0.002	2.05 (1.54-2.90)	0.21
IL-6 (T1-T0)	−0.06 (−0.56-0.39)	0.27 (−0.20-0.66)	0.02	0.12 (−0.29-0.70)	0.04	0.05 (−0.41-0.57)	0.05
IL-6 (T3-T0)	0.07 (−0.54-0.67)	0.23 (−0.19-0.80)	0.21	0.24 (−0.27-0.91)	0.19	0.16 (−0.42-0.76)	0.34
IL-6 (T1,3-T0[¶])	0.03 (−0.50-0.51)	0.29 (−0.13-0.61)	0.06	0.25 (−0.23-0.82)	0.04	0.10 (−0.33-0.58)	0.07

Abbreviations: IQR, interquartile range. NSAID, nonsteroidal anti-inflammatory drug; BMI, body mass index.

*Family history of colorectal cancer was defined as having ≥1 first-degree relative with colorectal cancer at baseline.

[†]All comparisons against the no adenoma recurrence group. P values for differences in proportions were calculated using Fisher's exact test. P values for differences in medians were calculated using Wilcoxon rank-sum test.

[‡]Regular dietary supplement use was defined as taking supplement ≥1 weekly. Regular medication use, including NSAIDs, was defined as taking medication ≥1 monthly. Hormone replacement therapy included unopposed estrogen and estrogen/progestin combinations.

[§]Physical activity was defined as self-reported time typically spent for any type of moderate or vigorous physical activity.

[‖]Other vegetables included 30% white onions, 5% red onions, 27% cucumbers, 16% celery, 12% radishes, and 10% pepper.

[¶]T1,2,3: mean values of the first 3 y of the trial. T1,3 = geometric mean of year 1 and year 3 values. T1,3-T0 = T1,3 minus baseline values.

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Table 2. Association between serum IL-6 concentrations and flavonol intake during the trial (n = 872)

IL-6 (pg/mL)	Flavonol intake quartiles (mg/d)*				$P^{\dagger}$
	Q1: <21.2	Q2: 21.2-29.7	Q3: 29.8-40.0	Q4: >40.0	
	Median (IQR)	Median (IQR)	Median (IQR)	Median (IQR)	
Sample size	218	218	218	218	
Baseline (T0)	2.02 (1.41-2.99)	1.90 (1.38-2.97)	1.79 (1.32-2.75)	1.95 (1.31-2.69)	0.30
Year 1 (T1)	2.05 (1.41-3.23)	2.00 (1.37-3.00)	1.77 (1.26-2.46)	1.83 (1.26-2.72)	0.008
Year 3 (T3)	2.30 (1.62-3.46)	2.07 (1.58-3.25)	1.99 (1.41-2.76)	1.84 (1.35-2.64)	0.0002
Trial (T1,3‡)	2.20 (1.61-3.19)	2.07 (1.54-2.98)	1.84 (1.37-2.51)	1.80 (1.39-2.60)	0.0002
Change (T1,3-T0‡)	0.17 (–0.30-0.60)	0.19 (–0.54-0.67)	–0.01 (–0.40-0.44)	0.01 (–0.51-0.48)	0.07

*Participants were grouped in quartiles (Q1-Q4) by mean flavonol intake during the first 3 trial years.

$^{\dagger}P$ values for differences in medians among the flavonol intake quartiles were calculated based on the Kruskal-Wallis test. The P value for trend using a multiple regression model adjusting for age tertiles (<58, 58-66, >66 y), sex, average BMI (<25, 25.0-29.9, ≥30 kg/m^2), current smoking status, and average energy intake (continuous) during the first 3 trial years was 0.01 for Trial (T1,3) and 0.10 for Change (T1,3-T0).

‡Trial (T1,3) = geometric mean of year 1 and year 3 values. Change (T1,3-T0) = T1,3 minus baseline values.

The association between flavonol intake (in quartiles) and IL-6 concentrations (linear) during the trial was evaluated using the Kruskal-Wallis test and multiple linear regression models. The same analyses were done within each baseline IL-6 tertile to determine whether participants with higher baseline IL-6 benefited from higher flavonol intake; a formal test for interaction was done using multiple linear regression modeling.

The association between flavonol intake during the trial and colorectal adenoma recurrence (no adenoma versus advanced, high-risk, or any adenoma) was measured with odds ratios (OR) and 95% confidence intervals (95% CI)

Table 3. Association between serum IL-6 concentrations and flavonol intake during the trial, stratified by baseline IL-6 concentrations (n = 872)

IL-6 (T0) tertiles* (pg/mL)	Flavonol intake quartiles (mg/d)*				$P^{\dagger}$
	Q1: <21.2	Q2: 21.2-29.7	Q3: 29.8-40.0	Q4: >40.0	
	Median (IQR)	Median (IQR)	Median (IQR)	Median (IQR)	
IL-6 (T0) <1.53*					
Sample size	65	71	79	76	
Trial (T1,3‡)	1.47 (1.15-1.84)	1.49 (1.19-1.90)	1.35 (1.01-1.72)	1.44 (1.07-1.75)	0.22
Change (T1,3-T0‡)	0.34 (0.00-0.58)	0.36 (0.02-0.69)	0.20 (–0.05-0.49)	0.30 (0.01-0.58)	0.15
IL-6 (T0) 1.53-2.37*					
Sample size	72	73	73	73	
Trial (T1,3‡)	2.07 (1.68-2.52)	2.12 (1.79-2.69)	1.93 (1.62-2.49)	1.90 (1.52-2.50)	0.29
Change (T1,3-T0‡)	0.22 (–0.17-0.51)	0.33 (–0.22-0.76)	0.12 (–0.31-0.49)	–0.08 (–0.37-0.42)	0.13
IL-6 (T0) >2.37*					
Sample size	81	74	66	69	
Trial (T1,3‡)	3.39 (2.45-4.66)	3.14 (2.15-4.90)	2.72 (2.09-4.11)	2.63 (1.77-3.56)	0.01
Change (T1,3-T0‡)	–0.26 (–1.15-0.66)	–0.90 (–2.31-0.26)	–0.76 (–1.91-0.15)	–0.75 (–1.65-0.01)	0.05

*Participants were grouped in tertiles by their baseline IL-6 concentrations (T0) and in quartiles (Q1-Q4) by mean flavonol intake during the first 3 trial years.

$^{\dagger}P$ values for differences in medians among the flavonol intake quartiles within baseline IL-6 tertiles were calculated based on the Kruskal-Wallis test. The P value for trend using a multiple regression model adjusting for age tertiles (<58, 58-66, >66 y), sex, average BMI (<25, 25.0-29.9, ≥30 kg/m^2), current smoking status, and average energy intake (continuous) during the first 3 trial years was 0.008 for Trial (T1,3) and 0.02 for Change (T1,3-T0) for the highest baseline IL-6 tertile.

‡Trial (T1,3) = geometric mean of year 1 and year 3 values. Change (T1,3-T0) = T1,3 minus baseline values.

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Table 4. Association between flavonol intake during the trial and colorectal adenoma recurrence among intervention group participants of the PPT (*n* = 872)

Flavonol quartiles* (mg/d)	Adenoma recurrence (T4)						
	No	Advanced		High-risk		Any	
	n (%)	*n* (%)	OR (95% CI)†	*n* (%)	OR (95% CI)†	*n* (%)	OR (95% CI)†
<21.2	128 (58.7)	25 (11.5)	1.00‡	40 (18.3)	1.00‡	90 (41.3)	1.00‡
21.2-29.7	134 (61.5)	11 (5.0)	0.35 (0.16-0.76)	22 (10.1)	0.49 (0.27-0.90)	84 (38.5)	0.89 (0.60-1.33)
29.8-40.0	124 (56.9)	8 (3.7)	0.32 (0.13-0.77)	19 (8.7)	0.54 (0.28-1.01)	94 (43.1)	1.16 (0.78-1.72)
>40.0	138 (63.3)	5 (2.3)	0.17 (0.06-0.50)	19 (8.7)	0.51 (0.26-0.98)	80 (36.7)	0.96 (0.63-1.46)
P-trend§			0.0002		0.02		0.87

*Flavonol intake in quartiles (Q1-Q4) by the mean consumption during the first 3 trial years.

†Multivariate OR and 95% CI models were adjusted for age tertiles (<58, 58-66, >66 y), sex, average BMI (<25, 25.0-29.9, ≥30 kg/m^2), and average energy intake (continuous) during the first 3 trial years.

‡Reference category.

§The ln-transformed median intakes (in mg/d) of each quartile were used as a continuous score variable to determine the *P* value for trend.

using logistic regression. ORs were estimated within quartiles of flavonol intake, using the first quartile as the reference category. A test for trend was done by treating log-transformed median values of each quartile as a continuous variable in a logistic regression model. A similar analysis was used to estimate the association between IL-6 concentration and colorectal adenoma recurrence except that the highest quartile was used as the reference category as compared with the lowest quartile. For examining the combined effect of flavonol intake and IL-6 concentrations (during the trial and at baseline) on colorectal adenoma recurrence, the data were stratified by the

Table 5. Association between serum IL-6 concentrations and colorectal adenoma recurrence among intervention group participants of the PPT (*n* = 872)

IL-6 quartiles* (pg/mL)	Adenoma recurrence (T4)						
	No	Advanced		High-risk		Any	
	n (%)	*n* (%)	OR (95% CI)†	*n* (%)	OR (95% CI)†	*n* (%)	OR (95% CI)†
Trial (T1,3)							
Q1: >2.80	125 (57.6)	15 (6.9)	1.00	35 (16.1)	1.00	92 (42.4)	1.00
Q2: 1.99-2.80	127 (58.3)	12 (5.5)	0.79 (0.34-1.81)	26 (11.9)	0.71 (0.39-1.27)	91 (41.7)	1.01 (0.68-1.49)
Q3: 1.47-1.98	131 (60.1)	16 (7.3)	1.19 (0.53-2.63)	26 (11.9)	0.82 (0.45-1.48)	87 (39.9)	1.01 (0.68-1.49)
Q4: <1.47	141 (64.4)	6 (2.7)	0.51 (0.19-1.41)	13 (5.9)	0.45 (0.22-0.92)	78 (35.6)	0.95 (0.63-1.42)
P-trend‡			0.38		0.05		0.80
Change (T1,3-T0)							
Q1: >0.55	123 (56.7)	14 (6.5)	1.00	33 (15.2)	1.00	94 (43.3)	1.00
Q2: 0.06-0.55	129 (59.2)	17 (7.8)	1.39 (0.63-3.06)	24 (11.0)	0.76 (0.42-1.41)	89 (40.8)	0.98 (0.66-1.45)
Q3:-0.43-0.05	131 (60.1)	10 (4.6)	0.83 (0.34-2.00)	24 (11.0)	0.79 (0.43-1.45)	87 (39.9)	0.98 (0.66-1.45)
Q4: <–0.43	141 (64.4)	8 (3.7)	0.47 (0.19-1.18)	19 (8.7)	0.44 (0.23-0.84)	78 (35.6)	0.72 (0.48-1.06)
P-trend‡			0.06		0.02		0.09

*IL-6 concentrations in quartiles (Q1-Q4) of the geometric mean values of year 1 and year 3 values [Trial (T1,3)] and of the change in IL-concentrations from baseline [Change (T1,3-T0)].

†Multivariate OR and 95% CI models were adjusted for age tertiles (<58, 58-66, >66 y), sex, average BMI (<25, 25.0-29.9, ≥30 kg/m^2), and current smoking status during the first 3 trial years.

‡The ln[ln(value + 1)]–transformed median concentrations of each quartile were used as a continuous score variable to determine the *P* value for trend for the IL-6 concentrations at T1,3. The median concentrations of each quartile were used to determine the *P* value for trend for the change in IL-6 concentrations (T1,3-T0).

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median values of flavonol intake and IL-6 concentrations (≤median, >median).

For all logistic and multiple regression models, potential confounders (listed in Table 1) were added to the models in a stepwise fashion; if a confounder changed the association by >10%, was associated with both study variables, and had a χ^2 P value ≤0.20, it remained in the model. All P values correspond to two-sided tests. Statistical tests were considered to be significant when $P \leq 0.05$ and considered to be a trend toward significance when $0.05 < P \leq 0.10$.

Results

At the end of the 4-year trial, 39.9% of participants had ≥1 adenoma, 11.5% had high-risk adenoma, and 5.6% had ≥1 advanced adenoma (Table 1). Adenoma recurrence was more common in men, older individuals, and individuals who consumed more fat during the first 3 years of the trial, and less common in women who used hormone therapy. High-risk and advanced adenoma recurrence were associated with lower fruit and vegetable, flavonol, isorhamnetin, kaempferol, quercetin, dry bean, and other vegetable intakes during the first 3 years of the trial. High-risk adenoma recurrence was also associated with lower fiber consumption. The intervention increased consumption of flavonols (change in medians: 14.6-29.7 mg/d), fiber (17.1-31.5 g/d), fruits and vegetables (3.5-5.7 servings/d), and especially of dry beans, the primary flavonol contributor (7.5-30.5 g/d), and decreased the percentage of calories from fat consumed (35.6-22.6% kcal). Serum IL-6 concentrations during the trial were strongly correlated ($r_{T1,3}$ = 0.86) and were combined using the geometric mean.

Intake of flavonols, especially of isorhamnetin, kaempferol, and quercetin, and flavonol-rich foods was inversely associated with serum IL-6 concentrations (highest versus lowest flavonol intake quartile, 1.80 versus 2.20 pg/mL; Table 2; Supplementary Tables S1 and S2); these associations were more pronounced in participants with the highest baseline IL-6 tertile (for flavonols, 2.63 versus 3.39 pg/mL; Table 3: Supplementary Tables S3 and S4). Flavonol intake was associated with caloric intake (r = 0.39), percent of calories from fat (r = −0.45), and fiber (r = 0.60), fruit and vegetable (r = 0.53), and dry bean intakes (r = 0.89); except for caloric intake, all of these were associated with serum IL-6 during the trial (Supplementary Table S2). Flavonol and dry bean intakes were the only dietary factors associated with change in IL-6 from baseline (Supplementary Table S2). Dietary factors did not confound the association between

Table 6. Association between flavonol intake by serum IL-6 concentrations and colorectal adenoma recurrence among intervention group participants of the PPT (n = 872)

Flavonol* (mg/d)	IL-6† (pg/mL)	Adenoma recurrence (T4)						
		No	Advanced		High-risk		Any	
		n (%)	*n* (%)	OR (95% CI)‡	*n* (%)	OR (95% CI)‡	*n* (%)	OR (95% CI)‡
Mean (T1,2,3)	*Baseline (T0)*							
Low ≤29.7	High >1.90	131 (58.0)	16 (7.1)	1.00	38 (16.8)	1.00	95 (42.0)	1.00
	Low ≤1.90	131 (62.4)	20 (9.5)	1.57 (0.75-3.27)	24 (11.4)	0.81 (0.45-1.48)	79 (37.6)	0.94 (0.63-1.40)
High >29.7	High >1.90	128 (61.2)	6 (2.9)	0.42 (0.15-1.15)	15 (7.2)	0.47 (0.24-0.93)	81 (38.8)	0.97 (0.65-1.45)
	Low ≤1.90	134 (59.0)	7 (3.1)	0.68 (0.25-1.86)	23 (10.1)	0.99 (0.52-1.89)	93 (41.0)	1.30 (0.87-1.95)
Mean (T1,2,3)	*Trial (T1,3)*							
Low ≤29.7	High >1.99	144 (58.5)	20 (8.1)	1.00	43 (17.5)	1.00	102 (41.5)	1.00
	Low ≤1.99	118 (62.1)	16 (8.4)	1.22 (0.58-2.54)	19 (10.0)	0.65 (0.35-1.20)	72 (37.9)	0.99 (0.66-1.47)
High >29.7	High >1.99	108 (57.1)	7 (3.7)	0.51 (0.20-1.31)	18 (9.5)	0.64 (0.33-1.23)	81 (42.9)	1.19 (0.80-1.79)
	Low ≤1.99	154 (62.3)	6 (2.4)	0.40 (0.15-1.07)	20 (8.1)	0.64 (0.34-1.19)	93 (37.7)	1.11 (0.75-1.64)
Mean (T1,2,3)	*Change (T1,3-T0)*							
Low ≤29.7	High >0.06	138 (58.2)	22 (9.3)	1.00	36 (15.2)	1.00	99 (41.8)	1.00
	Low ≤0.06	124 (62.3)	14 (7.0)	0.72 (0.35-1.51)	26 (13.1)	0.78 (0.43-1.40)	75 (37.7)	0.86 (0.58-1.27)
High >29.7	High >0.06	114 (57.6)	9 (4.6)	0.62 (0.26-1.49)	21 (10.6)	0.90 (0.47-1.72)	84 (42.4)	1.20 (0.80-1.80)
	Low ≤0.06	148 (62.2)	4 (1.7)	0.19 (0.06-0.58)	17 (7.1)	0.51 (0.27-0.99)	90 (37.8)	0.98 (0.67-1.45)

*Flavonol intake below or above the median of the mean intake during the first 3 trial years (T1,2,3).

†IL-6 concentrations below or above the median baseline IL-6 concentration (T0), of the median trial IL-6 concentration (T1,3), and of the median change in IL-6 concentrations from baseline [Change (T1,3-T0)].

‡Multivariate OR and 95% CI models were adjusted for age tertiles (<58, 58-66, >66 y), sex, average BMI (<25, 25.0-29.9, ≥30 kg/m^2), current smoking status, and average energy intake (continuous) during the first 3 trial years.

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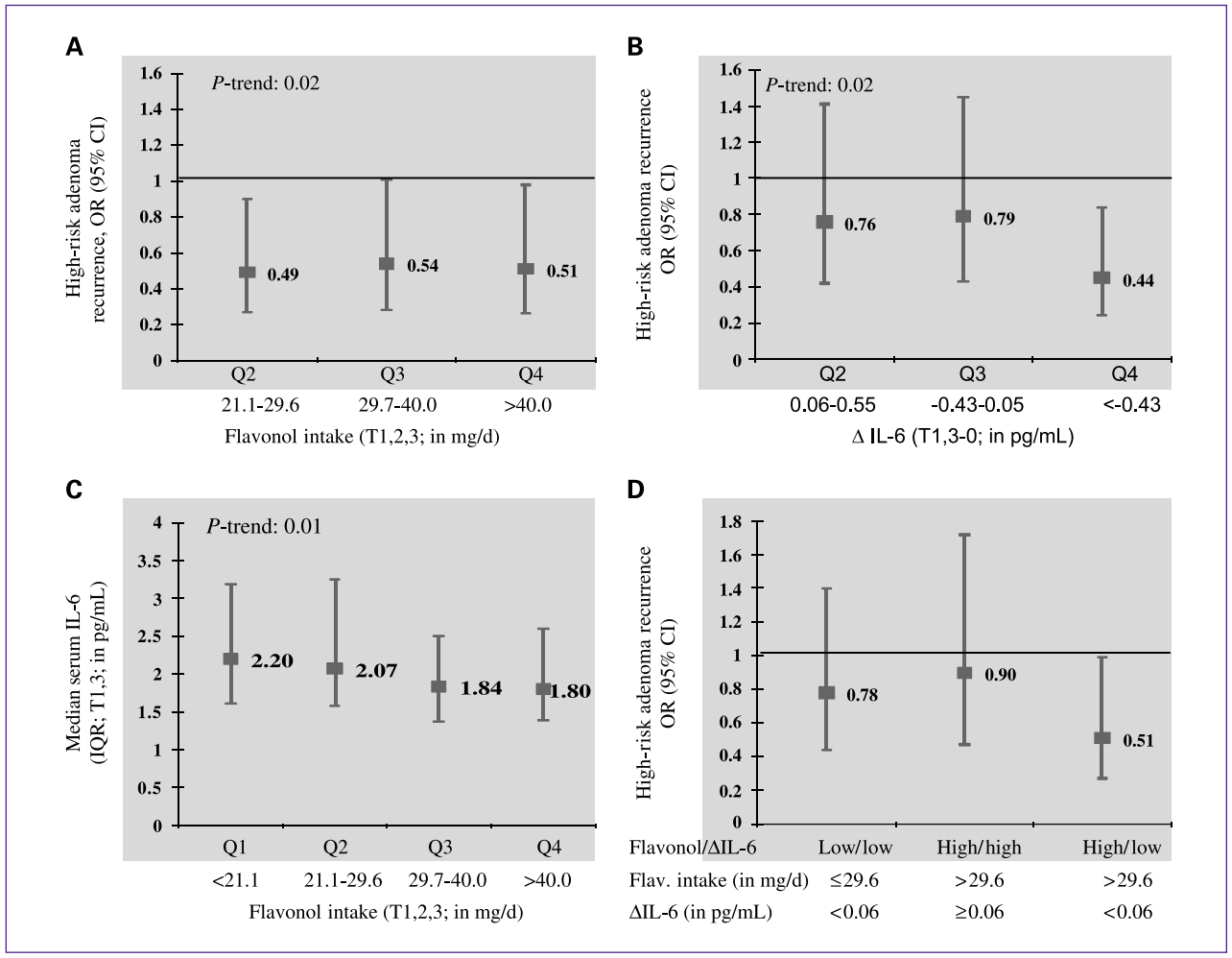

Fig. 1. Summary of the association between flavonol intake, serum IL-6 concentration, and high-risk adenoma recurrence. Higher flavonol intake (A) and a decrease in serum IL-6 concentrations during the trial (B) were both associated with decreased incidence of high-risk adenoma recurrence and with each other (C), with the greatest reduction of high-risk adenoma recurrence when both were combined (D).

flavonol intake and adenoma recurrence. Higher flavonol intakes were inversely associated with advanced adenoma (4th versus 1st quartile: OR, 0.17; 95% CI, 0.06-0.50; *P*-trend = 0.0002) and high-risk adenoma recurrence (OR, 0.51; 95% CI, 0.26-0.98, *P*-trend = 0.02; Table 4). A decrease in IL-6 concentration during the trial was inversely associated with high-risk adenoma recurrence (OR, 0.44; 95% CI, 0.23-0.84; *P*-trend = 0.02) and suggestively with advanced (OR, 0.47; 95% CI, 0.19-1.18, *P*-trend = 0.06) and any adenoma recurrence (OR, 0.72; 95% CI, 0.48-1.06, *P*-trend = 0.09; Table 5). Similar risks were observed for IL-6 concentrations during the trial (Table 5).

Higher flavonol intakes were inversely associated with high-risk adenoma recurrence in participants with above median baseline IL-6 concentrations (above versus below median flavonol intake: OR, 0.47; 95% CI, 0.24-0.93; *P* = 0.03; Table 6), but not in participants with equal or below median baseline IL-6 concentrations (*P*-interaction = 0.04). Individuals with above median flavonol intake and equal or below median IL-6 change had the lowest risk of recurrence of advanced (OR, 0.19; 95% CI, 0.06-0.58) and high-risk adenomas (OR, 0.51; 95% CI, 0.27-0.99) compared to individuals with equal or below median flavonol intake and above median serum IL-6 concentrations (Table 6). Estimates of flavonol intake and change in IL-6 concentration on advanced or high-risk adenoma recurrence remained similar after mutually adjusting for each other. The main finding of this study, shown in Fig. 1, is that higher flavonol intakes and a reduction in serum IL-6 concentrations during the trial were associated both with decreased incidence of high-risk adenoma recurrence and with each other, with the greatest reduction in high-risk adenoma recurrence when the two were combined.

Discussion

There is a need for biomarkers that can identify individuals at increased risk or in early stages of colorectal

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carcinogenesis and that can be modified by diet. We examined whether serum IL-6 was associated with both colorectal adenoma recurrence and flavonol intake and thus may serve as an indicator of dietary response. A reduction in serum IL-6 concentrations during the trial tended to be associated with a decreased risk of adenoma recurrence and higher flavonol intake. The greatest risk reduction for the more progressive forms of adenoma recurrence (high-risk and advanced adenoma) was observed in participants that had higher flavonol intake and a decreased IL-6 concentration during the trial. Thus, our results suggest that IL-6 may serve as a risk indicator and as a response indicator to dietary flavonols for colorectal cancer prevention.

Previously, we had shown that higher flavonol intake was associated with a 76% decreased risk of advanced adenoma recurrence in the PPT (5). The risk reduction was even greater in the intervention arm, which had a greater range of flavonol intake (5); thus, we restricted this serum study to samples from intervention-arm participants. For the first time, we examined the effect of flavonol intake on high-risk adenoma recurrence. Higher flavonol intakes during the trial were associated with a decreased risk of advanced and high-risk adenoma recurrence, suggesting that high flavonol consumption may primarily inhibit adenoma progression. Similarly, vegetable intake is more strongly associated with colorectal cancer than with adenoma risk (25), suggesting that more progressed tumor cells are more sensitive to flavonols, as shown in cell culture (26, 27).

Serum and tumor tissue IL-6 concentrations are primarily associated with stage and progression of advanced colorectal cancer (28–31). Similar to our findings, high plasma IL-6 concentration was positively associated with colorectal adenoma in a colonoscopy-based cross-sectional study in North Carolina (16). Three cohort studies with limited power previously prospectively examined the association between circulating IL-6 concentration and risk of colorectal cancer. Similar to our findings, high serum IL-6 tended to be positively associated with colorectal cancer (continuous OR for unit change on the log scale, 1.44; 95% CI, 0.90-2.31) in the U.S. Health Aging and Body Composition Cohort (2,438 men and women, 40 cases; ref. 17). In contrast, no association was observed in two British studies (4,286 women, 32 cases; 2,398 men, 30 cases; ref. 32). IL-6 may play a key role in inflammation and colorectal carcinogenesis, as it coordinates and orchestrates the innate and adaptive immune response and promotes the survival of premalignant epithelial cells (11–13). Bacteria, viruses, and cytokines, such as IL-1β and tumor necrosis factor α, induce expression and secretion of IL-6, which induces the production of immunoglobulins and acute-phase proteins such as C-reactive protein (13). Thus, circulating IL-6 concentrations may be a potential indicator for increased risk or early stages of colorectal carcinogenesis.

The lowest risk for higher-grade adenomas was observed in participants that had higher flavonol intake and had decreased their IL-6 concentrations during the trial. Previously, we had shown in an animal model that nondetectable serum IL-6 concentrations were associated with a high dry bean diet, a major source of flavonols in the PPT, and the absence of preneoplastic lesions (33). Using the same USDA flavonol food database (23), Chun et al. (15) had shown that high flavonol consumption was associated with lower serum C-reactive protein concentrations in 8,809 U.S. adults. In contrast, plasma IL-6 and C-reactive protein concentrations were not associated with flavonol consumption in 344 nondiabetic women from the U.S. Women's Health Study (14); however, the authors used an older, smaller flavonol database. There is a general trend that diets rich in fruits and vegetables, thus rich in flavonols, and low in meat and fat are associated with lower IL-6 and C-reactive protein concentrations in blood (refs. 34, 35, and this study); thus, IL-6 may also be useful as an indicator for dietary colorectal cancer prevention.

Flavonols attenuated inflammation-induced IL-6 gene expression and release of IL-6 at dietary concentrations of 0.1% in animal models (9) and at concentrations of 1 μmol/L or more in cell culture (36–38). The effects of flavonols on the immune system were not reported to be specific to IL-6, but also not a general physiologic effect (9, 36–38), which is similar to our observations. In humans, flavonol supplementation decreased *ex vivo* tumor necrosis factor-α and IL-8 secretion after an inflammatory challenge more strongly in individuals with elevated cytokine concentrations than in healthy controls (39). In agreement, we showed that higher flavonol intake especially helped participants with higher baseline IL-6 concentrations to decrease their IL-6 concentrations and to reduce their risk of advanced and high-risk adenoma recurrence. Therefore, higher flavonol consumption may especially benefit persons with elevated IL-6 concentrations for prevention of colorectal cancer. This does not mean that flavonols are exclusively protective at high IL-6 concentrations as shown in cell culture (40) and with the decreased incidence of advanced adenoma recurrence in individuals with low baseline IL-6 concentrations in this study.

A major strength of this study was the information on adenomas from complete colonoscopies performed at baseline, year 1, and year 4, as well as histologic characteristics noted by two pathologists independently, decreasing the risk of misclassification. A second strength of this study included the prospectively collected and repeated serum measures; this is one of the first prospective studies to examine repeated measures, and thus changes, in IL-6 on adenoma recurrence. A third strength of this study was the prospective assessment of dietary flavonol intake using a dietary questionnaire that was developed specifically for this study to focus on vegetable and fruit consumption (21, 22). Furthermore, the dietary questionnaire was administered annually and reviewed by registered dietitians, which further improved its accuracy (21, 24), and was linked to the recently released validated USDA flavonoid database (23). Other strengths of this study include the large range of flavonol consumption in this population (5).

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There are, however, several limitations to our study. Our study findings may not apply to the general population because all participants had a history of adenomas and a flavonol intake often greater than what is commonly consumed in the United States (30 mg/d in our study versus 8-12 mg/d in the general U.S. population)[6] and most individuals engaged in a health-promoting lifestyle. Dietary exposure measurement error related to the exposure assessment method (e.g., no direct measure of dietary flavonol exposure) and the flavonol food database are likely to be present and could lead to inaccurate risk estimates. The FFQ was not specifically designed to estimate flavonol intake and the flavonol estimates from the FFQ have not been validated by other means of dietary assessment. Participants in the intervention arm of the study were aware what dietary patterns were expected from them and might have answered the FFQ during the trial according to the expectation of the registered dietitian (41). Limitations in the flavonol food database and variation in the growing, harvesting, storage, and preparation methods and food quantities may cause measurement error and might have led to attenuated risk estimates. Another weakness of this study include the high coefficients of variation for IL-6, which could be because many IL-6 values were close to the detection limit (33) and because heterophilic antibodies can lead to too high or too low IL-6 values (42–45). To counteract this limitation, which is also observed in other studies (14, 16, 17, 33, 46), we analyzed each sample in duplicate, used the same plate lot for all samples, combined serum IL-6 concentrations from years 1 and 3, and ran all serum samples of each participant on the same plate.

Flavonols may exert their anticarcinogenic effects through multiple mechanisms (5), some of them inflammation associated and only one of them being IL-6 inhibition. Furthermore, flavonols may not directly affect IL-6 levels but rather the presence of a higher-grade adenoma, although results in cell culture would suggest otherwise (40). Serum IL-6 concentrations are not specific to the location, strength, and type of inflammation, they fluctuate during the day, and they may be influenced by multiple factors (18). Combining IL-6 with other inflammatory markers in serum may provide a more complete picture of inflammation-associated colorectal carcinogenesis. Foods and other food components that are colinear with flavonol consumption may also be associated with serum IL-6 (refs. 34, 35; Supplementary Tables S1-S4). The most likely scenario is that multiple bioactive food components act synergistically on serum IL-6 concentration and on adenoma recurrence, as IL-6 plays a critical role in inflammation-promoted carcinogenesis (13). Thus, we cannot confirm that serum IL-6 may be a good indicator of just flavonol cancer prevention.

In conclusion, our results suggest that high flavonol intakes and a decrease in IL-6 concentrations during the trial reduced the risk of more progressive forms of adenoma recurrence, with the greatest benefit in those with both. Verification of these results in other prospective cohorts with high-quality dietary, biomarker, and IL-6 measures is needed to clarify the role of serum IL-6 concentrations (total and change) as one of possibly several indicators for cancer prevention by dietary flavonols and other chemopreventive foods or food components.

Appendix

The members of the Polyp Prevention Study Group participated in the conduct of the PPT. However, the data presented in this article and the conclusions drawn from them are solely the responsibility of the above-listed co-authors.

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Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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